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THE CLASSIFICATION OF SYMPATRIC FORMS OF BOTTLENOSE DOLPHINS (GENUS *TURSIOPS*) IN CHINESE WATERS

By

JOHN YU-CHAO WANG, M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

THE CLASSIFICATION OF BOTTLENOSE DOLPHINS (GENUS *TURSIOPS*)

In memory of

Dr. David E. Gaskin,

who believed in me

and gave me the opportunity to pursue my interests in biology

and

Dr. J. Stephen Leatherwood,

who provided energy, help and endless encouragement

during times of frustration

Descriptive Note

DOCTOR OF PHILOSOPHY (1999) **McMaster University**

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TITLE. The Classification of Sympatric Forms of Bottlenose Dolphins (Genus: *Tursiops*) in Chinese Waters

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Abstract

The classification within bottlenose dolphins (genus: *Tursiops*) is controversial. The prevalent view is that of a single species. To test this hypothesis, osteological, molecular and external morphological data from two sympatric forms of bottlenose dolphins in Chinese waters were examined.

Multivariate cluster analysis of meristic osteological characters identified two distinct groups that were consistent with the field classifications of the specimens into two forms. The main contributor to the separation of the two groups was total number of vertebrae. Cluster analyses of principal components scores for cranial morphometric data resulted in two distinct groups that agreed completely with the meristic analysis and field classifications. A series of discriminant analyses revealed highly significant differences in cranial characters between the two forms, helped to identify important characters for an identification key and generated classification functions for separating the two forms.

A portion of the mitochondrial DNA control region (386 base pairs) from 47 specimens was analysed. Separation of the haplotypes of the two forms was revealed independently by maximum likelihood, neighbor-joining and maximum parsimony analyses. Separation of the two forms in Chinese waters was also supported by bootstrap and consensus values, a large divergence of ~4.4%, and seven fixed site differences.

Congruence between the osteological and molecular data provided strong evidence that reproductive isolation between the two sympatric forms exists. According to the Biological Species Concept, the two forms in Chinese waters represent separate species. Furthermore, the results also satisfied the criteria of other species concepts.

To determine if these species can be differentiated externally, several morphological characters were subjected to a discriminant analysis. The results were highly significant. Examining individual characters revealed non-overlapping distributions in rostrum length and rostrum length as a proportion of total body length or snout-to-eye length. These characters are potentially useful for identifying free-ranging dolphins.

Preface

This thesis contains five chapters. The first and last chapters are a general introduction and discussion, respectively, that were written by the candidate. The other three chapters were written with co-authors, were formatted for submission to peer-reviewed scientific journals and are self-contained manuscripts with individual reference sections. An appendix at the end of the thesis lists all the specimens that were examined and their classifications.

Chapter 2: Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters.

Authors: John Y. Wang, Lien-Siang Chou and Bradley N. White

Status: Submitted to the Journal of Zoology for review.

Contribution: Specimens and data were collected mainly by J.Y.W. Data analysis and the writting of this paper were performed by J.Y.W. L.-S. Chou provided logistic and financial support. The research was conducted under the supervision and guidance of B.N.W.

Chapter 3: Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters

Authors: John Y. Wang, Lien-Siang Chou and Bradley N. White

Status: Submitted to Molecular Ecology for review.

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Chapter 4: Differences in the external morphology of two sympatric species of bottlenose dolphins (genus:

Tursiops) in Chinese waters

Authors: John Y. Wang, Lien-Siang Chou and Bradley N. White

Status: Submitted to the Journal of Mammalogy for review.

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As with any long-term and effort-intensive project, there are numerous people to thank. However, the lengthier a project and the greater the number of people involved, the more likely someone will be excluded accidentally. Furthermore, the larger the list of acknowledged people, the less appreciated those that are omitted will feel. So I would like to start by thanking those that I may have forgotten below, not because their contributions were not significant, but because substantial ageing during this degree has taken a toll on my memory.

I am most grateful to my supervisor, Bradley White, who provided me with the freedom and independence to conduct research that was of interest to me. His trust and belief in my abilities and our discussions about science and politics as peers gave me the strength and confidence to tackle many difficult issues during my studies. Spending much time among people in an educational system of a nation with very different cultural and political backgrounds has made me more appreciative of my relationship with my supervisor and the educational system in Canada. I would also like to thank my other committee members, Jim Quinn and Lisle Gibbs, who have expressed real interest in my work and offered much useful advice.

My thesis research would not have been possible without Lien-Siang Chou. Her tenacity for getting logistical problems resolved, especially with Taiwan's bureaucracy and government authorities, helped me tremendously in data and sample collection. Although we follow different paths, I believe our ultimate goal is similar.

My gratitude to the numerous people who participated in data and sample collection can not be over-emphasized. Many worked through foul weather, poor facilities, stressful situations, long hours for many consecutive days, screwed-up biorhythms (eating and sleeping at unusual times), physically and mentally strenuous expeditions, necropsy "festivals", the "flavourful" task of preparing skeletons, and sea-sickness. A full appreciation of the conditions under which they had to work can only be gained by experience. In

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I also have numerous people to thank in our department. All my labmates, especially Joyce Marsolais, Brent Murray, Diana Polley and Monica Marcinko-Kuehn. Joyce was always there for me. Brent helped with the lab work of some of my many other interests and was always eager to discuss science. Diana was always happy to see me after I returned from a long trip and dealt with some of my paperwork while I was away. Monica always got my mind off thesis writing (a good thing!) at least for a short time by discussing orchid cultivation. In addition, Dr. S. Dudley provided invaluable statistical advice, encouragement, direction and reviews of manuscripts; F. Yazdani was incredibly generous with his advice on molecular analyses and friendship; Dr. W.F. Perrin provided a greatly appreciated thorough review of the thesis as the external examiner; and M. Bassoi provided several original illustrations. All the secretaries in the main office (especially Kathy G.) and P. Hayward were incredibly helpful regardless of the bureaucratic mess I found myself in and they have never yelled at me nor kicked me out of the office.....yet!

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John Y. Wang

January 10, 1999

(~10,000m over the North Pacific Ocean)

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Chapter 2

Scope of Thesis

How organisms are classified not only reflects our understanding of biology but also influences how we perceive and interpret biological information. Classification has also become the basis for the conservation of biodiversity. Therefore, objectivity in the process of recognizing taxa, especially species, has been debated for a long time and has been scrutinized by conservation biologists. Bottlenose dolphins (genus *Tursiops*) are one group of marine mammals that has suffered from a lack of objectivity in classification. This thesis is an examination of the classification within bottlenose dolphins (genus *Tursiops*). To avoid the pitfalls of previous studies, the hypothesis that sympatric forms of bottlenose dolphins in Chinese waters represent a single species will be tested under the theoretical framework of the Biological Species Concept by comparing morphological and molecular characters. Congruent results from independent data sets would suggest reproductive isolation between the sympatric forms, and under the criterion of the Biological Species Concept (the most widely accepted species definition), the two forms would represent distinct species. As a result, the accepted view of a monotypic genus would not be supported. In addition, the data will be considered under other species concepts to determine whether the *Tursiops* forms also satisfy other definitions of species.

Confident identification of specimens is important for research and conservation policy (e.g., if two species are not easily distinguishable, there would be difficulties in law enforcement, monitoring of exploitation, setting quotas, etc.). To facilitate differentiating the two forms, each of the three main chapters in this thesis highlights the characters that are important in the discrimination of the species. In the osteology study (Chapter 2), a key and the classification functions from a discriminant analysis are presented. For molecular data, fixed diagnostic site differences between the two forms are shown (Chapter 3). And for situations where osteological and molecular data are not available (e.g., free-ranging dolphins, photographs, etc.) or where rapid identification of specimens is needed, diagnostic external characters are summarized in Chapter 4.

Chapter 1

General Introduction

Importance of species

Defining the boundaries of species is one of the most fundamental tasks in biology. It reflects and influences our perceptions and understanding of organisms, their evolutionary histories and biological processes. In addition, it has also become integral to conservation since most legislation either affords protection or assigns higher importance to species than to other discrete but subordinative entities (O'Brien, 1994). Because there is no consensus on the definition of species and the process of determining species status is being perceived with increasing scrutiny (Rojas, 1992; Sites and Crandall, 1997), it is likely that more species designations will be debated and decided in a judicial setting, especially in nations where conservation legislation allow for civil suits. Therefore, not only do interpretations of biological data have to be within reasonable scientific uncertainties but they also have to be legally justifiable.

For scientifically and legally defendable species, testing of clearly-defined *a priori* hypotheses based on the criterion(a) of a specific species concept is required. By doing so, the task of validating species becomes objective and divorced from the more esoteric issue of what is the "best" concept. Resolutions to the latter question will likely be more subjective and dependent upon specific applications (e.g., the Biological Species Concept is widely accepted for sexually reproducing species but is inappropriate for asexual or extinct organisms). In fact, many concepts were formulated by researchers who found that existing definitions were inadequate for their needs. There are numerous definitions of species (for an overview, see King, 1993) but some never gained serious consideration. Here, I review briefly the definitions and limitations of the most commonly encountered concepts. However, I return to the Biological Species Concept (BSC) and explain why this definition was chosen for examining the classification within bottlenose dolphins (genus *Tursiops*).

Species definitions

The longest-enduring species definition is the Typological (also known as the Linnaean or Morphological) Species Concept. The basis of this concept is that morphological differences between species are clearly defined and unchanging over time. With this idea, Linnaeus also introduced the binomial system for naming species. Subsequently, rules and the International Commission of Zoological (or Botanical) Nomenclature were established formally to regulate the scientific naming of species under Linnaean's system and remain little changed today. The concept provided an organized method of categorizing and identifying organisms. However, it has serious limitations in its treatment of polymorphic and sibling (or morphologically indistinguishable) species.

The Biological Species Concept (BSC) defines species as "groups of actually or potentially interbreeding natural populations which are also reproductively isolated from other such groups" (Mayr, 1942). For sexually reproducing organisms, the BSC remains the dominant and most pervasive definition. It offers testability (Sites and Crandall, 1997) and is perceived to reflect natural divisions (King, 1993). The main criticisms of this definition include: 1) its treatment of allopatric populations; 2) using morphological differences to infer reproductive isolation and the fact that even if mating experiments were performed, the results would provide little to no information regarding organisms *in situ*; 3) inapplicability to asexual or extinct organisms; 4) difficulties with hybridization; and 5) the fact that it is a concept that is static in time and confined to the present (actually, the very recent past).

There are two major versions of the Evolutionary Species Concept (ESC) and both were designed to include asexual and extinct organisms. The first was formulated by Simpson (1961) to account for the continuity in the evolutionary process. Simpson's definition was a reflection of successional changes within a lineage or phyletic evolution and as such was dismissed by present phylogeneticists who view speciation as a dichotomous phenomenon. Wiley's (1978) definition was very different in that phyletic evolution and the need for morphological or phenetic differences was eliminated. Presently, Wiley's ESC is most commonly acknowledged and used. Both definitions, however, are vague in specified criteria for recognizing evolutionary species and thus operationally difficult for objective testing of potential species (see King, 1993; Sites and

Crandall, 1997). While theoretical debates of species definitions may not require consideration for operational criteria, conservation and applied research needs (and the general public) would have little use and acceptance of any concept that does not allow species to be defined operationally.

The Phylogenetic Species Concept (PSC) is a lineage-based definition originating from cladistic theory. The version encountered most commonly is accredited to Cracraft (1983) who described a phylogenetic species as, "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent". In other words, species are defined by the sharing of diagnostic, derived characters that are also inheritable. Vogler and DeSalle (1994) advocated this species concept and also suggested the same definition for the Evolutionary Significant Unit (ESU) but recommended that the ESU not be equated to "species". Their reasons for this recommendation was unclear. By their own interpretation of the PSC, if a cluster of organisms is "diagnosably distinct", it is both a phylogenetic species and an ESU. Therefore, their recommended approach for recognizing ESUs (and its limitations) should also be applicable to delimiting phylogenetic species.

In addition to the operational limitations of cladistic methodology (which is beyond the scope of this thesis), there are other serious problems with the PSC and approach recommended by Vogler and DeSalle (1994). To rectify the problem of having to designate every lineage as a distinct species under the phylogenetic species definition, Vogler and DeSalle distinguished between "characters", which are phylogenetically informative, and "traits", which differentiate individuals in a tokogenetic system. Only "characters" were considered important for determining conservation units. "Characters" were defined by the criterion of diagnosability (Davis and Nixon, 1992) or in the words of Vogler and DeSalle as "only those attributes that are shared by all members in a potential conservation unit to the exclusion of other units". By defining an ESU as a population with "characters" and then defining "characters" as diagnostic attributes that belong to an ESU, Vogler and DeSalle created a tautology.

Another major problem is the suggestion of Vogler and DeSalle to use population aggregation analysis (Davis and Nixon, 1992) to assess ESUs and distinguish "characters" from "traits". In this process, the variation of many attributes of individuals of multiple populations are scored. Populations that are

diagnosably distinct from all others are considered ESUs and populations that contain any individuals with shared attributes are homogenized into one ESU. The attributes that are diagnostic are "characters" and all others are "traits". This process is problematic because ESU status would be highly dependent upon sampling on at least three levels: sampling of attributes, sampling of individuals within populations and sampling of populations (see Sites and Crandall, 1997). Inadequate sampling of attributes would in general result in recognizing fewer ESUs whereas under sampling of individuals would consistently increase the number of ESUs, and the effects of under sampling populations on the number of ESUs can vary.

The Cohesion Species Concept (CSC) define a species as, "the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms" (Templeton, 1989). The main contribution appears to be that the focus of this concept is on mechanisms that draw together rather than isolate organisms. This concept has been interpreted differently. King (1993) claimed this concept has "no worthwhile difference" from the BSC but Sites and Crandall (1997) considered it to be an operational, lineage-based concept. This may be the result of a later modification that required a potential cohesion species to represent a single evolutionary lineage (Templeton, 1994). To satisfy the cohesion species definition, the independent lineages must also be either genetically or demographically exchangeable. Although the criteria for determining genetic and demographic exchangeability were clear, the criteria for identifying distinct evolutionary lineages were not as apparent. This is a common problem among lineage-based concepts.

Sites and Crandall (1997) also suggested the Concordance Principles Concept (CPC) of Avise and Ball (1990) as an operational lineage-based definition. This concept emphasises operationality by defining the boundaries of species with concordant unlinked and independent markers. Although Avise and Ball (1990) focused on molecular characters, I would interpret that phenotypic markers would be acceptable as well. Their idea is that concordance should be observed for groups of organisms that have been isolated from each other for a long time. Although this concept includes asexual organisms, it faces great difficulty in application to extinct taxa. However, the major problem with this definition is that concordant support may not be observed for recently evolved species since markers evolve at different rates and there were no clear provisions for dealing with incomplete congruence objectively.

The Biological Species Concept and the bottlenose dolphin

Sites and Crandall (1997) identified the flaw of most taxonomic studies determining species status of organisms as being the failure to present a testable hypothesis based on a species concept. Since there is no universal species definition and all contain limitations, selecting a concept to test species status must be a conscious decision based on weighing strengths and weaknesses. The choice will likely depend on individual philosophy, previous knowledge of the organisms and operationality. For sexually reproducing organisms, the BSC remains a very useful concept in conservation biology (O'Brien and Mayr, 1991a,b). Given the appropriate conditions (e.g., sympatry), the weaknesses of the BSC can be reduced or eliminated. In the marine environment, obligate asexual organisms are rare and sympatric sibling species (or species which are similar morphologically) are relatively common compared to the terrestrial environment (Knowlton, 1993). Thus the BSC appears suited for testing species status of many marine organisms and has been important for recognizing sibling species (Knowlton, 1993). The sympatric forms of bottlenose dolphins in Taiwan presented an opportunity where conditions were ideal for testing species status under the BSC criterion of reproductive isolation. First, bottlenose dolphins are both sexually reproducing and extant organisms so they are within the scope of the BSC. Second, the problem of allopatry is not present. Indirect evidence suggests that reproductive activity is occurring within each of the two forms during their sympatric existence near the Penghu Islands. Studies of captive individuals revealed that females are spontaneous ovulators and can be receptive throughout the year (Schroeder, 1990). Limited data on foetus length (n = 5; J.Y. Wang, unpublished data) suggest a spring calving season for both forms in Chinese waters. This is consistent with other populations, which show prolonged calving seasons with peaks in the spring (Mead, 1975; Mead and Potter, 1990) and in some populations, a second peak occur in the autumn (Harrison and Ridgway, 1971; Odell, 1975; Hohn, 1980; Scott et al., 1990). With a gestation period of about 12 months for *Tursiops* (Leatherwood and Reeves, 1983; Perrin and Reilly, 1984; Kasuya, 1985), mating coincides approximately with the calving period. Therefore, the two sympatric forms in Chinese waters are potentially interbreeding and offer a natural experiment for examining whether reproductive isolation exists. To address the criticism that the BSC uses morphological differences as a proxy for reproductive isolation, morphological characters and an independent

molecular marker (mitochondrial DNA control region sequence data) will be compared. Congruence between these two independent lines of evidence would provide strong inference for reproductive isolation. One problem that might exist is the potential of hybridization because bottlenose dolphins are notorious among cetaceans for intergeneric hybridization (Sylvestre and Tasaka, 1985). However, given the prevalence of previous hybridization events involving bottlenose dolphins, the lack of recorded hybrids between these two forms would provide even stronger support for reproductive isolation. Finally, the limitation of being a temporally static concept is of little importance to conservation biology at present. Fortunately, we do not yet need to rank the relative "importance" of species for prioritizing conservation decisions. By having the conditions to address the major criticisms of the BSC, I believe this concept is an appropriate selection. Also, using the BSC has two other benefits. By demonstrating reproductive isolation from the congruence of morphological and molecular characters, the evidence would also satisfy, or at least be consistent with, many other concepts. Secondly, this concept has the widest acceptance and is easily understood by most people.

A brief description of the bottlenose dolphin and its importance to humans

Bottlenose dolphins have been studied extensively and their biology is the best known for all cetaceans. They are small, robust cetaceans reaching a maximum total length of almost 400 cm and a mass of about 300 kg (Leatherwood and Reeves, 1983). Their colouration is generally dark grey on the dorsal field with a light grey to white, sometimes even pink, ventral surface and there can be a distinct stripe extending posteriorly from the gape of the mouth to the anterior insertion of the flipper. The dorsal fin is generally tall and falcate and is located at approximately the halfway point of the body length. Bottlenose dolphins have a cosmopolitan distribution, inhabiting all but sub-polar and polar waters. They represent the "typical" dolphin to most people and are responsible for the popularity of dolphins.

Bottlenose dolphins still represent an important source of protein and oil for maritime residents of many poor nations, but in developed regions consumption of this resource is now scarce. They have been the subject of much research in physiology, evolution, hydrodynamics, acoustics and psychology. The plasticity of their behaviour, habitat, food preferences and rapid training have not only made them ideal for research and

display animals but also for military use as naval mine-sweepers, underwater sentinels and object retrievers. More recently, bottlenose dolphins have gained therapeutic value for autistic patients and contributed to the increased public consciousness of environmental issues (see Donoghue and Wheeler, 1990).

Bottlenose dolphin classification

With the amount of research conducted on bottlenose dolphins, it is surprising that the classification within this genus remains highly controversial. Due to the great amount of phenotypic variation, many species of *Tursiops* have been described (Hershkovitz, 1966). Currently, all variants are considered to be synonymous with *Tursiops truncatus* (Montagu, 1821) because there has yet to be convincing evidence for other species (Tomilin, 1957; Mitchell, 1975; Walker, 1981; Hersh and Duffield, 1990; Mead and Potter, 1990; Ross and Cockcroft, 1990; Gao et al., 1995).

There is much uncertainty surrounding the nominal species *T. aduncus* (Ehrenberg, 1832). This species was first described based on a specimen that stranded on Belhosse Island in the Red Sea but no type specimen exists (see Hershkovitz, 1966). In general, *T. aduncus* is smaller than *T. truncatus*, has proportionately larger extremities and develops ventral spotting pigmentation with age (Yang, 1976; Ross, 1977, 1984; Zhou, 1987; Miyashita, 1993). Its distribution appears to be limited to the tropical, coastal waters of the Indo-Pacific from the eastern Cape of South Africa in the west and extending throughout the Indian Ocean to the continental shelf waters of the western tropical Pacific Ocean in the east. Its most northern and southern range is southern Japan and somewhere along the Pacific coast of Australia, respectively (for more details of distribution, see Ross, 1977, 1984; Zhou and Qian, 1985; Ross and Cockcroft, 1990; Miyashita, 1993).

In a study of bottlenose dolphins in South African waters, Ross (1977) decided that two species exist, *T. truncatus* and *T. aduncus*. But after studying Australian *Tursiops*, this conclusion was recanted (Ross and Cockcroft, 1990). Similarly, the same two species were described from Chinese waters (Zhou and Qian, 1985; Zhou, 1987). But after an examination of more specimens with multivariate analysis, this claim was also rejected (Gao et al., 1995; note: Zhou was a co-author of this study).

However, I contend that the species status of *T. aduncus* remains unresolved because fundamental flaws exist in the study designs of both Ross and Cockcroft (1990) and Gao et al. (1995). The absence of testable hypotheses or criteria for defining species in these studies is the basis of the flaws (Sites and Crandall, 1997). In Ross and Cockcroft (1990), body and skull lengths (size measurements) were correlated with environmental parameters or geographic location and clinal differences were found. From this finding, they concluded that "there was no morphological differentiation of taxonomic entities at the species level within the sample". However, the size of any character, especially when correlated with environmental differences (e.g., temperature, food availability, etc.), offers little to no taxonomic information. In fact, environmental or geographical effects often obscure "studies regarding alpha-level taxonomy" (Heyning and Perrin, 1994). Therefore, the conclusion by Ross and Cockcroft (1990) was inappropriate and should not be considered taxonomic evidence for monotypy in *Tursiops*.

The study of Gao et al. (1995) also suffered from a serious experimental design problem. They selected geographic location as the basis of the *a priori* classification for their discriminant analyses. Given that sympatry exists in Chinese waters (Yang, 1976; Zhou and Qian, 1985), geographic location was inappropriate. The arbitrary division of specimens into northern and southern populations ignores the importance of sympatry, particularly in the Taiwan Strait. Even with this design flaw, non-overlapping separation of the two populations was still found in external morphology and cranial measurements using discriminant analyses. However, a single species was concluded because, "the northern/southern variation in bottlenose dolphin in Chinese waters seemed no larger than that among the three populations of finless porpoises". Since none of the widely accepted species concepts contain a criterion involving comparisons between taxa, the interpretation of the results by Gao et al. (1995) can only be viewed as being arbitrary and subjective.

Conservation problems with bottlenose dolphins

Misguided or unresolved taxonomy not only affects our understanding of biology but it can also have dire consequences for conservation. For example, concluding that one species exists when there are more than

one reduces the perceived seriousness of threats and influences our assignment of conservation status. This is particularly harmful if the species are experiencing different levels or types of exploitation.

In Chinese and adjacent waters, there are directed catches of bottlenose dolphins for food, display, and to reduce the perceived or real competition with commercial fishermen for resources (Miyashita, 1993; Zhou et al., 1995; UNEP, 1996). Furthermore, an astounding number of gillnets (> 3.5 million) are used in Chinese waters (Zhou and Wang, 1994). Although the magnitude of the incidental catches in gillnets is poorly documented in this region, this problem is likely to be substantial since it is the greatest immediate threat to small cetaceans in most other regions (Perrin et al., 1994). The above problems faced by bottlenose dolphins are additional to the general threats faced by all coastal marine species (e.g., habitat destruction, pollution, prey reduction, etc.). Even though there is evidence of differential exploitation of bottlenose dolphin forms (see Chapter 3 and 4), it is presently impossible to determine the seriousness of the threats to each form because the practice of reporting mortality for each form separately has been inconsistent and unreliable. This inattentiveness towards separating the two forms is the product of the taxonomic uncertainties about bottlenose dolphins. Recently, this problem was recognized formally, and resolving the classification within bottlenose dolphins was identified as requiring urgent attention, especially in Southeast Asia (UNEP, 1996).

Objectives

The main objective of this thesis is to provide some resolution to the taxonomic controversy about *Tursiops*. To accomplish this objective, I examined bottlenose dolphin specimens collected from Chinese waters, especially around the Penghu Archipelago where two forms are sympatric and frequently in mixed schools. The sympatric forms offered a rare opportunity where the conditions exist to test species status under the theoretical framework of the BSC with minimal environmental or geographic differences. The null hypothesis is that the two forms represent a single species with no reproductive isolation. Morphological (skeletal and external) characters were examined using multivariate analyses and a portion of the mtDNA control region was sequenced and analysed using three independent methods of phylogeny reconstruction. The presence of congruent separation of the two forms in morphology (which is the phenotypic expression of

nuclear genes) and mitochondrial DNA would provide strong inference for reproductive isolation.

A secondary objective was to identify important differences between the two forms so that classification of new specimens can be facilitated. Confident identification of each form is essential for further research as well as conservation. For example, conservation policy and actions may depend on the practicality of enforcement or monitoring of threats, both of which are completely dependent on the ability to identify each form. Osteological, genetic and external morphological characters were examined for diagnostic differences that can be used to differentiate each form regardless of the condition of the specimens.

Chapter 2

Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters

(With a few minor exceptions for the purposes of thesis presentation, this manuscript was prepared in the format of the *Journal of Zoology* and is currently being reviewed for publication)

Osteological differences between two sympatric forms of bottlenose dolphins (genus *Tursiops*) in Chinese waters

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Abstract

Although there has been extensive research on bottlenose dolphins (genus *Tursiops*), much controversy about the classification within this genus still exists. Even though many morphological variants occur, the prevailing view is that of a single species, *Tursiops truncatus* (Montagu, 1821). To evaluate this view, two sympatric forms of bottlenose dolphins inhabiting Chinese waters were examined using multivariate statistical analyses of osteological data for two meristic and 18 cranial morphometric characters from a total of 78 specimens. Cluster and principal components analyses revealed clear osteological separation of the two forms. Furthermore, the distributions of the total number of vertebrae and several proportions of cranial characters were non-overlapping between the two forms. These results provided strong evidence that the two sympatric forms of *Tursiops* in Chinese waters are isolated reproductively and do not support the current view of a monotypic genus. Classification functions of a discriminant analysis and a key of several characters were presented to help researchers identify new specimens. Provisional names (*T. truncatus* and *T. aduncus*) were suggested but a formal taxonomic revision of this genus is still required. However, regardless of the nomenclature, immediate amendments to all present wildlife conservation legislation are strongly urged.

Introduction

The bottlenose dolphin (genus *Tursiops*) has been the subject of extensive research. Thus, it is somewhat surprising that the classification within this genus is still plagued with considerable controversy. Due to the cosmopolitan distribution of and great regional morphological variation within this genus, many species have been described (see Hershkovitz, 1966). Nevertheless, most researchers consider these species names to be junior synonyms of *Tursiops truncatus* (Montagu, 1821) and the predominant view is still of a monotypic genus (Tomilin, 1957; Walker, 1981; Hersh & Duffield, 1990; Mead & Potter, 1990, 1995; Ross & Cockcroft, 1990; Gao, Zhou & Wang, 1995). However, one form of bottlenose dolphin appears to be restricted to the warm, coastal waters of the Indo-Pacific. Distinctive pigmentation (spotting on the ventrum), small size and other morphological characters led some researchers to suggest that this form was also a valid species; they referred it to *Tursiops aduncus* (Ehrenberg, 1932) (Pilleri & Gihr, 1972; Ross, 1977, 1984; Zhou & Qian, 1985; Zhou, 1987). But due to inconclusive evidence, *T. aduncus* has not gained wide acceptance. For example, in South African waters, Ross (1977) concluded that both *T. truncatus* and *T. aduncus* were valid species, but in a later study on *Tursiops* in Australian waters, Ross & Cockcroft (1990) decided that "there is little doubt that they [the two forms of bottlenose dolphins from South Africa] should be treated as a single species, *T. truncatus*, and that Australian bottlenose dolphins should be assigned to this species also".

From the Taiwan Strait (between Taiwan and China), two forms of *Tursiops* have also been reported (Yang, 1976; Zhou & Qian, 1985; Zhou, 1987; Gao et al., 1995). The larger, more northern form which is found generally in deep, offshore waters was referred to *T. gilli*[*i*] by Yang (1976) but Zhou & Qian (1985) suggested this form should be named *T. truncatus* because no thorough taxonomic examination of these dolphins had been conducted. Therefore, in this study, we will take the conservative approach by referring to this form as the *truncatus*-type. The smaller, more southern form which inhabits shallow, tropical, coastal waters resembles the description of *T. aduncus* (Yang, 1976; Zhou & Qian, 1985) and will be referred to as the *aduncus*-type in this study.

Ross (1977, 1984) reported that the two forms in South African waters, being associated with different currents or bodies of water, were "essentially allopatric". In contrast, the two forms of *Tursiops* in Chinese waters are sympatric, particularly around the Penghu Archipelago (Yang, 1976; Zhou & Qian, 1985; J.Y.Wang, unpublished data) which is situated off the western coast of Taiwan in the Taiwan Strait. Furthermore, in this region, the two forms often occur in mixed schools that frequently include other species of dolphins as well (see Zhou & Qian, 1985). Presently, this region represents the only area where sympatric forms of bottlenose dolphins occur consistently and offers a rare opportunity to examine the hypothesis that *Tursiops* is composed of a single species.

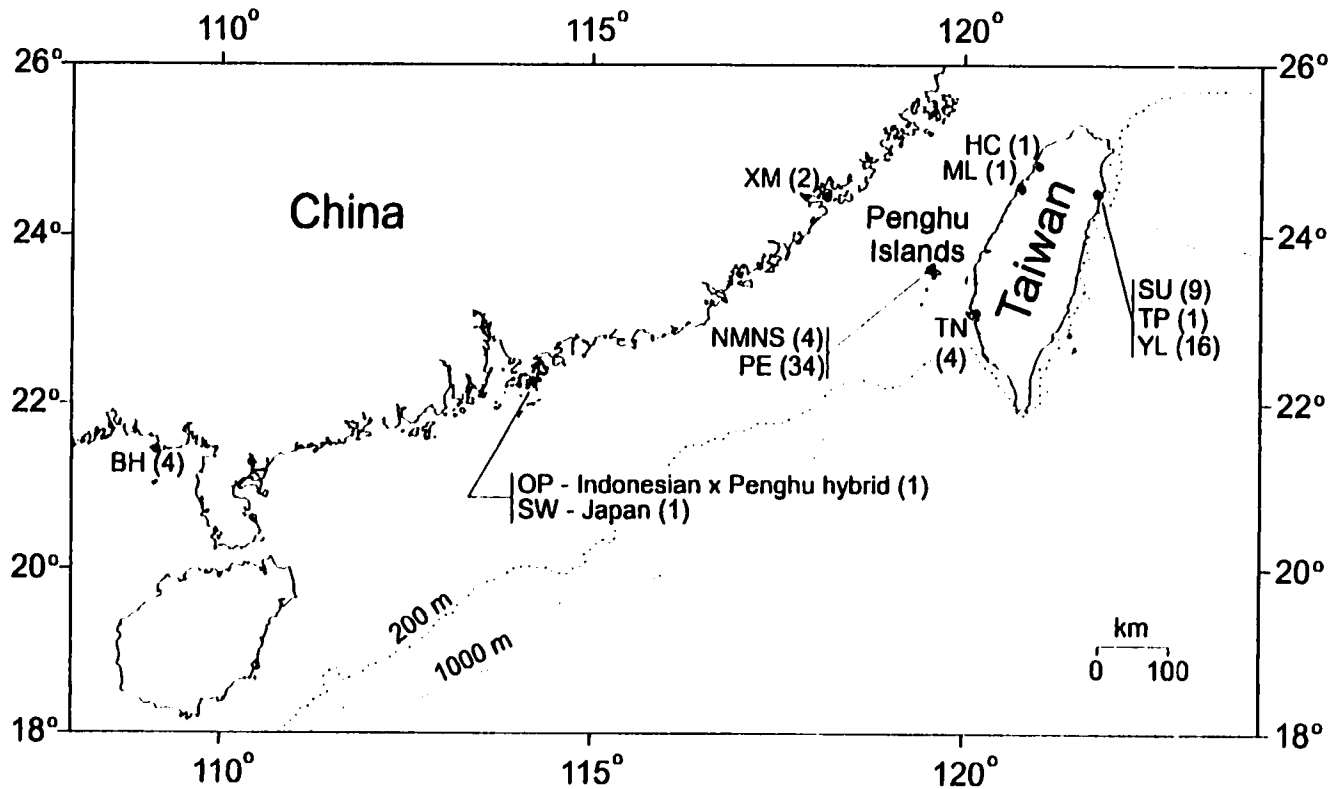
In the present study, we analysed skeletal material of specimens collected from Chinese waters to determine if osteological evidence supports the recognition of two distinct species. Also, since the ability to classify the two forms confidently has important scientific and conservation applications, we suggest skeletal characters that may offer rapid and accurate identification of specimens.

Materials and Methods

Specimens and measurements

Skeletal material of 78 specimens was examined. Most specimens were collected during late winter to early spring between January 1994 and March 1998 from the coastal waters of Taiwan and southern to south-central China (Fig. 1). Exceptions include: dolphins being sold illegally and confiscated by Taiwan police (all YL- specimens) - based on fragmentary information, the capture location of these animals is very likely northeastern Taiwan but the capture dates are unknown; a calf (PE-94-12) that was born in captivity from a dolphin which was caught around the Penghu Islands in the winter of 1992/93; a sun-bleached skull (PE-94-01) found on the Penghu Islands with an unknown date of death; a dolphin (SW-95-01) that was captured from Japanese waters in 1975 for display at Ocean Park, Hong Kong; and an offspring (OP-95-05) of a mating between two *aduncus*-type dolphins, one from Indonesia and the other from Taiwan (S. Leatherwood, Ocean Park, Hong Kong, personal communication) (see Appendix I for more details of specimens). With the exception of display dolphins, all specimens were obtained opportunistically from stranding events or fishery

Figure 1. Sampling locations of bottlenose dolphins. Letters represent the first part of the specimen code (see **Appendix I**) and numerals in parentheses indicate sample size.


110°
115°
120°
26°
24°
22°
20°
18°
China
Penghu
Islands
Taiwan
XM (2)
HC (1)
ML (1)
SU (9)
TP (1)
YL (16)
TN
(4)
NMNS (4)
PE (34)
BH (4)
OP - Indonesian x Penghu hybrid (1)
SW - Japan (1)
200 m
1000 m
km
0
100

interactions. Specimens were classified in the field as *truncatus*-type or *aduncus*-type based on external morphology, pigmentation and collection location (the latter only for specimens collected from the east coast of Taiwan where *aduncus*-type dolphins do not exist - J.Y. Wang, unpublished data).

The total number of vertebrae of 39 specimens were counted with the small, triangular terminal caudal element being counted as two fused vertebrae. Only specimens with complete or nearly complete vertebral columns (i.e., missing 5 or fewer elements) were included; the number of missing vertebrae for nearly intact specimens were estimated by comparison to complete specimens. Small bones were lost for many of the specimens collected early in the study as a result of handling or preparation. Therefore, whenever possible, vertebrae of later specimens were counted during necropsy. The total number of teeth was obtained for 70 specimens by counting alveoli.

Thirty-three cranial characters were measured in the present study. Twenty-eight were measured as described in Perrin (1975): condylobasal length (CBL), length of rostrum (LR), tip of rostrum to external nares (TREN), width of rostrum at the base (WRB), width of rostrum at 1/4 length of rostrum (WRQ), width of rostrum at 1/2 length of rostrum (WRH), width of rostrum at 3/4 length of rostrum (WRT), greatest preorbital width (GPRW), least supraorbital width (LSOW), greatest postorbital width (GPOW), greatest width of external nares (GWEN), greatest width of premaxillaries (GWPM), greatest width of the parietal (GPW), length of upper left toothrow (LUTR), greatest length of pterygoid (GLP), greatest width of internal nares (GWIN), greatest width across the zygomatic process (GWZP), length of the antorbital process of the left lacrimal (LAPL), length of left orbital (LLO), greatest length of left ramus (GLLR), greatest height of left ramus (GHLR), length of left lower toothrow (LLTR), length of mandibular fossa (LMF), length of basihyal at midline (LBH), greatest width of basihyal (GWBH), greatest proximal width of left thyrohyal (GWTH), greatest length of left thyrohyal (GLTH), and alveolar tooth width measured at mid-rostrum (ATW). Two were measured following Walker (1981): greatest length and width of mandibular condyle (GLMC and GWMC, respectively). Three measurements (greatest width of basisphenoid - GWBS; width of alisphenoid at the suture with the basisphenoid - WAS; and tip of rostrum to the apex of the premaxillary convexity - TPC) were included after an initial examination of several skulls (note: we discovered later that Ross (1977) had also found differences

between the two forms in the premaxillae so his terminology, "premaxillary convexity", was adopted; also some acronyms in this study are different from other studies). All measurements were taken by J.Y.W. with vernier calipers to the precision of 0.05 mm.

Data analyses

Meristic characters

Teeth and vertebrae numbers were analysed separately from the other metric characters since they are independent of size and the data are categorical. A two-means cluster analysis was used to divide 37 specimens with both teeth and vertebrae data into two groups.

Cranial morphometrics

Due to the state of some specimens, not all characters could be measured reliably. Since multivariate analyses are sensitive to missing data, a subset of the specimens and characters was selected for analyses. The selection of data began by including specimens with complete measurements. Then characters were eliminated from the data set if they were missing, or could not be measured reliably, in 5 or more specimens. Finally, specimens were omitted if they contained missing data for the remaining characters. In total, 18 characters (see Fig. 2a-c) and 57 specimens were subjected to multivariate analyses. Field classifications identified 38 and 14 of these specimens as *truncatus*-type and *aduncus*-type, respectively. Field classifications for five specimens (NMNS-863, PE-94-11, PE-95-08, PE-96-02 and PE-96-03) were not available due to the condition of the animals.

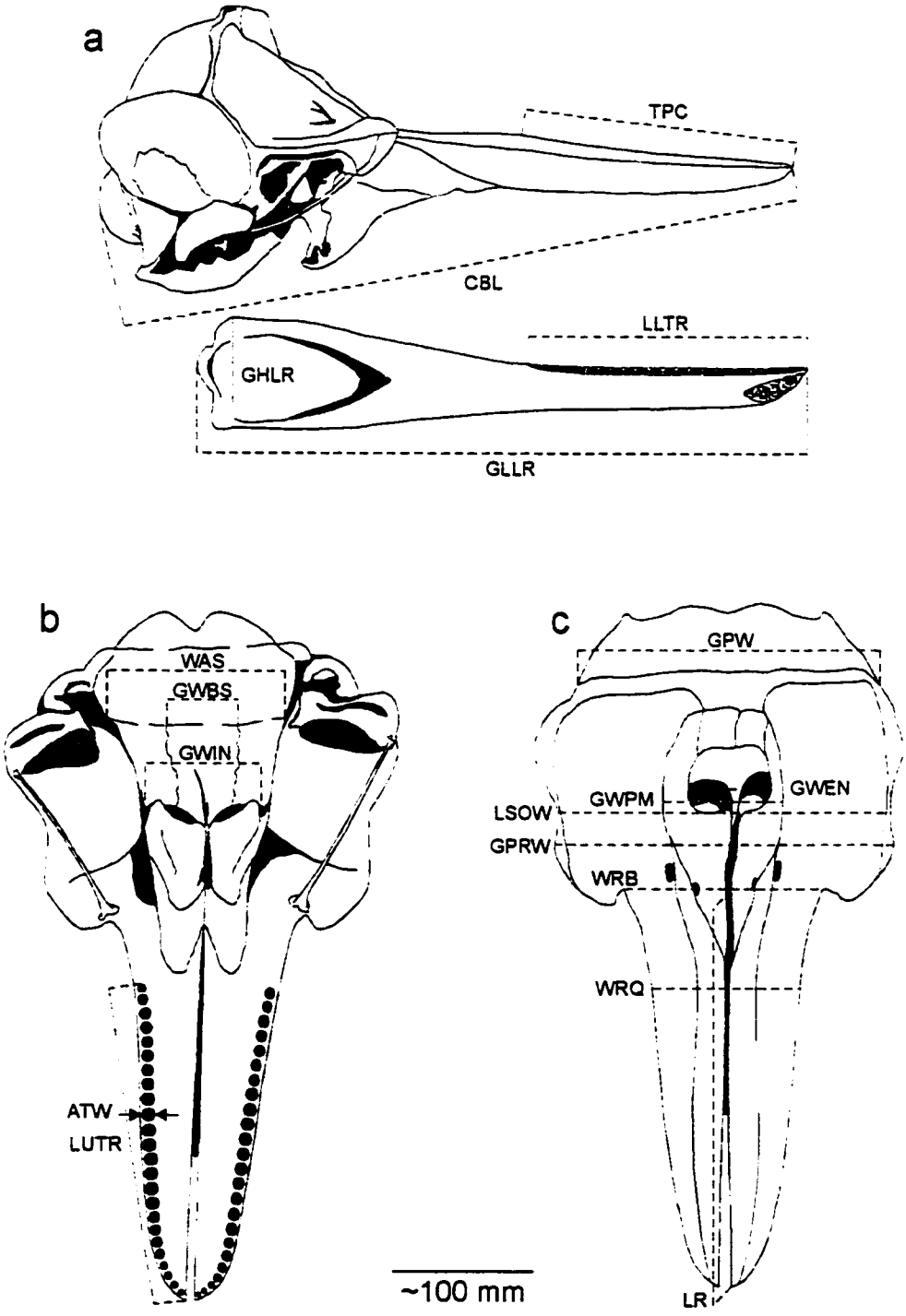
All data analyses were performed with *STATISTICA*™ version 5.0 by StatSoft®.

Results

Meristic characters

A two-means cluster analysis of the meristic characters showed two clear groups (for teeth, $F_{1,35} = 43.64$, $p << 0.0001$; for vertebrae, $F_{1,35} = 292.45$, $p << 0.0001$) whose means were separated by a Euclidean

Figure 2. Generalized bottlenose dolphin skulls illustrating the landmarks for measuring 18 cranial characters used in a discriminant analysis. The skull is shown in right lateral (a), ventral (b) and dorsal (c) views. The medial view of the left dentary is depictd in (a).

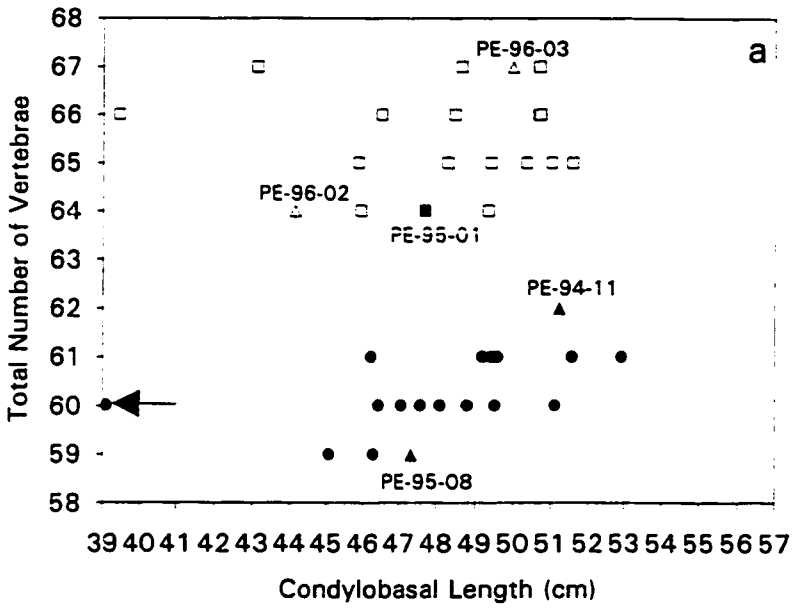

a
TPC
CBL
LLTR
GHLR
GLLR
b
WAS
GWBS
GWIN
ATW
LUTR
c
GPW
GWEN
GWPM
LSOW
GPRW
WRB
WRQ
LR
~100 mm

distance of 1.7. Scatterplots of each character versus CBL showed that the separation of the groups was due mainly to the non-overlapping distributions of the total number of vertebrae (Fig. 3a). Although the group centroids of the total number of teeth differed, there was substantial overlap in the distributions of the specimens (Fig. 3b). All specimen memberships were congruent with their field identifications except PE-95-01, which was identified in the field as *aduncus*-type but clustered with *truncatus*-type specimens (Appendix I). For the specimens that had no field classifications, PE-96-02 and PE-96-03 were classified as *truncatus*-type and PE-94-11 and PE-95-08 were classified as *aduncus*-type. The total number of teeth of NMNS-863 (=86) indicated that they belonged with the *truncatus*-type specimens (vertebrae data wase not available for this specimen).

Cranial morphometrics

A principal components analysis was performed on the correlation (standardized) matrix with the varimax rotation, and three principal components (PCs) were extracted. Extraction of three PCs was supported by a scree plot and the Kaiser criterion; furthermore, only two residual correlations, between GWEN and ATW and GWEN and GWBS, had absolute values greater than 0.1. All width measurements (except GWBS and GWEN) had high loadings on PC 1 while PC 2 had high loadings from all length measurements (except TPC); PC 3 had a single high loading from GWEN (Table I). PCs 1, 2 and 3 accounted for 87.1% of the total variation (51.4%, 27.9% and 7.8%, respectively). The PC scores were then subjected to a two-means cluster analysis with distances sorted and taken at constant intervals. Since most measurements are some function of size (length or width), correlations between measurements can be substantial. The contribution of length or width may be inflated not only by the strength of the correlations between measurements but also by the number of each type of measurement (i.e., length or width) included in the data set. Therefore, performing a cluster analysis on the original data set may over-inflate the importance of size in the grouping of specimens by the analysis. In contrast, PCs are orthogonal "characters" so a cluster analysis of PC scores would avoid this problem.

Figure 3. The relationship between the total number of vertebrae and condylobasal (skull) length (a) and the total number of teeth and condylobasal (skull) length (b). □ and ● represent *truncatus*-type and *aduncus*-type dolphins, respectively, as identified by a two-means cluster analysis; ■ represents PE-95-01 (a specimen whose field and osteological classifications conflicted); △ and ▲ represent specimens which were classified *a posteriori* as *truncatus*-type and as *aduncus*-type dolpins, respectively (specimen codes are shown in the figures). The arrow points to a nursing calf specimen (BH-95-02) with most of its teeth being unerupted and a condylobasal length less than 39 cm (but could not be measured accurately due to the fragmentary nature of the soft cranium).


a
PE-96-03
PE-96-02
PE-95-01
PE-94-11
PE-95-08
Total Number of Vertebrae
68
67
66
65
64
63
62
61
60
59
58
39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57
Condylobasal Length (cm)

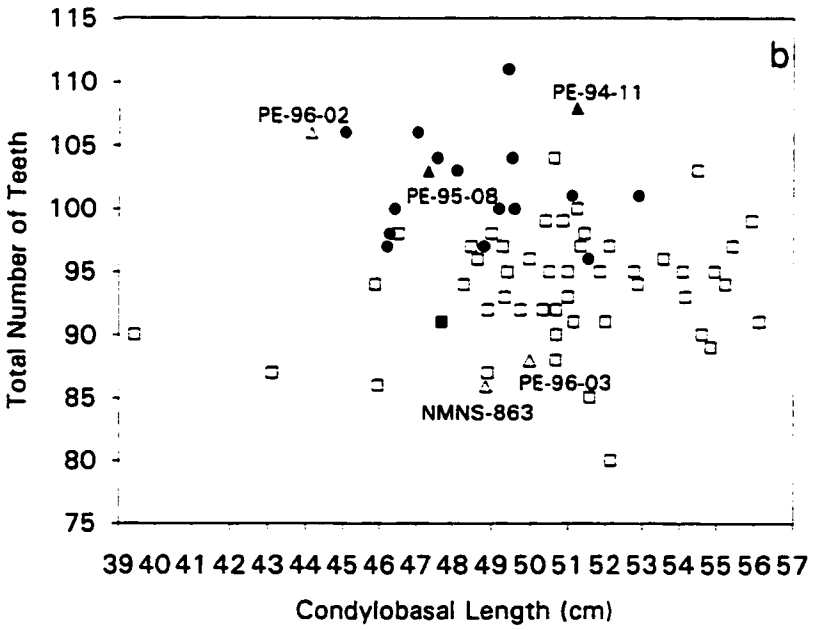

b
PE-94-11
PE-96-02
PE-95-08
PE-96-03
NMNS-863
Total Number of Teeth
115
110
105
100
95
90
85
80
75
39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57
Condylobasal Length (cm)

TABLE I

Loadings and coefficients (in parentheses) of the first three principal components (PC) under varimax rotation for 18 cranial characters of bottlenose dolphins (for character abbreviations, see ***Materials and Methods****). *, loadings > 0.700.*

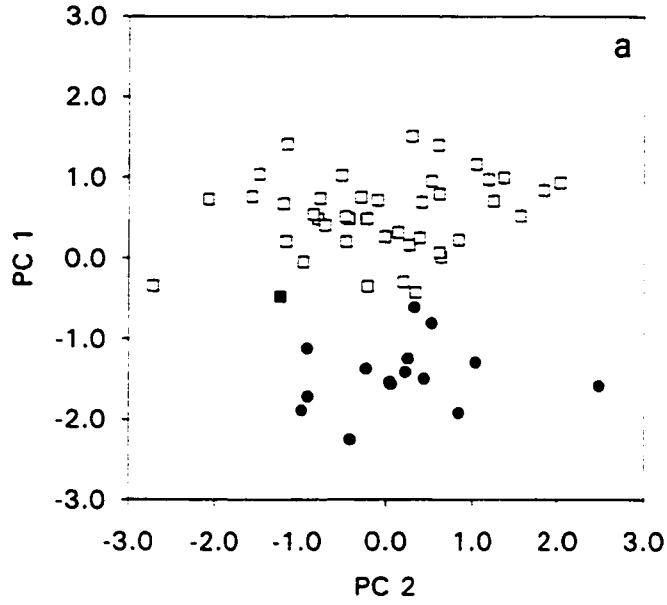
Cranial Characters	PC 1	PC 2	PC 3
Length Measurements:			
CBL	0.595	*0.770	0.139
LR	0.282	*0.925	0.179
LUTR	0.384	*0.888	0.073
GLLR	0.649	*0.723	0.149
LLTR	0.305	*0.887	0.076
TPC	-0.443	0.682	0.429
Width Measurements:			
WRB	*0.884	0.323	0.060
WRQ	*0.913	0.275	0.062
GPRW	*0.894	0.355	0.160
LSOW	*0.861	0.400	0.209
GWPM	*0.736	0.383	0.389
GPW	*0.936	0.071	0.091
GWIN	*0.915	0.250	-0.029
GHLR	*0.836	0.390	-0.005
ATW	*0.756	0.174	0.076
WAS	*0.918	0.237	0.144
GWBS	0.628	0.112	0.167
GWEN	0.199	0.272	*0.877
Eigenvalues	9.3	5.0	1.4
% Total Variance	51.4	27.9	7.8
Cumulative % Variance	51.4	79.3	87.1

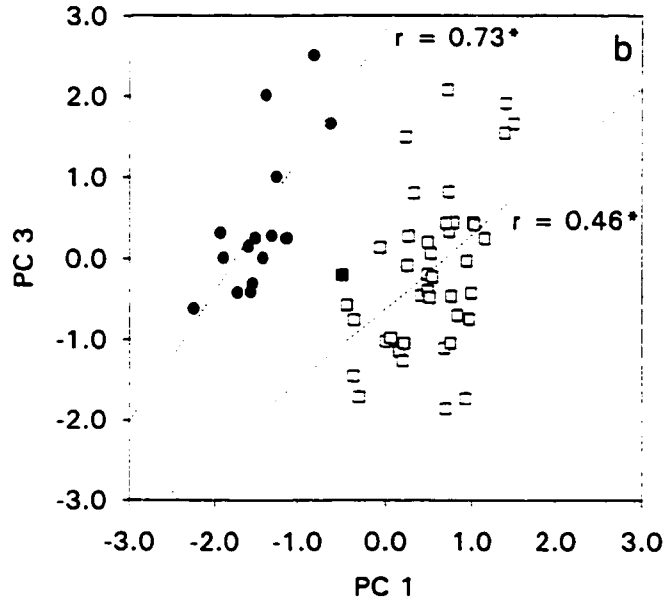
The division of the specimens by the cluster analysis of the PC scores was consistent with the field and meristic classifications (for PC 1, $F_{1,55} = 187.66$, $p << 0.0001$; for PC 2, $F_{1,55} = 0.73$, $p = 0.397$; for PC 3, $F_{1,55} = 4.38$, $p = 0.041$; Euclidean distance between the clusters = 1.2). Again, PE-95-01 was clustered with other *truncatus*-type specimens as in the meristic analysis. The clustering of specimens without field classifications were consistent with their meristic classifications. The scatterplot of PC 1 versus PC 2 showed no obvious relationship between the PCs within, and no overlap between, the clusters (Fig. 4a). However, the scatterplot of PC 3 versus PC 1 (Fig. 4b) showed significant, positive, linear correlations between these principal components within each group (for *aduncus*-type: $r = 0.73$, $p < 0.002$, $n = 15$; for *truncatus*-type: $r = 0.46$, $p < 0.002$, $n = 42$).

If present, sexual dimorphism may affect the results of the main analyses. However, reports of sexual dimorphism of *Tursiops* from different regions have been inconsistent (e.g., Ross, 1977; Hersh et al., 1990; Read et al., 1993; Tolley et al., 1995). Therefore, to determine if any of the characters analysed were sexually dimorphic, a one-way multivariate analysis of variance (MANOVA) for the *truncatus*-type (17 males, 14 females) was performed. No statistically significant sex effect was found (Wilks' $\Lambda = 0.53$; Rao's $R = 0.59$; $df_1 = 18$; $df_2 = 12$; $p = 0.852$). For the *aduncus*-type, there were too few specimens of known sex (six males and six females) to allow a MANOVA to be performed on 18 characters. But a one-way MANOVA was performed on a subset of the 18 characters (i.e., the nine 'most important" characters as determined later in this study) and there was no significant sex effect (Wilks' $\Lambda = 0.09$; Rao's $R = 3.32$; $df_1 = 9$; $df_2 = 3$; $p = 0.176$). Analysis of eight external morphometric characters also revealed no sex effects within each form (Chapter 4). Furthermore, there was no obvious bias in the sex composition of the members in either cluster; one cluster contained six males and six females while the other cluster had 17 males and 14 females. These results are consistent with Ross (1977) who found no indications of sexual dimorphism in osteological characters within the *truncatus*-type or the *aduncus*-type dolphins of South Africa. Therefore, pooling male and female specimens within each form of *Tursiops* for the main analyses was supported.

The main analyses were useful in demonstrating clear separation between the dolphin specimens in this study. However, since principal components are linear combinations of several characters, they are not

Figure 4. Relationships between principal components (PCs) 2 and 1 (a) and PCs 3 and 1 (b). □ - *truncatus*-type; ● - *aduncus*-type; and ■ - PE-95-01 (a specimen whose field and osteological classifications conflicted). *, $p < 0.002$.


a
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
PC 1
-3.0 -2.0 -1.0 0.0 1.0 2.0 3.0
PC 2

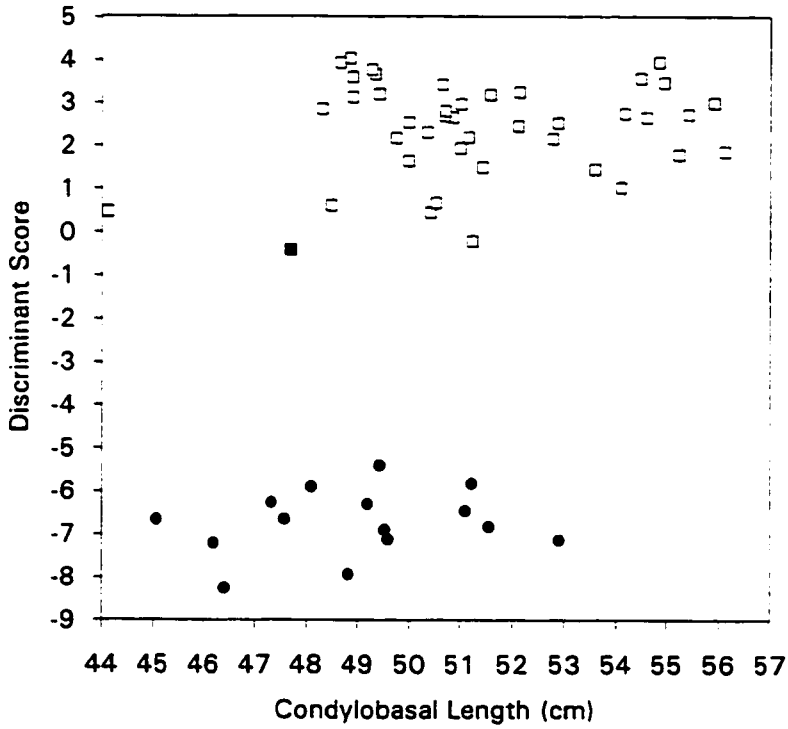

b
r = 0.73*
r = 0.46*
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
PC 3
-3.0 -2.0 -1.0 0.0 1.0 2.0 3.0
PC 1

interpreted easily nor are they directly useful for understanding how the two forms may differ with regards to measurable characters. Furthermore, cluster analysis does not allow new specimens to be classified directly and objectively. Therefore, to determine characters important for distinguishing the two forms and the classification of new specimens, a series of discriminant analyses were performed on the standardized data set (group membership of the specimens, as determined by the cluster analysis, provided the *a priori* classification for the discriminant analyses). PE-95-01 was omitted from the discriminant analyses since its field and cluster analysis classifications were inconsistent, but it was re-examined afterwards (note: inclusion of this one specimen had little affect on the discriminant analyses).

A discriminant analysis of the 18 cranial characters showed highly significant differences between the two groups (Wilks' $\Lambda = 0.06$; $\sim F_{18,37} = 35.23$; $p << 0.0001$) which were separated by a Mahalanobis distance of 87.4 ($F_{18,37} = 34.50$; $p << 0.0001$) and the *a posteriori* classifications of the specimens were 100% correct. However, since these were *post hoc* analyses, the results were not surprising. The pertinent results from this analysis were: 1) the largest contribution to the separation of the two forms was clearly from TPC ($F_{1,37} = 17.13$; $p < 0.0002$); 2) the functions for classifying new specimens; and 3) all *aduncus*-type specimens had highly negative discriminant scores (i.e., < -4) while all *truncatus*-type specimens, except PE-97-08 (-0.213), had positive values (Fig. 5).

Although the 18-character discriminant analysis identified TPC as the most important character, the contributions of other characters independent of TPC may be obscured. Also, obtaining measurements for all 18 characters required by the functions may not be practical nor possible for some specimens. Therefore, we wanted to reduce the number of characters required to classify specimens to only those that are important contributors to the discrimination of the two forms of dolphins but still maintain a minimum overall correct classification of 95% (prespecified by the authors for this study). To identify characters that are important for the separation of the two forms, an analysis resembling a forward stepwise discriminant analysis was performed. However, rather than having a prespecified $F_{\text{to enter}}$ criterion, the character with the highest F value was removed from further steps and added to a list of "most important" characters if the correct overall

Figure 5. Relationship of bottlenose dolphin specimen scores from a discriminant analysis of 18 osteological characters and condylobasal (skull) length. □ - *truncatus*-type; ● - *aduncus*-type; and ■ - PE-95-01 (a specimen whose field and osteological classifications conflicted).


Discriminant Score
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
44 45 46 47 48 49 50 51 52 53 54 55 56 57
Condylobasal Length (cm)

classification of specimens was ≥95%. This was continued until the correct overall classification of specimens dropped below 95%; the remaining characters were not examined further. A final discriminant analysis using the "most important" characters was performed.

Nine characters were determined to be important by the modified forward stepwise discriminant analysis. They included, in the order of identification: TPC, GWEN, GPW, LUTR, WRB, GPRW, GWIN, WAS and WRQ. Only after omitting all these characters did the overall correct classification drop to below 95%. Discriminant analysis of these nine characters also produced the same major results as with 18 characters (i.e., highly significant separation of the two species (Wilks' $\Lambda = 0.08$; $p << 0.0001$) and 100% correct *a posteriori* classification) (Table II & Fig. 6).

Key for Tursiops *identification*

Multivariate analyses are much more powerful at detecting structure in data sets with many variables than a series of univariate analyses. However, the benefits gained from multivariate analyses also have limitations such as: non-applicability to specimens with incomplete data, the time required to collect the data and the experience level of the measurer. Therefore, the nine "most important" characters were examined for simple proportions that may be useful in a key to the identification of the two forms by inexperienced measurers or for specimens with very limited data. Examination of these characters revealed that each of the ratios: TPC/CBL, TPC/LR, TPC/LUTR and GWEN/GPW had non-overlapping distributions between the groups. Each ratio alone was sufficient to correctly identify specimens with great accuracy and a key using these characters was constructed (see Appendix II).

Classification of PE-95-01 and incomplete specimens

Specimen PE-95-01 was classified initially in the field as *aduncus*-type but was classified as *truncatus*-type by the cluster analyses of cranial characters and meristic data. Based on the discriminant

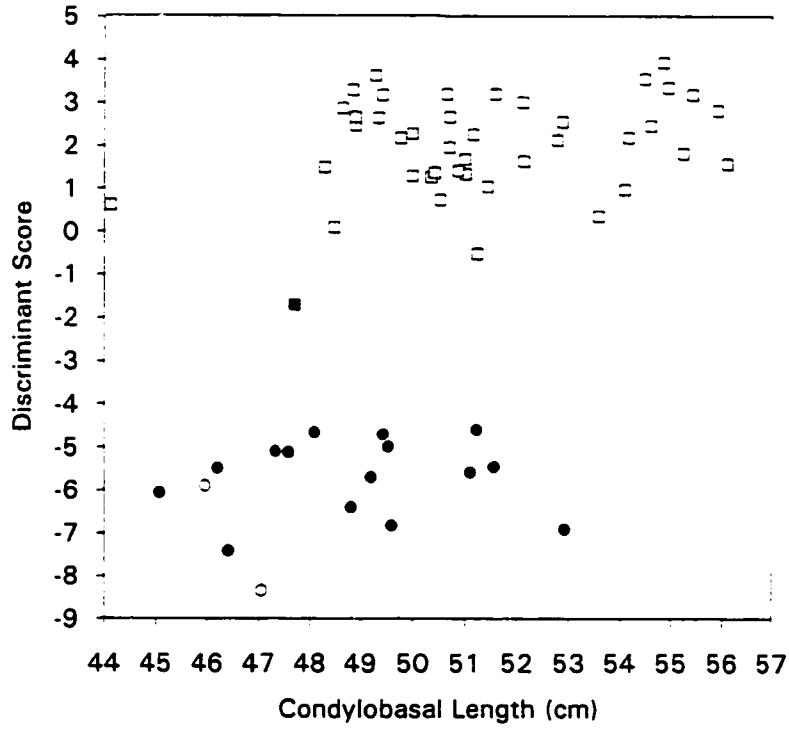
TABLE II

Specimen sums for nine cranial characters of bottlenose dolphins and the equation coefficients required to calculate discriminant and classification scores for new specimens. For character abbreviations, see ***Materials and Methods.*** ***, $p << 0.0001$.*

Cranial Characters	Specimen Sums	Standardized Coefficients	Coefficients for Classification Functions*	
			Tursiops aduncus	*Tursiops truncatus*
WRB	738.806	0.497	-4.365	1.597
WRQ	529.803	-0.298	2.543	-0.930
GPRW	1276.720	0.410	-3.298	1.207
GWEN	327.030	-0.595	3.370	-1.233
GPW	1111.095	0.576	-6.198	2.268
LUTR	1376.180	0.502	-2.948	1.078
GWTN	417.558	0.406	-3.709	1.357
WAS	443.234	0.045	-0.389	0.142
TPC	923.189	-1.353	9.995	-3.657
Constants			-17.550	-2.485
Eigenvalue		12.32		
Canonical R		0.96		
Wilks' Λ		0.08**		
df		9		

*To classify new specimens, standardize the new measurements for each character by using the specimen sums (based on n = 56 specimens) (i.e., new value - [specimen sum + new value]/n'; where n' = 56+1). Then scores for the functions of each species are calculated using the new (standardized) values in the equations: $A = -17.550 + \Sigma c_i x_i$ and $T = -2.485 + \Sigma k_i x_i$, where A and T = *T. aduncus* and *T. truncatus* function scores, respectively; c_i and k_i = coefficients of the ith character for *T. aduncus* and *T. truncatus* functions, respectively; and x_i = the standardized value of the ith character for the new specimen. The new specimen is classified as the species whose function produces the highest score (e.g., A > T, new specimen = *T. aduncus*). Discriminant scores for new specimens are calculated using the standardized coefficients.

Figure 6. Relationship of bottlenose dolphin specimen scores from a discriminant analysis of nine osteological characters and condylobasal (skull) length. □ - *truncatus*-type; ● - *aduncus*-type; ■ - PE-95-01 (a specimen whose field and osteological classifications conflicted); and ○ - *aduncus*-type specimens with incomplete data for the 18 character discriminant function analysis.


Discriminant Score
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
44 45 46 47 48 49 50 51 52 53 54 55 56 57
Condylobasal Length (cm)

analyses of both the 18- and nine-character data sets, PE-95-01 belonged within the *truncatus*-type group (discriminant scores were -0.413 and -1.744, respectively; see Fig. 5 & 6). The best estimate of vertebrae number for PE-95-01 (=64) was greater than the maximum observed for other *aduncus*-type specimens but within the *truncatus*-type distribution. Also, the total number of teeth for PE-95-01 (=91) was well within the *truncatus*-type distribution but represented the lower extreme of the *aduncus*-type distribution. This result was the same for the number of rostral teeth. However, if only mandibular teeth were considered, PE-95-01 (=44) was well within the limits of *truncatus*-type (39-54) but fell below the lower limit for *aduncus*-type (47-56) PC scores also indicated a closer affinity of PE-95-01 with other *truncatus*-type specimens (see Fig. 4a & b).

Two other specimens, PE-94-01 and XM-95-03, had sufficient measurements to be classified using the 9-character discriminant analysis functions. Both were consistent with their field identifications as *aduncus*-type. The total number of vertebrae for the latter specimen (= 60) also supported the *aduncus*-type classification. Nine specimens which did not have enough data for classification using the 9-character discriminant analysis functions (NMNS-4, NMNS-334, PE-94-12, PE-94-15, PE-95-05, PE-97-07, PE-97-09, PE-97-13 and SU-94-85) and seven specimens newly collected from around the Penghu archipelago (PE-97-17, PE-98-01, PE-98-03, PE-98-05, PE-98-07, PE-98-08 and PE-98-09) were used for testing the key characters. The key identifications of the specimens with field data matched exactly with the field classifications (i.e., *T. truncatus* - PE-94-12, PE-95-05, PE-97-07, PE-97-09, PE-97-13, PE-98-01, PE-98-03, PE-98-05 and SU-94-85; *T. aduncus* - PE-94-15, PE-97-17 and PE-98-09). Four specimens without field identifications were identified as *T. truncatus*: NMNS-4, NMNS-334, PE-98-07 and PE-98-08. Although each of the key characters alone was sufficient to correctly classify the specimens in this study, we recommend using as many of these characters as possible for new specimens, especially when the values for these characters are near the adjacent ends of the groups' distributions.

Discussion

Tursiops *is not monotypic*

Studies of sympatric forms of bottlenose dolphins in Chinese waters have provided some resolution to some of the taxonomic controversies within this genus. In this study, congruence between cluster analyses of meristic and cranial morphometric data and field identifications (with the exception of one specimen but see later) indicated clear osteological separation between the two sympatric forms. In a concurrent study analysing mitochondrial DNA (mtDNA) control region sequences, a clear division into two lineages was also found (Chapter 3). Where specimens were common to both studies (i.e., having osteological and molecular data), the classification of these specimens were always consistent (Appendix I). The congruence of molecular and morphological data is "strong evidence that the underlying historical pattern has been discovered" (Hillis, 1987). Furthermore, an analysis of external morphological differences between the two forms also separated the specimens clearly and resulted in non-overlapping discriminant analysis scores (Chapter 4). These results would not be expected if the two forms represented a single reproductive entity with unrestricted gene flow. The clear separation, lack of intermediate specimens and diagnostic differences (in osteology, mtDNA control region sequence and external morphology) between the two forms reflect the completeness of the reproductive isolation and suggest that the barrier(s) to gene flow has(have) been effective for a substantial period.

Further support for reproductive isolation comes from hybridization evidence. The interspecific promiscuity of bottlenose dolphins is well known and reports of hybridization between *Tursiops* and other delphinoids are more prevalent than for all other cetaceans combined. Hybrids with the rough-toothed dolphin (*Steno bredanensis*), Risso's dolphin, (*Grampus griseus*), false killer whale (*Pseudorca crassidens*) and short-finned pilot whale (*Globicephala macrorhynchus*) were produced in captivity (Sylvestre & Tasaka, 1985) while three possible wild *T. truncatus* × *G. griseus* hybrids were reported by Fraser (1940, as cited by Sylvestre & Tasaka, 1985). Therefore, it is surprising that only a single hybrid between the two forms of *Tursiops* (which was produced in a Japanese oceanarium) has ever been reported (Sylvestre & Tasaka, 1985). Other institutions that have maintained both forms of *Tursiops* for many years (Ocean Park, Hong Kong; Ocean

World Taipei, Taiwan) have never reported hybridization. Among South African specimens studied, none were thought to be hybrids of the two forms (Ross, 1977, 1984). In the present study, PE-95-01 may represent a free-ranging hybrid of the two forms since its field and osteological classifications conflict. But more likely, its field classification (by J.Y.W.) was incorrect due to the advanced state of decomposition of this specimen (post-mortem time: ~2 months). Initial attempts at extracting DNA from this specimen have been unsuccessful so confirmation with molecular evidence is currently not available. Therefore, hybridization of these sympatric species among free-ranging individuals remains uncertain. Regardless, the evidence indicates that the reproductive barrier(s) between the sympatric forms of *Tursiops* in Chinese waters is(are) effectively complete.

The two forms in Chinese waters also differ ecologically. Preliminary examination of differences in prey and habitat preferences revealed that the *aduncus*-type prefers the coastal, shallow waters of the continental shelf (i.e., < ~ 200m) and preys upon benthic or reef-dwelling fish and cephalopods while the *truncatus*-type feeds primarily on schooling epipelagic and mesopelagic species (J.Y. Wang, unpublished data). Whereas the latter species is also found in shelf waters, the former has never been observed in the deep waters of the east coast of Taiwan (J.Y. Wang, unpublished data). These patterns are similar to those found by Ross (1977, 1984). Ecological differences between the two species were also indicated by: differences in carbon and nitrogen stable isotope ratios found in bone and skin tissues (J.Y. Wang, unpublished data); and differential survivorship in captivity, with the *aduncus*-type having greater longevity than the *truncatus*-type (Reeves et al., 1994).

Since the two forms of *Tursiops* in Chinese waters are sympatric and occur in mixed schools, the maintenance of reproductive isolation satisfies the criterion of the Biological Species Concept (Mayr, 1942) as distinct species. As noted by Heyning & Perrin (1994), Wiley (1981) (who defined the current and most widely cited version of the Evolutionary Species Concept) also accepts the occurrence of phenotypically distinct populations in sympatry as "prima facie evidence for two species". Furthermore, the present results are also consistent with other popular species definitions such as: the Phylogenetic; the Concordance Principles; and the Cohesion species concepts (for an overview, see King, 1993; Sites & Crandall, 1997). Therefore,

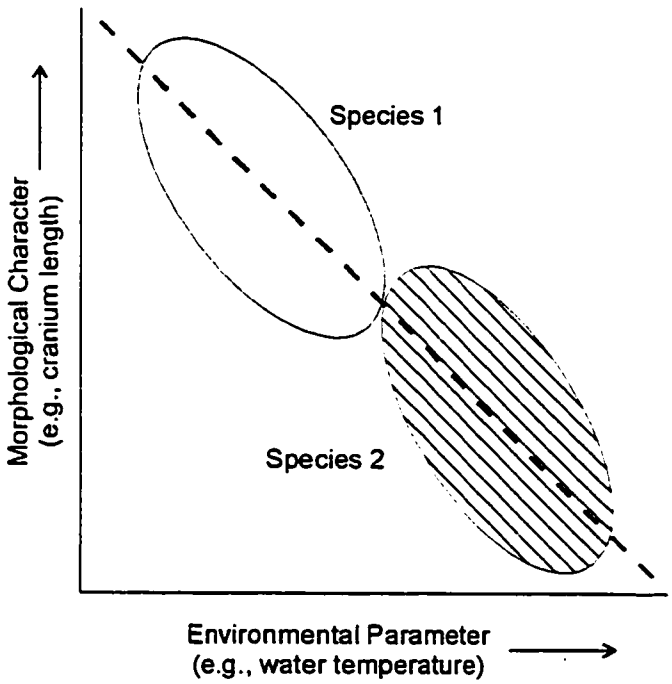
under the criteria of all these major species concepts, the monotypic hypothesis for *Tursiops* was not supported by the present study.

The results of the present study are consistent with previous studies. However, our conclusion conflicts with those of Ross & Cockcroft (1990) and Gao et al. (1995). Since Ross & Cockcroft (1990) correlated environmental parameters or geographic location with morphology (e.g., water temperature and total length; locality and condylobasal length), their study would be inappropriate for detecting separate species in sympatry. For example, as with most mammals, both species may have responded to living in a cooler environment with an increase in body size (see Fig. 7). However, such correlations reveal little about phylogenetic histories and taxonomic relationship. In fact, variation resulting from adaptation to different environments may obscure features that reveal ancestry and should be eliminated whenever possible in phylogenetic and taxonomic treatments. By studying sympatric forms, the effects of geographical and environmental variation can be reduced greatly (Heyning & Perrin, 1994). Interestingly, Ross & Cockcroft (albeit with limited samples) also found that vertebral counts revealed two non-overlapping distributions that were very similar to those found in the present study for *aduncus*-type and *truncatus*-type forms, respectively.

In the study of Gao et al. (1995), geographic sampling location was used as the basis for the *a priori* classification for their discriminant analysis. Given that sympatry and even mixed schools exists in Chinese waters (Zhou & Qian, 1985; Zhou, 1987), geographic location is inappropriate for *a priori* classifications. Nevertheless, separation of the two groups was still found by the authors. This is a reflection of the level of differentiation between the two species and the sampling of specimens outside the sympatric region. Also, their conclusion that the differences were not great enough to warrant separate species classification was based, in part, on a comparison with the amount of differentiation observed among subspecies of the finless porpoise (*Neophocaena phocaenoides*). Species validation for *Tursiops* forms, or any other taxa, should not be based on a comparison of the level of differentiation observed within or between other taxa. None of the species concepts has this type of comparison as a criterion for defining species.

Since some specimens in the present study were likely subadults (maturity information was not available for most specimens), ontogenetic effects may present problems. However, very young animals were

Figure 7. A graph illustrating how a correlation between an environmental parameter (water temperature) and a morphological character (cranium length) can obscure taxonomic relationships.


Species 1
Species 2
Morphological Character
(e.g., cranium length)
Environmental Parameter
(e.g., water temperature)

omitted automatically from the data set of cranial morphometrics since many characters could not be measured reliably due to the loose articulation of the cranial bones. Also, the total number of teeth and vertebrae appear to be independent of age / size (see Fig. 3a & b) with the latter being a diagnostic character for these species. Furthermore, the cluster analysis of cranial characters was performed on PC scores which reduced the influence of size on the analysis and there appeared to be no obvious relationship between PC 1 and PC 2 (the width and length components) within groups. Finally, discriminant scores of the specimens (both for 18 and nine characters) showed no overlap and no obvious relationship with the size of dolphins as measured by condylobasal length (Fig. 5 & 6). Therefore, the inclusion of subadults in the present analyses appears to have had no obvious effect on the outcome.

Finally, it is interesting that other examples of sympatric *Tursiops* forms have not been reported. With the great amount of variation found in Australian *Tursiops*, it would be surprising not to find the two forms in sympatry once better information on distribution and external appearance is available and more specimens are analysed. In South African waters, even though the two forms have been described as being basically allopatric, sympatry appears to occur along the southeast coast (Ross, 1977). Miyashita (1993) also reported that both forms occur in the waters of the Ryukyu Archipelago of southern Japan. However, the degree and frequency of sympatry in these latter regions need further examination.

Nomenclature

Although the present study provided convincing evidence for separate species of *Tursiops* in Chinese waters, names are still needed for addressing and referring to these species (e.g., for conservation legislation, etc.). However, due the lack of comparisons with type specimens of nominal species in this study, proper scientific names can not be assigned. However, provisional names can be suggested pending a formal taxonomic revision. There is little argument that at least the larger form from Chinese and South African waters is *T. truncatus* (see Ross, 1977; Zhou, 1987; Gao et al., 1995). In this study, the vertebrae number of this form was similar to the number reported previously for specimens from the waters of China / Taiwan (Zhou, 1987; Gao et al., 1995), South Africa (Ross, 1977, 1984), Australia (Ross & Cockcroft, 1990) and

Britain (see Ross, 1977) (note: the last is the type locality for *T. truncatus*). Comparisons of other osteological characters between Chinese *T. truncatus* specimens and specimens from South African (Table IIIa & b) and British (see Ross, 1977) waters revealed minimal differences. Also, a recent mtDNA analysis did not reveal any obvious differences between the *truncatus*-type dolphins from Chinese waters and *T. truncatus* from the North or South Atlantic oceans (Chapter 3). Therefore, there is no evidence to suggest that the large form in Chinese waters should be given a name other than *T. truncatus*.

The external appearance of the *aduncus*-type dolphin resembles closely that of the Atlantic spotted dolphin, *Stenella frontalis*. However, the vertebral and dental formulae of these species are non-overlapping and very disparate. The *T. aduncus* formulae are more typical of, but slightly lower than, the formulae of *T. truncatus* (Barnes, 1990; Rommel, 1990) and much lower than *S. frontalis* (Perrin et al., 1987; Perrin, Caldwell & Caldwell, 1994) or any other *Stenella* species (see Perrin & Gilpatrick, 1994; Perrin & Hohn, 1994; Perrin, Wilson & Archer, 1994; Perrin & Mead, 1994). In addition, the largest *S. frontalis* skull reported was smaller than those of the non-calf *T. aduncus* specimens examined. Therefore, the superficial resemblance between *T. aduncus* and *S. frontalis* was not reflected in osteology. Furthermore, Ross (1977) found no convincing evidence that other related species named from the Indian Ocean (i.e., *T. catalania*, *T. absulam* and *Delphinus gadamu*) were more than junior synonyms of *T. aduncus*. Since the *aduncus*-type specimens in Chinese waters were consistent with those from South Africa in osteology (Table IIIa & b), external morphology, ventral spotting pigmentation and ecology (Ross, 1977, 1984; J.Y. Wang, unpublished data), the evidence favours maintaining the provisional name, *T. aduncus*, until further studies show otherwise.

Conclusions

The osteological, molecular, external morphological and preliminary ecological data provided overwhelming support for two species of *Tursiops* in Chinese waters and thus resolution to some of the taxonomic controversies within this genus. This finding of two species reveals the inadequacy of the current legislation to provide legal protection for bottlenose dolphins. Since different levels of exploitation of these

TABLE IIIa.

Basic statistics of cranial characters (in mm and % of CBL) for bottlenose dolphins from Chinese and South African waters. See text for character abbreviations.

	Chinese Waters								South African Waters*							
Cranial	*Tursiops truncatus*				*Tursiops aduncus*				*Tursiops truncatus*				*Tursiops aduncus*			
Character	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.
CBL	50	506.2	33.35	394.4-561.1	18	485.1	22.23	450.7-529.1	9	545.8	26.21	504.0-578.0	33	472.7	16.14	433.0-507.0
LR	49	283.8	23.44	203.5-319.6	18	282.0	14.96	258.0-317.4	9	309.1	18.02	283.2-334.6	33	271.9	12.03	250.0-297.0
(%CBL)	(49)	(55.9)	(1.44)	(51.6-59.1)	(18)	(58.0)	(1.20)	(55.9-60.0)	(9)	(56.6)	(0.82)	(55.6-58.3)	(33)	(57.5)	(0.99)	(54.7-59.4)
TREN	49	335.2	26.72	243.5-375.2	14	328.5	17.56	298.4-366.1	9	364.5	18.17	337.2-386.9	33	316.9	12.83	294.0-343.0
(%CBL)	(49)	(66.0)	(1.41)	(61.7-68.9)	(13)	(66.9)	(1.28)	(64.9-69.2)	(9)	(66.8)	(0.63)	(65.8-67.5)	(33)	(67.0)	(0.95)	(65.0-68.7)
WRB	49	134.5	11.17	98.1-154.1	19	115.8	7.84	103.4-134.0	9	142.8	10.81	127.0-157.8	33	112.3	6.13	100.9-125.0
(%CBL)	(49)	(26.6)	(1.14)	(23.9-29.3)	(18)	(23.9)	(1.20)	(22.0-26.0)	(9)	(26.1)	(0.92)	(24.5-27.3)	(33)	(23.8)	(1.20)	(21.4-27.7)
WRH	46	84.0	8.92	54.5-101.5	18	64.2	5.03	56.3-71.3	9	88.6	10.45	73.1-105.8	32	64.9	3.97	56.0-74.9
(%CBL)	(46)	(16.5)	(1.02)	(13.8-18.5)	(17)	(13.3)	(0.83)	(12.1-14.9)	(9)	(16.2)	(1.30)	(14.5-18.3)	(32)	(13.8)	(0.81)	(11.6-15.6)
WRT	47	64.3	7.75	44.7-80.4	17	50.3	6.40	41.0-60.6	9	66.9	9.74	55.9-85.0	33	48.8	5.37	34.0-59.9
(%CBL)	(47)	(12.6)	(1.04)	(10.2-14.7)	(16)	(10.5)	(1.18)	(8.8-12.9)	(9)	(12.2)	(1.33)	(10.8-14.7)	(33)	(10.3)	(1.08)	(7.3-12.8)

GPRW	49	231.8	18.55	171.5-262.5	18	201.9	14.24	177.0-230.1	9	253.4	15.54	229.8-276.9	32	203.4	8.16	180.1-219.8
(%CBL)	(49)	(45.8)	(1.57)	(42.2-49.5)	(18)	(41.6)	(1.51)	(38.1-44.6)	(9)	(46.4)	(1.57)	(43.2-48.4)	(32)	(43.0)	(1.32)	(41.0-46.2)
LSOW	50	224.5	18.17	163.2-254.1	18	199.5	13.83	175.3-226.3	6	250.4	16.74	228.8-269.8	33	207.3	7.53	187.1-225.0
(%CBL)	(50)	(44.3)	1.49)	(40.3-46.8)	(18)	(41.1)	(1.50)	(37.8-43.9)	(6)	(46.4)	(1.54)	(44.2-48.1)	(33)	(43.9)	(1.25)	(42.0-47.3)
GPOW	50	254.6	19.47	187.2-286.6	14	223.4	14.46	200.0-245.3	9	277.3	17.10	253.9-301.1	32	230.2	9.15	202.2-251.0
(%CBL)	(50)	(50.3)	(1.70)	(46.2-53.4)	(14)	(46.0)	(1.58)	(42.8-48.4)	(9)	(50.8)	(1.89)	(47.7-53.0)	(32)	(48.7)	(1.43)	(46.6-52.3)
GWEN	50	57.7	3.75	49.5-67.6	18	58.7	4.26	54.0-70.3	6	62.2	4.95	54.9-68.2	33	54.4	2.60	50.0-61.0
(%CBL)	(50)	(11.4)	(0.71)	(10.2-13.3)	(18)	(12.1)	(0.74)	(11.1-13.6)	(6)	(11.5)	(0.41)	(10.9-11.9)	(33)	(11.5)	(0.56)	(10.6-12.6)
GWPM	50	93.5	6.93	76.5-107.4	18	86.2	5.35	77.1-100.0	9	101.2	5.83	88.2-108.1	33	83.4	3.46	76.8-90.1
(%CBL)	(50)	(18.5)	(0.77)	(16.9-20.5)	(18)	(17.8)	(0.88)	(16.5-19.5)	(9)	(18.5)	(0.86)	(17.2-19.6)	(33)	(17.7)	(0.72)	(16.6-19.0)
LUTR	49	243.6	20.45	172.0-277.7	19	236.9	13.45	209.4-265.9	6	242.4	45.76	154.0-276.9	31	224.8	9.64	208.0-245.0
(%CBL)	(48)	(48.0)	(1.36)	(43.6-50.6)	(18)	(48.7)	(1.25)	(45.6-50.3)	(6)	(45.0)	(8.06)	(28.6-50.1)	(31)	(47.6)	(1.09)	(45.5-49.9)
GWZP	50	257.3	20.47	188.6-290.0	13	230.6	13.5	209.0-251.3	9	282.3	19.46	257.0-313.0	30	229.6	10.73	197.9-251.0
(%CBL)	(50)	(50.8)	(1.88)	(46.7-54.0)	(13)	(47.0)	(1.27)	(45.2-49.1)	(9)	(51.7)	(2.07)	(48.6-55.1)	(30)	(48.5)	(1.52)	(45.7-52.3)
LAPL	49	60.0	6.43	40.8-71.9	14	46.1	3.15	40.1-51.2	8	63.7	6.42	52.9-71.0	21	44.8	3.97	38.1-52.8
(%CBL)	(49)	(11.8)	(0.80)	(10.2-13.3)	(12)	(9.4)	(0.40)	(8.7-10.0)	(8)	(11.7)	(0.91)	(10.1-12.6)	(21)	(9.52)	(0.69)	(8.2-11.0)
GLLR	51	434.3	29.57	340.8-480.8	17	415.0	20.77	385.9-460.9	9	466.1	26.74	425.9-498.2	30	399.6	14.48	372.8-422.0
(%CBL)	(50)	(86.1)	(1.39)	(82.7-89.7)	(16)	(85.1)	(1.25)	(83.2-87.1)	(9)	(85.4)	(1.05)	(83.8-87.1)	(30)	(84.8)	(1.19)	(82.5-87.0)

GHLR	51	91.4	7.80	61.0-104.4	17	82.6	4.40	76.9-92.7	9	100.1	6.42	90.1-109.8	30	83.2	4.30	71.9-90.2
(%CBL)	(50)	(18.1)	(0.79)	(15.5-19.5)	(16)	(16.9)	(0.53)	(16.1-18.0)	(9)	(18.3)	(0.73)	(17.0-19.3)	(30)	(17.7)	(0.66)	(16.3-18.9)
LLTR	51	243.4	18.19	187.7-278.7	18	243.8	10.24	228.2-267.6	5	249.8	12.13	237.9-269.2	30	226.9	9.46	212.0-248.0
(%CBL)	(50)	(48.2)	(1.33)	(45.0-50.8)	(17)	(50.0)	(0.80)	(48.5-51.5)	(5)	(47.0)	(0.28)	(46.8-47.4)	(30)	(48.2)	(1.39)	(46.6-53.1)

* from Ross (1977; 1984).

Note: Specimens from Chinese waters contain known juveniles while those from South African waters had 4 or more dentine layers in the teeth.

TABLE IIIb

Basic statistics of meristic characters for bottlenose dolphins form Chinese and South African waters.

Meristic	Chinese Waters								South African Waters*							
	Tursiops truncatus				*Tursiops aduncus*				*Tursiops truncatus*				*Tursiops aduncus*			
Character	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.	N	Mean	SD	Min.-Max.
Teeth:																
UL	54	23.9	1.39	21-27	20	25.2	1.11	23-27	9	24.2	0.67	23-25	33	25.8	1.09	24-28
UR	54	23.8	1.42	20-27	20	25.4	1.23	24-28	9	23.8	0.83	22-25	33	25.3	1.04	24-28
LL	54	23.1	1.54	19-27	19	25.7	1.49	23-28	9	22.6	0.73	22-24	29	25.9	1.28	23-28
LR	54	23.0	1.54	20-27	19	25.6	1.17	24-28	9	22.6	1.01	21-24	30	26.1	1.27	23-29
Total	54	93.9	5.09	80-106	19	102.0	4.32	96-111	9	93.1	2.37	88-96	29	102.9	3.66	97-111
Vertebrae	20	65.5	1.15	64-67	19	60.2	0.86	59-62	4	64.5	0.58	64-65	9	60.6	0.88	59-62

* from Ross (1977; 1984).

UL = upper left; UR = upper right; LL = lower left; LR = lower right.

species may occur in Chinese waters (J.Y. Wang, unpublished data), both species must be recognized as separate entities and considered independently.

For assessing and monitoring the levels of non-natural mortality experienced by each species, accurate identifications are necessary. To help researchers with identifications, we have summarized the osteological differences between these species in two ways: classification functions from a discriminant analysis of nine characters (Table II); and a key (Appendix II) that was tested successfully on many specimens not included in the main analyses. The usefulness and scope of the classification functions and key will be known only after being tested with many more specimens and from other areas. One data set available for testing the key is that of South African specimens (Ross, 1977, 1984). Unfortunately, only total vertebrae number could be used in the key. There were no discrepancies between the classifications by Ross and the key, but Ross (1984) reported the upper limit of the vertebrae number for *T. aduncus* as 63. Since the data required by the classification functions or by other key characters were not available, further examination could not be performed.

Our understanding of *Tursiops* in Chinese waters is in its infancy and many more studies are needed to obtain the information necessary for directing effective conservation programs for these popular and highly-exploited species. The results of this and other studies reinforce the urgent need for a formal taxonomic revision of this genus (UNEP, 1996). This enormous task will require much resources, time and cooperation among researchers. However, since bottlenose dolphins, like other cetaceans, are long-living organisms with low recruitment rates and therefore especially vulnerable to exploitation, it is strongly urged that amendments to wildlife conservation laws recognize more than a single species of *Tursiops* be considered immediately and not be delayed by unresolved nomenclature; provisional names can be used to refer to these species in the interim. Legal recognition is an essential first step towards the conservation of any species.

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References

Barnes, L. G. (1990). The fossil record and evolutionary relationships of the genus *Tursiops*. In *The Bottlenose Dolphin*: 3-26. Leatherwood, S. & Reeves, R. R. (Eds). San Diego: Academic Press.

Gao, A., Zhou, K. & Wang, Y. (1995). Geographical variation in morphology of bottlenose dolphins (*Tursiops sp.*) in Chinese waters. *Aquat. Mammals* **21(2):** 121-135.

Hersh, S. L. & Duffield, D. A. (1990). Distinction between Northwest Atlantic offshore bottlenose dolphins based on hemoglobin profile and morphometry. In *The Bottlenose Dolphin*: 129-139. Leatherwood, S. & Reeves, R. R. (Eds). San Diego: Academic Press.

Hersh, S. L., Odell, D. K. & Asper, E. D. (1990) Sexual dimorphism in bottlenose dolphins from the east coast of Florida. *Mar. Mamm. Sci.* **6:** 305-315.

Hershkovitz, P. (1966). Catalog of living whales. *U.S. National Museum Bulletin* **246:** 1-259.

Heyning, J. E. & Perrin, W. F. (1994). Evidence for two species of common dolphins (genus *Delphinus*) from the eastern North Pacific. *Contr. Sci. (Los Angeles)* **442:** 1-35.

Hillis, D. M. (1987). Molecular and morphological approaches to systematics. *Ann. Rev. Ecol. Syst.* **18**: 23-42.

Mayr, E. (1942). *Systematics and the origin of species*. New York: Columbia University Press.

Mead, J. G. & Potter, C. W. (1990). Natural history of bottlenose dolphins along the central Atlantic coast of the United States. In *The Bottlenose Dolphin*: 165-195. Leatherwood, S. & Reeves, R. R. (Eds). San Diego: Academic Press.

Mead, J. G. & Potter, C. W. (1995). Recognizing two populations of the bottlenose dolphin (*Tursiops truncatus*) off the Atlantic coast of North America: morphologic and ecologic considerations. *IBI Reports* **5:** 31-44.

Perrin, W. F. (1975). Variation of spotted and spinner porpoise (genus *Stenella*) in the eastern tropical Pacific and Hawaii. *Bull. Scripps Inst. Oceanogr.* **21:** 1-206.

Perrin, W. F. & Gilpatrick, J. W., Jr. (1994). Spinner dolphin *Stenella longirostris* (Gray, 1828). In *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins*: 99-128. Ridgway, S. H. & Harrison, R. (Eds). San Diego: Academic Press.

Perrin, W. F. & Hohn, A. A. (1994). Pantropical spotted dolphin *Stenella attenuata*. In *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins*: 71-98. Ridgway, S. H. & Harrison, R. (Eds). San Diego: Academic Press.

Perrin, W. F. & Mead, J. G. (1994). Clymene dolphin *Stenella clymene* (Gray, 1846). In *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins*: 161-171. Ridgway, S. H. & Harrison, R. (Eds). San Diego: Academic Press.

Perrin, W. F., Mitchell, E. D., Mead, J. G., Caldwell, D. K., Caldwell, M. C., van Bree, P. J. H. & Dawbin, W. H. (1987). Revision of the spotted dolphins, *Stenella* spp. *Mar. Mamm. Sci.* **3**: 99-170.

Perrin, W. F., Caldwell, D. K. & Caldwell, M. C. (1994). Atlantic spotted dolphin *Stenella frontalis* (G. Cuvier, 1829). In *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins*: 173-190. Ridgway, S. H. & Harrison, R. (Eds). San Diego: Academic Press.

Perrin, W. F., Wilson, C. E. & Archer II, F. I. (1994). Striped dolphin *Stenella coeruleoalba* (Meyen, 1833). In *Handbook of Marine Mammals, Volume 5: The First Book of Dolphins*: 129-159. Ridgway, S. H. & Harrison, R. (Eds). San Diego: Academic Press.

Pilleri, G. & Gihr, M. (1972). Contribution to the knowledge of the cetaceans of Pakistan with particular reference to the genera *Neomeris*, *Sousa*, *Delphinus*, and *Tursiops* and description of a new Chinese porpoise *Neomeris asiaeorientalis*). *Invest. Cetacea* **4**: 108-162.

Read, A. J., Wells, R. S., Hohn, A. A. & Scott, M. D. (1993). Patterns of growth in wild bottlenose dolphins, *Tursiops truncatus*. *J. Zool. Lond.* **231:** 107-123.

Reeves, R. R., DeMaster, D. P., Hill, C. L. & Leatherwood, S. (1994). Survivorship of odontocete cetaceans at Ocean Park, Hong Kong, 1974-1994. *Asian Mar. Biol.* **11:** 107-124.

Rommel, S. (1990). Osteology of the bottlenose dolphin. In *The Bottlenose Dolphin*: 29-49. Leatherwood, S. & Reeves, R. R. (Eds). San Diego: Academic Press.

Ross, G. J. B. (1977). The taxonomy of bottlenosed dolphins *Tursiops* in South African waters, with notes on their biology. *Ann. Cape Prov. Mus. (nat. Hist.)* **11(9):** 135-194.

Ross, G. J. B. (1984). The smaller cetaceans of the south east coast of southern Africa. *Ann. Cape Prov. Mus. (nat. Hist.)* **15(2):** 173-410.

Ross, G. J. B. & Cockcroft, V. G. (1990). Comments on Australian bottlenose dolphins and the taxonomic status of *Tursiops aduncus* (Ehrenberg, 1832). In *The Bottlenose Dolphin*: 101-128. Leatherwood, S. & Reeves, R. R. (Eds). San Diego: Academic Press.

Sites, Jr., J. W. & Crandall, K. A. (1997). Testing species boundaries in biodiversity studies. *Conserv. Biol.* **11**: 1289-1297.

Sylvestre, J. & Tasaka, S. (1985). On the intergeneric hybrids in cetaceans. *Aquat. Mammals* **11:** 101-108.

Tolley, K. A., Read, A. J., Wells, R. S., Urian, K. W., Scott, M. D., Irvine, A. B. & Hohn, A. A. (1995). Sexual dimorphism in wild bottlenose dolphins (*Tursiops truncatus*) from Sarasota, Florida. *J. Mammal.* **76:** 1190-1198.

Tomilin, A. G. (1957). *Mammals of the U.S.S.R. and adjacent countries Volume IX. Cetacea.* Translated from Russian by the Israel Program for Scientific Translations, Jerusalem.

UNEP (1996) Report of the workshop on the biology and conservation of small cetaceans and dugongs of southeast Asia. UNEP(W)/EAS WG.1/2. United Nations Environmental Programme, Bangkok.

Walker, W. A. (1981). *Geographical variation in morphology and biology of bottlenose dolphins (Tursiops) in the eastern North Pacific.* U.S. National Marine Fisheries Service Administrative Rep. LJ-81-03C.

Wiley, E. O. (1981). *Phylogenetics: the theory and practice of phylogenetic systematics*. New York: Wiley & Sons.

Yang, H. C. (1976). Studies on the whales, porpoises and dolphins of Taiwan. *Ann. Rep Science, Taiwan Museum (Taipei)* **19:** 131-178.

Zhou, K. (1987). Notes on two species of dolphins of the genus *Tursiops* in Chinese waters. *Acta Theriologica Sinica* **7:** 246-254. (in Chinese with English abstract).

Zhou, K. & Qian, W. (1985). Distribution of the dolphins of the genus *tursiops* in the China Seas. *Aquat. Mammals* **1:** 16-19.

APPENDIX I

*List of bottlenose dolphin specimens examined with collection information (see **Fig. 1** for map of locations), available material and classifications based on different analyses and characters. A* – Tursiops aduncus; *T* – T. truncatus.

						Classifications					
Original Code	NMNS Code[a]	Sex	Approximate Date of Death	Collection Location	Available Material[b]	Cluster Analysis	Discrim. Analysis	Osteo. Key	Osteo. Field	MtDNA[c]	Ext. Morph.[d]
BH-95-02	---	M	Dec.16/94	Gulf of Tonkin, China	CS	---	---	A	A	---	A
BH-95-03	---	M	Dec.21/94	Gulf of Tonkin, China	CS	A	A	A	A	A	A
BH-95-04	---	M	Jan.20/95	Gulf of Tonkin, China	CS	A	A	A	A	A	A
BH-95-05	---	F	Dec.26/94	Gulf of Tonkin, China	CS	A	A	A	A	A	A
HC-96-01	1297	M	Mar.-Apr./96	Hsinchu, Taiwan	CS	A	A	A	A	A	A
ML-97-01	1393	M	~Feb./97	Miaoli, Taiwan	CS	A	A	A	A	---	---
---	3	---	Mar./91	Penghu Islands, Taiwan	CS	A	A	A	---	---	---
---	4	---	Mar./91	Penghu Islands, Taiwan	CS	---	---	T	---	---	---
NMNS-334	1316[e]	---	Jun./92	Penghu Islands, Taiwan	CD	---	---	T	---	---	---
---	863	---	---	Penghu Islands, Taiwan?	Sk	T	T	T	---	---	---
OP-95-05	---	---	---	Captive-born[f]	CD	---	---	A	A	---	---
PE-94-01	1135	---	---	Penghu Islands, Taiwan	Cranium	---	A	A	---	---	---
PE-94-02	629	---	~late Jan./94	Penghu Islands, Taiwan	Sk+	T	T	T	T	---	---

PE-94-11	591	---	Jul.-Aug./94	Penghu Islands, Taiwan	~CS	A	A	A	---	---	---
PE-94-12	738	F	died: Oct.19/94[a]	Captive-born	CS	---	---	T	T	T	T
PE-94-13	736	F	Dec.12/94	Penghu Islands, Taiwan	~CS	A	A	A	A	A	A
PE-94-14	740	M	Dec.12/94	Penghu Islands, Taiwan	~CS	A	A	A	A	A	A
PE-94-15	731	F	Dec.12/94	Penghu Islands, Taiwan	~CS	---	---	A	A	A	A
PE-95-01	730	---	~Dec.23-24/94	Penghu Islands, Taiwan	~CS	T	T	T	A	---	---
PE-95-02	739	F	Feb.16/95	Penghu Islands, Taiwan	CS	A	A	A	A	A	A
PE-95-03	815	F	Feb.15/95	Penghu Islands, Taiwan	CS	A	A	A	A	A	A
PE-95-04	737	---	~Feb.18/95	Penghu Islands, Taiwan	CS	T	T	T	T	T	---
PE-95-05	932	M	~Mar.20/95	Penghu Islands, Taiwan	CS	---	---	T	T	T	T
PE-95-08	1051	---	Aug.22/95	Penghu Islands, Taiwan	~CS	A	A	A	---	---	---
PE-96-01	1298	F	Mar.19/96	Penghu Islands, Taiwan	CS	T	T	T	T	T	T
PE-96-02	1287	M	early 1996	Penghu Islands, Taiwan	CS	T	T	T	---	---	---
PE-96-03	1289	---	early 1996	Penghu Islands, Taiwan	~CS	T	T	T	---	---	---
PE-97-01	1374	F	~Jan.02-03/97	Penghu Islands, Taiwan	CS	T	T	T	T	---	T
PE-97-02	1375	F	~Jan.02-04/97	Penghu Islands, Taiwan	CS	T	T	T	T	---	T
PE-97-03	1376	M	~Jan.05-06/97	Penghu Islands, Taiwan	CS	T	T	T	T	---	T
PE-97-04	1379	M	~Jan.06-07/97	Penghu Islands, Taiwan	CS	T	T	T	T	---	T
PE-97-05	1395	F	~Jan.12-14/97	Penghu Islands, Taiwan	CS	A	A	A	A	---	A

PE-97-06	1389	F	~Jan. 29/97	Penghu Islands, Taiwan	CS	A	A	A	A	---	A
PE-97-07	1392	F	~Feb.10-13/97	Penghu Islands, Taiwan	CS	---	---	T	T	---	T
PE-97-08	1394	---	late Feb./97	Penghu Islands, Taiwan	Sk+	T	T	T	T	---	---
PE-97-09	1391	F	late Feb./97	Penghu Islands, Taiwan	Sk+	---	---	T	T	---	---
PE-97-10	2037	M	early Mar./97	Penghu Islands, Taiwan	CS	T	T	T	T	---	---
PE-97-13	2052	---	early 1997	Penghu Islands, Taiwan	Sk	---	---	T	T	---	---
PE-97-17[h]	2380	F	Oct.25/97	Penghu Islands, Taiwan	Sk	---	---	A	A	---	---
PE-98-01[h]	2369	M	Jan.06/98	Penghu Islands, Taiwan	Sk	---	---	T	T	---	---
PE-98-03[h]	2376	M	Jan.13/98	Penghu Islands, Taiwan	Sk	---	---	T	T	---	---
PE-98-05[h]	2370	M	Jan.22/98	Penghu Islands, Taiwan	Sk	---	---	T	T	---	---
PE-98-07[h]	2379	---	Feb.16/98	Penghu Islands, Taiwan	Sk	---	---	T	---	---	---
PE-98-08[h]	2378	---	Feb.26/98	Penghu Islands, Taiwan	Sk	---	---	T	---	---	---
PE-98-09[h]	2377	M	Mar.26/98	Penghu Islands, Taiwan	Sk	---	---	A	A	---	---
SU-94-07	532	F	~Jan.23/94	Nan Fang Ao, Taiwan	Sk	T	T	T	T	T	T
SU-94-85	735	F	Sept.10/94	Nan Fang Ao, Taiwan	Sk	---	---	T	T	---	---
SU-96-03	1377	---	Sep.13/96	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SU-96-05	1370	F	Dec./96	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SU-96-06	1369	---	Sep.15/96	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SU-97-02	1381	F	Dec./96-Jan./97	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---

SU-97-03	1382	F	Dec./96-Jan./97	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SU-97-12	1385	M	Mar./97	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SU-97-13	1386	F	Mar./97	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	---
SW-95-01	---	F	Jul./75	Southern Japan	CD	---	---	T	T	---	---
TN-97-03	---	M	~Jan.18/97	Tainan, Taiwan	~CS	T	T	T	T	---	T
TN-97-05	---	F	~mid-Jan./97	Tainan, Taiwan	~CS	T	T	T	T	---	---
TN-97-08	1387	M	Mar.12/97	Tainan, Taiwan	CS	T	T	T	T	---	---
TN-97-09	1388	---	Mar.12/97	Tainan, Taiwan	Sk	T	T	T	T	---	---
TP-96-03	1305	---	~Jun./96	Nan Fang Ao, Taiwan	Sk	T	T	T	T	---	T
XM-95-03	---	F	Mar.27/95	~Penghu Islands, Taiwan	CS	---	A	A	A	A	A
XM-95-07	---	M	Apr.09/95	~Penghu Islands, Taiwan	CS	A	A	A	A	A	A
YL-94-20	1355	---	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-07	1362	F	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-08	1352	M	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-09	1366	M	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-10	1363	F	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-11	1358	M	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-12	1359	M	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T
YL-96-13	1360	F	---	Nan Fang Ao, Taiwan'	Sk	T	T	T	T	---	T

YL-96-14	1364	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-15	1361	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-31	1353	F	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-35	1354	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-45	1365	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-65	1356	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-66	1357	M	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T
YL-96-67	1367	F	---	Nan Fang Ao, Taiwan[i]	Sk	T	T	T	T	---	T

[a]National Museum of Natural Science of Taiwan code.

[b]CS - complete skeleton; ~CS - near-complete specimen; CD - complete display specimen; Sk+ - skull & incomplete skeleton, Sk - skull only.

[c]from Chapter 3.

[d]from Chapter 4.

[e]NMNS code was changed.

[f]captive-born offspring of dolphins captured from Penghu Islands (Taiwan) and Indonesia for display at Ocean Park, Hong Kong.

[g]dolphin was born in semi-captive enclosure; mother was captured during the winter of 1992-93.

[h]new specimens not in original data set.

[i]most likely capture location.

APPENDIX II

Osteological key to the identification of bottlenose dolphin species (Tursiops truncatus *and* Tursiops aduncus) *in Chinese waters.*

1) Total number of vertebrae (note: the terminal, triangular element is counted as two vertebrae):

A. 59 to 62 ..... *T. aduncus*

B. 64 to 67 ..... *T. truncatus*

C. 63 ..... use other characters

2) Greatest width of external nares divided by greatest parietal width (GWEN/GPW):

A. ≥ 0.313 ..... *T. aduncus*

B. ≤ 0.306 ..... *T. truncatus*

C. 0.313 > GWEN/GPW > 0.306 ..... use other characters

3A. The premaxillary convexity from lateral view (Fig. 8a) and premaxillary "pinch" at ~ 1/3 rostral length from dorsal view are obvious (Fig. 9a) 4

3B. Premaxillary convexity or "pinch" of the rostrum not obvious (Fig. 8b & 9b) ..... *T. truncatus*

4) Tip of rostrum to the apex of the premaxillary convexity (see Fig. 2a) divided by condylobasal length (TPC/CBL):

A. ≥ 0.352 ..... *T. aduncus*

B. ≤ 0.346 ..... *T. truncatus*

C. 0.352 > TPC/CBL > 0.346 ..... use other characters

5) Tip of rostrum to the apex of the premaxillary convexity divided by length of left upper toothrow (TPC/LUTR):

A. ≥ 0.723 ..... *T. aduncus*

B. ≤ 0.719 ..... *T. truncatus*

C. 0.723 > TPC/LUTR > 0.719 ..... use other characters

6) Tip of rostrum to the apex of the premaxillary convexity divided by length of rostrum (TPC/LR):

A. ≥ 0.607 ..... *T. aduncus*

B. ≤ 0.606 ..... *T. truncatus*

Figure 8. Lateral views of representative skulls from bottlenose dolphin species *T. aduncus* (a); and *T. truncatus* (b). The arrows indicate the premaxillary convexity.

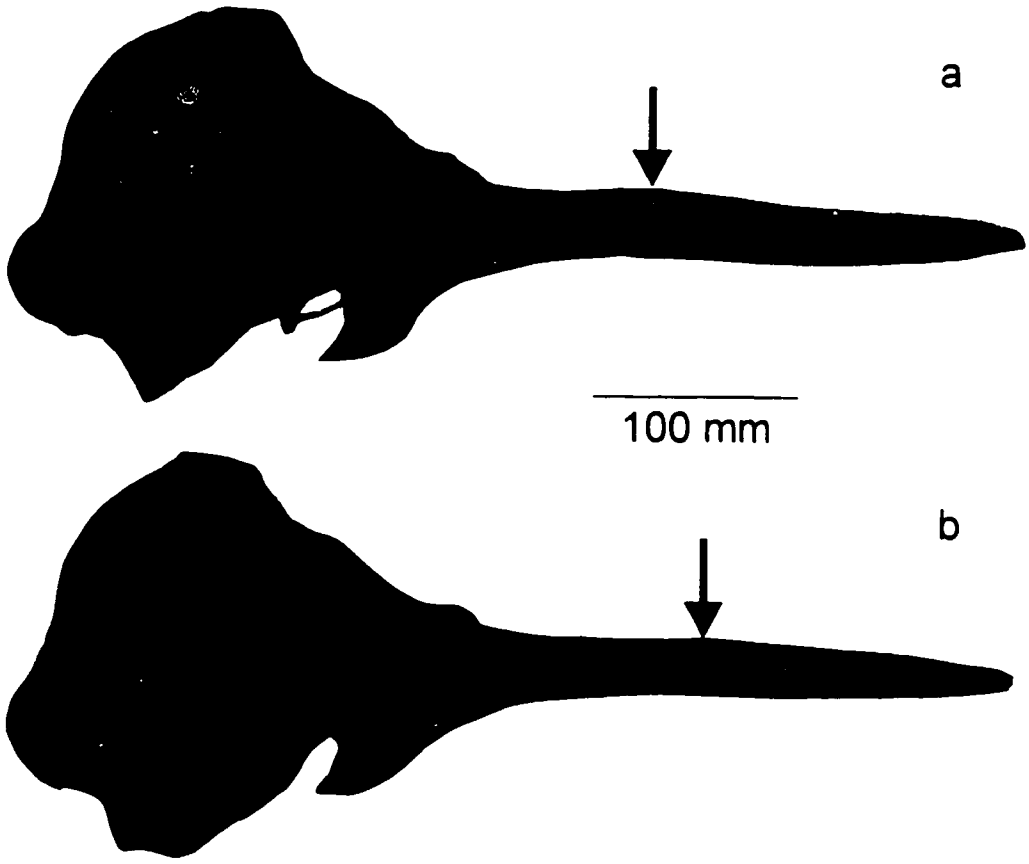
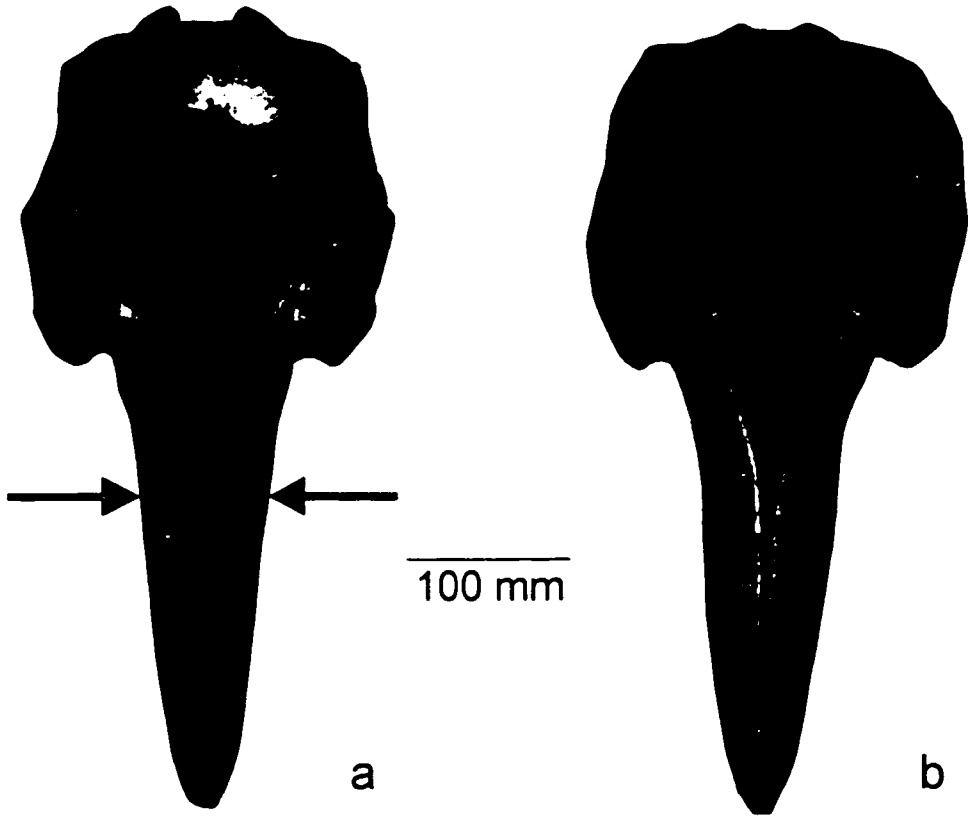

a
100 mm
b

Figure 9. Dorsal views of representative skulls from bottlenose dolphin species *T. aduncus* (a), and *T. truncatus* (b). The arrows indicate the narrowing (or "pinch") in the premaxillae of *T. aduncus*.


100 mm
a
b

Chapter 3

Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters

(With a few minor exceptions for the purposes of thesis presentation, this manuscript was prepared in the format of the journal *Molecular Ecology* and is currently being reviewed for publication)

Mitochondrial DNA analysis of sympatric morphotypes of bottlenose dolphins (genus: *Tursiops*) in Chinese waters

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Abstract

The classification within the bottlenose dolphin (genus *Tursiops*) is controversial. Although many morphological variants exist, most authors have concluded that the genus is composed of a single species, *Tursiops truncatus* (Montagu, 1821). Two distinct morphotypes of bottlenose dolphins, which have been referred to as *T. truncatus* and *T. aduncus*, exist in sympatry in Chinese waters. Comparisons of a 386 base pair fragment of the mitochondrial DNA control region (n=47) indicated that the two sympatric morphotypes were genetically distinct, with seven fixed site differences and a sequence divergence of approximately 4.4%. Furthermore, phylogenetic analyses using maximum likelihood, neighbor-joining and maximum parsimony approaches showed that the *truncatus*-type dolphins from Chinese waters were related more closely to Atlantic Ocean *truncatus*-type than to the sympatric *aduncus*-type dolphins. The Atlantic *truncatus*-type dolphins also shared the same diagnostic sites that separated Chinese *truncatus*-type from *aduncus*-type dolphins. The molecular data agreed completely with the morphological classifications of the specimens. This congruence is strong evidence that the sympatric morphotypes in Chinese waters are reproductively isolated and comprise two distinct species. These findings have important implications for the conservation of bottlenose dolphins in Chinese waters.

Introduction

Classification within the bottlenose dolphin (genus *Tursiops*) is plagued with considerable controversy. Although many species have been proposed (see Hershkovitz 1966), the predominant view is of a single species, *Tursiops truncatus* (Montagu, 1821) (Tomilin 1957; Mitchell 1975; Walker 1981; Hersh & Duffield 1990; Mead & Potter 1990, 1995; Ross & Cockcroft 1990). Many studies have demonstrated that at least two forms of *Tursiops* (coastal and offshore) exist in the waters of the United States that differ in distribution, morphology, haemoglobin profiles, parasitology and diet (e.g., Walker 1981; Duffield *et al.* 1983; Hersh & Duffield 1990; Mead & Potter 1990, 1995). However, none of these researchers recognized more than one species. For South African waters, Ross (1977) concluded that two species, *T. truncatus* and *T. aduncus* (Ehrenberg, 1832) exist, but in a later study on *Tursiops* in Australian waters, Ross & Cockcroft (1990) decided that "there is little doubt that they [the two forms of bottlenose dolphins of South Africa] should be treated as a single species, *T. truncatus*, and that Australian bottlenose dolphins should be assigned to this species also".

Although two morphotypes of *Tursiops* exist in these areas, the degree of overlap in their distributions is unknown. Habitat partitioning between these forms may make the occurrence of sympatry, if at all present, infrequent and inconsistent (see Ross 1977; Kenney 1990; Scott & Chivers 1990). Two morphotypes of *Tursiops* have also been described in Chinese waters (Yang 1976; Zhou & Qian 1985, Zhou 1987; Gao *et al.* 1995) but in contrast, they are known to be sympatric, particularly around the Penghu Archipelago which is situated midway between Taiwan and China in the Taiwan Strait (Yang 1976; Zhou & Qian 1985; J.Y.Wang unpublished data) Furthermore, in this region, these two morphotypes often occur in mixed schools that frequently include other species of dolphins as well (Zhou & Qian 1985).

Recent osteological analysis of the two sympatric morphotypes of *Tursiops* in Chinese waters (see Chapter 2) revealed clear separation and non-overlapping distributions in some cranial characters and the total number of vertebrae. One form resembled *T. truncatus* while the other was similar to the description of *T. aduncus*. These results suggested that the two forms are reproductively isolated in sympatry, and therefore the hypothesis of a monotypic genus was unsupported.

Congruence between morphology and molecular data is strong evidence that the underlying history has been found (Hillis 1987). For example, consistent differences in morphology and mitochondrial DNA (mtDNA) sequences between two sympatric morphotypes of common dolphins (genus *Delphinus*) in the waters off southern California led to the wide acceptance that this genus is not monotypic (see Heyning & Perrin 1994, Rosel *et al.* 1994). Two species (*Delphinus delphis* and *D. capensis*) are now recognized.

The sympatric forms of *Tursiops* in Chinese waters offer a rare opportunity to examine the classification within another highly controversial genus. Clear osteological differentiation has already been demonstrated (Chapter 2). Therefore, in this study, we analysed mtDNA control region sequences to determine: 1) if direct genetic evidence for the separation of the sympatric morphotypes of *Tursiops* in Chinese waters exist; and 2) whether this evidence is congruent with the osteological data.

Materials and Methods

Sample collection and location

Muscle, skin or blood was obtained from 40 bottlenose dolphins collected from Chinese, Indonesian, Brazilian and North African waters (see Fig. 1 and Table 1). Tissue samples were obtained primarily from dolphins killed by fisheries interactions and beach-cast dolphins that died of undetermined cause(s). Blood samples were taken from six living, captive-display dolphins held at Ocean Park, Hong Kong. Muscle and skin tissues were preserved in a solution of 0.25 M disodium EDTA, 20% DMSO and saturated with NaCl (Seutin *et al.* 1991) while blood samples were stored in vacutainers with 1.5 mg of potassium-EDTA per ml of blood and then subsequently frozen at -20°C.

Assignment to the different forms of *Tursiops* was determined *a priori* (by J.Y.Wang) based on pigmentation, external morphology (Chapter 4) and osteology (Chapter 2). For living or freshly dead dolphins, pigmentation or external morphology was sufficient for confident classification. For moderately to extremely decomposed dolphins, the initial classification in the field or prior to necropsy was later verified with skeletal characters. Among the dolphins of this study, 21 were identified as *truncatus*-type and 19 as *aduncus*-type (dolphins from Brazil and North Africa were grouped into the former). There was no uncertainty

Figure 1. Locations where bottlenose dolphins were sampled. Numerals within the square and circle symbols represent the sample size for *Tursiops truncatus*-type and *T. aduncus*-type, respectively. BH - Beihai, Guangxi province, China; BRZ - Rio Grande do Sul, Brazil; DO, XI and XM - Xiamen, Fujian province, China; HC - Hsinchu, Taiwan; HK - Hong Kong; INDO - Indonesia; NAF - Mauritania; PE - Penghu (Pescadores) Islands, Taiwan; SU - Nan Fang Ao, Taiwan; TG - Tungkang, Taiwan. Note: sampling locations do not necessarily reflect the origin of the dolphins (see Table 1).

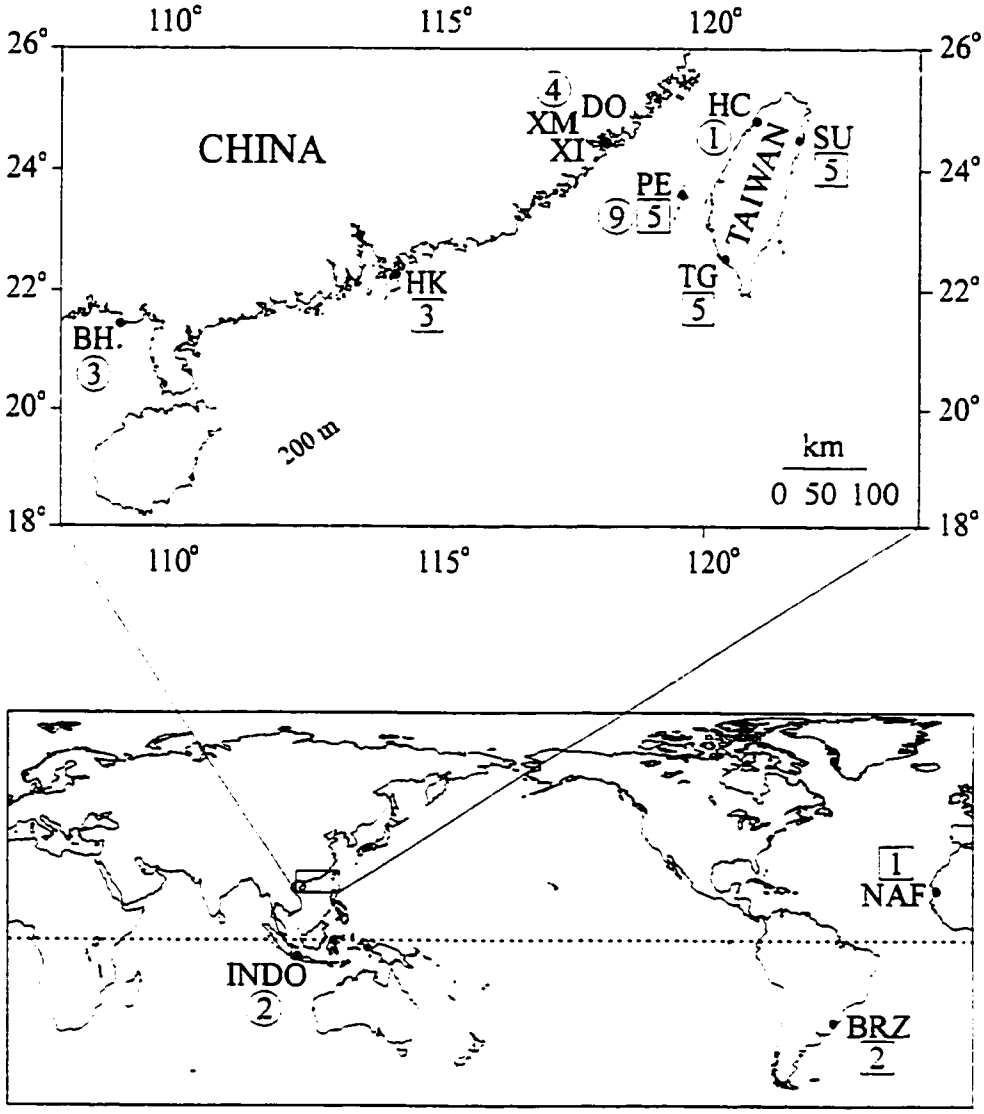

110°
115°
120°
26°
24°
22°
20°
18°
CHINA
4
DO
XM
XI
HC
1
SU
5
PE
5
9
TAIWAN
TG
5
HK
3
BH.
3
200 m
km
0 50 100
INDO
2
1
NAF
BRZ
2

Table 1. Sampling information of bottlenose dolphins.

Specimen Code[a]	Sex	Collection Location	Morphotype	Haplotype	Event[b]
BRZ-92-03	F	Rio Grande do Sul, southern Brazil	*truncatus* [c]	T1	U/S
BRZ-92-05	M	Rio Grande do Sul, southern Brazil	*truncatus* [c]	T1	U/S
HK-94-03	M	Hong Kong	*truncatus*	T2	U/S
HK-94-04	F	Hong Kong	*truncatus*	T3	U/S
HK-95-02	F	Hong Kong	*truncatus*	T2	U/S
NAF-93-04	?	Mauritania, northern Africa	*truncatus* [d]	T4	U/S
PE-94-12	F	Penghu Islands, Taiwan	*truncatus*	T5	H/O
PE-95-01	?	Penghu Islands, Taiwan	*truncatus* [e]	----	U/S
PE-95-04	?	Penghu Islands, Taiwan	*truncatus*	T2	FI
PE-95-05	M	Penghu Islands, Taiwan	*truncatus*	T6	U/S
PE-96-01	F	Penghu Islands, Taiwan	*truncatus*	T7	U/S
SU-94-03	F	Nan Fang Ao, northeastern Taiwan	*truncatus*	T8	FI
SU-94-07	F	Nan Fang Ao, northeastern Taiwan	*truncatus*	T2	FI
SU-94-13	M	Nan Fang Ao, northeastern Taiwan	*truncatus*	T9	FI
SU-94-14	F	Nan Fang Ao, northeastern Taiwan	*truncatus*	T10	FI
SU-94-17	F	Nan Fang Ao, northeastern Taiwan	*truncatus*	T11	FI
TG-94-01	M	Unknown location, Taiwan	*truncatus*	T11	FI
TG-94-02	M	Unknown location, Taiwan	*truncatus*	T12	FI
TG-94-03	F	Unknown location, Taiwan	*truncatus*	T13	FI
TG-96-06	M	Unknown location, Taiwan	*truncatus*	T14	FI
TG-94-34	M	Unknown location, Taiwan	*truncatus*	T2	FI
BH-95-03	M	Beihai, southern China	*aduncus*	A1	FI
BH-95-04	M	Beihai, southern China	*aduncus*	A2	FI

BH-95-05	F	Beihai, southern China	*aduncus*	A2	FI
DO-94-01	?	~Penghu Islands, Taiwan	*aduncus*	A3	FI
HC-96-01	M	Hsinchu, northwestern Taiwan	*aduncus*	A4	FI
INDO-01 (J8)[f]	M	Indonesia	*aduncus*	A5	LDA
INDO-02 (J13)[f]	F	Indonesia	*aduncus*	A6	LDA
PE-01 (TA7804)[f]	F	Penghu Islands, Taiwan	*aduncus*	A7	LDA
PE-03 (TA7805)[f]	F	Penghu Islands, Taiwan	*aduncus*	A8	LDA
PE-04 (TA7901)[f]	M	Penghu Islands, Taiwan	*aduncus*	A8	LDA
PE-05 (TA8403)[f]	F	Penghu Islands, Taiwan	*aduncus*	A1	LDA
PE-94-13	F	Penghu Islands, Taiwan	*aduncus*	A9	FI
PE-94-14	M	Penghu Islands, Taiwan	*aduncus*	A10	FI
PE-94-15	F	Penghu Islands, Taiwan	*aduncus*	A9	FI
PE-95-02	F	Penghu Islands, Taiwan	*aduncus*	A8	FI
PE-95-03	F	Penghu Islands, Taiwan	*aduncus*	A7	FI
XI-95-01	?	~Penghu Islands, Taiwan	*aduncus*	A7	FI
XM-95-03	F	~Penghu Islands, Taiwan	*aduncus*	A7	FI
XM-95-07	M	~Penghu Islands, Taiwan	*aduncus*	A11	FI

[a]letters of the specimen code correspond to collection locations shown in figure 1 (except for TG - collection location unknown but most likely in Taiwan waters; and DO, XI and XM - origin of specimens is the waters of the Penghu Islands, Taiwan).

[b]U/S = undetermined / stranding; H/O = heart failure possibly resulting from severe osteomyelitis; FI = fishery interaction; LDA = living display animal.

[c]identified by E.R. Secchi, Museu Oceanográfico, Rio Grande, Brazil.

[d]identified by Dr. M.A. Vely, Tropical Oceans Mammals Services, Madagascar.

[e]identified as *truncatus*-type using osteological data but as *aduncus*-type in the field.

[f]provided by Ocean Park, Hong Kong (Ocean Park specimen code in parentheses).

in the morphological classification of any specimen except PE-95-01 which was classified in the field as *aduncus*-type but as *truncatus*-type using osteology (see Chapter 2).

DNA extraction and amplification

Total DNA was extracted from tissues following the methods used in Wang *et al.* (1996). For blood, 3.5 ml of the sample was added to an equal amount of lysis buffer of double concentration before proceeding to DNA extraction. A segment of the mtDNA which includes a portion of the highly variable control region was amplified by the polymerase chain reaction (PCR) using the primer of Strobeck (5'-TAATATACTGGTCTTGTAAACC-3') which was modified from primer L15926 of Kocher *et al.* (1989; see Murray *et al.* 1995) and H00034 (5'-TACCAAATGTATGAAACCTCAG-3') of Rosel *et al.* (1994). For the corresponding position in the human sequence of the former primer, see Anderson *et al.* (1981). The amplified product was isolated by gel electrophoresis (1.0% low-melting point agarose), then excised and eluted in 150 µl of distilled-deionized water. This product was then used as the template DNA for a second PCR amplification with the same primers. The product from the second amplification was purified following Malik *et al.* (1997). All amplifications were performed using a Perkin-Elmer-Cetus Thermal Cycler 480 in a total volume of 25 µl with 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 5 µmoles of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 1.5 U AmpliTaq DNA polymerase (Perkin-Elmer). In the first amplification reaction, 25 ng of template DNA were used and for the second amplification, 2 µl of the products of the first amplification (eluted in water) were used as the template. All amplification reactions were performed with the following temperature profile: three cycles at 94°C for three minutes, 55°C for one minute and 72°C for two minutes; and 30 cycles at 95°C for 15s, 55°C for 30s and 72°C for two minutes followed by an eight minute extension period at 72°C. Both the L- and H-strands of the amplified products were sequenced for all samples using the primer of Strobeck (above) and the internal primer H16498 (5'-CCTGAAGTAAGAACCAGATG-3') of Rosel *et al.* (1994), respectively. Cycle sequencing was performed with 150 to 300 ng of PCR product using the PRISM™ Ready Reaction Dye Deoxy Terminator Protocol (Applied Biosystems Inc., Foster City, CA). Sequencing

reactions were performed with a Perkin-Elmer-Cetus Thermal Cycler 480 and the 373A Stretch DNA Sequencing System (Applied Biosystems Inc.). DNA sequences were determined by the ABI PRISM™ Sequencing Analysis software version 2.1.1 and the ABI 373A Data Collection program version 1.2.1. In addition, several specimens of both morphotypes were sequenced twice to verify sequences.

The control region sequences of eight additional *Tursiops* samples were obtained from Genbank via accession numbers U20912 to U20917, U20919 and U20920. These *Tursiops* sequences were assigned to the *truncatus*-type since they were from U.S. Atlantic waters (L. Siemann, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA personal communication) where the *aduncus*-type does not exist. Control region sequences for two outgroups were also obtained from GenBank: the short-beaked common dolphin, *Delphinus delphis* (U02646) and the long-beaked common dolphin, *D. capensis* (U02661) of Rosel *et al.* (1994). The sequence for a third outgroup species, the pantropical spotted dolphin (*Stenella attenuata*) was obtained directly from Rosel *et al.* (1995a).

DNA analysis

Sequences were edited using SeqEd™ (ABI 1992) and aligned by eye. Phylogenetic relationships among haplotypes (unique sequences) were determined using the maximum likelihood method which has been shown to be superior to both neighbor-joining and maximum parsimony methods for reconstructing molecular phylogenies (Hasegawa *et al.* 1991; Tateno *et al.* 1994; Huelsenbeck 1995). However, neighbor-joining and maximum parsimony analyses were also performed for comparison. Maximum likelihood and maximum parsimony analyses were performed using the Phylogenetic Inference Package (PHYLIP) version 3.5c (Felsenstein 1995) with global rearrangement, "jumble" and "outgroup" (*D. delphis* in all cases) options and transitions were given the default weighting of 1:2 relative to transversions. Sequence data were bootstrapped 500 times using SEQBOOT and the majority-rule consensus tree was determined using CONSENSE (both are algorithms of PHYLIP version 3.55c) for maximum parsimony. The distance matrix for the neighbor-joining analysis was generated using the Tamura-Nei Gamma distance method of MEGA version 1.0 (Kumar *et al.*

1993) with the options: a=0.99 (following Rosel *et al.* 1995b; note: the results of using the default setting of a=0.5 were little different from a=0.99); pairwise deletion; and 500 bootstraps. The neighbor-joining analysis was also performed using MEGA with 500 bootstraps. The average number of substitutions per site within groups (nucleotide diversity) and between groups (nucleotide divergence) was estimated following Nei & Jin (1989) using the SENDBS program written by N. Takezaki (National Institute of Genetics, Mishima, Shizuoka, Japan). Computations were performed with the Tamura-Nei Gamma model of substitutions with a=1.0 (unchangeable default) and the standard errors were calculated with 500 bootstraps. Nucleotide diversity was estimated for Chinese *truncatus*-type and *aduncus*-type dolphins and nucleotide divergence estimates were obtained for all pairwise comparisons between Chinese *truncatus*-type, *aduncus*-type and the outgroups. Haplotypic diversity (Nei & Tajima 1981) for the two morphotypes of Chinese waters was also estimated.

Results

All samples except PE-95-01 yielded sufficient quantities of DNA for analysis (unfortunately, sampling of PE-95-01 occurred about two months after it was first reported stranded and dead on a beach). Of the 386 bp of control region examined for 47 *Tursiops* sequences (39 sequenced in the present study plus 8 from Genbank), 50 sites were variable, 46 sites were informative phylogenetically and 4 were autapomorphies (Fig. 2). When outgroups were considered, 56 sites were variable, which included 42 transitions, 8 transversions and 6 insertions / deletions. Seven site differences were fixed between the two morphotypes of *Tursiops*; three transitions (sites 22, 93 and 112), two tranversions (sites 214 and 273), one insertion / deletion (site 275) and one site that contained both a transition and a transversion (site 266). Site numbers correspond to positions in the control region sequence of *S. attenuata* (Rosel *et al.* 1995a).

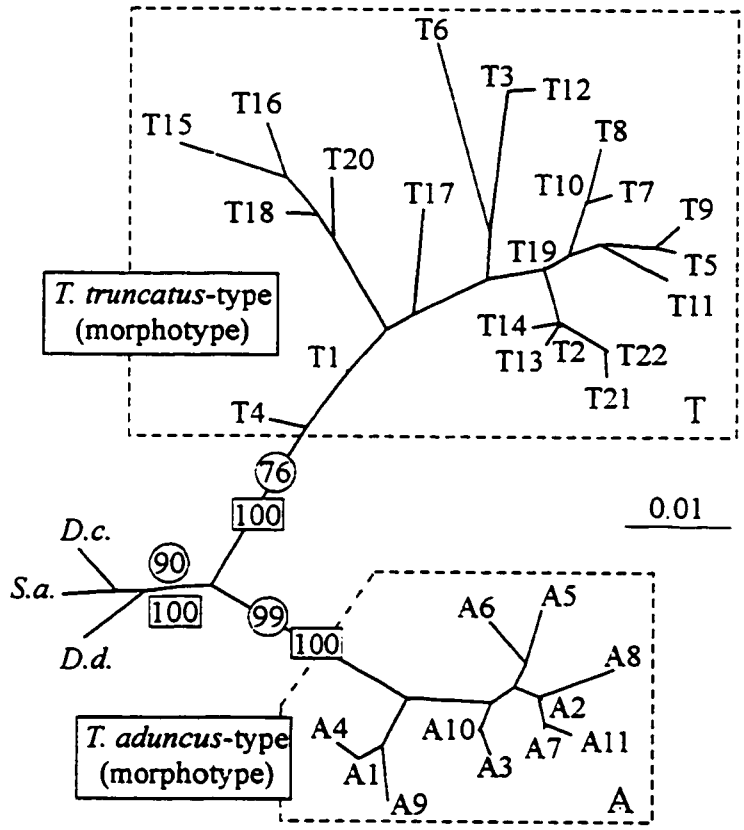
Molecular phylogeny and classification

The division of the two *Tursiops* morphotypes was supported independently by maximum likelihood, neighbor-joining and maximum parsimony analyses of the sequence data (Fig. 3). Most of the termini of the

Figure 2. Variable sites of 33 bottlenose dolphin mtDNA control region haplotypes (L-strand, 5' to 3' sequence). Site numbers correspond to positions in the control region sequence of the pantropical spotted dolphin (Rosel et al. 1995a). A dot indicates identity and a dash indicates a deletion relative to the top sequence (T1). The number of individuals (*n*) for each haplotype is shown. Complete haplotype sequences (386 base pairs) were deposited into GenBank under accession numbers: AF056219 to AF056243. Asterisks indicate fixed site differences between haplotypes of T and A lineages of figure 3.

Haplotype	111111111222222222222222222222333333333333 234446991114455679001144667777778888889001125799992425643912678019255649695603578901345654615681234 7 * * * * * **	n
T1	GTCCGTTTTGGATCATGTCCACCGTACACATTCTCCTCCGTTTATCCCTT	2
T2	.C.....C....T.....T..T..C.....C.......A....CT....	5
T3	..........A..T........T.CC....CCT.T....A....CT.T..	1
T4	.......C...........T.....................TA..	1
T5	.C.....C....TG.......TA.C.....C.......A.C..CTT...	1
T6	............T.....T.....T......TCTT...A...GCT..C.	1
T7	.C.T....C....T.....T.....C.....C...T...A....CTT...	1
T8	.CGT.C..C....T...........C.....C...T...A....CTT...	1
T9	.C.....C....TG.......T..C.....C.......A.CC.CTT...	1
T10	.C.T....C....T...........C.....C...T...A....CTT...	1
T11	.CG.....C....T........TA.C.....C..T....A....CTT...	2
T12	..G.....T.A..T........T.CC....CCT.T....A....CT.T..	1
T13	.C.....C....T.....T..T..C.............A....CT....	1
T14	.CG.....C....T.....T..T..C.....C.......A....CT....	1
T15	....C..CT..-.....C....T...T..-.CT.T..-T......T....	1
T16	....T...T..-.......T..T........CT.....T......T....	1
T17	...TA.....-...................-.C..T...T.....CT...-	1
T18	.C..T.....-.....C....T.......-.CT...........CT....	1
T19	.C.....C..-.T...........C.....C.......A....CT...-	1
T20	.C.....C...-.....C....T..C.....CT...........T....	1
T21	.C.....C..-.T.....T..T...T....C..T..-.A....CT....	1
T22	.C.....C..-.T.....T..T..CT....C..T..-.A....CT....	1
A1	A..T..CCTA...T....T.TT...G.T-...TC..C...........C.	2
A2	A..T..CCTAA..T.C....TT...G.T-...TCT.C..AC....T....	2
A3	A..T..CCTAA.CT......TT...G.T-...TC.....AC....T....	1
A4	A..T..CCTA...T....T.TT...G.T-...TC..C..A........C.	1
A5	A..T..CCCAA..T.CA.T.TT...G.T-...TC..C..AC....T....	1
A6	A..T..CCTAA..T.C..T.TT...GTT-...TC.....AC....T....	1
A7	A..T..CCTAA..T.C....TT...G.T-...TCT.C..AC....T.T..	4
A8	A..T..CCTAA..T.C....T.T..G.T-...TCT.C..A.....T....	3
A9	A..T..CCTA...T....T.TTT..G.T-...TC..C.......CT..C.	2
A10	A..T..CCTAA.CT......TT...G.T-...TC..C..AC....T....	1
A11	A..T..CCTAA..T.C....TT...G.T-...TCT....AC....T.T..	1

Figure 3. Maximum likelihood tree of bottlenose dolphin haplotypes based on mitochondrial DNA control region sequences. "T" and "A" represent lineages that correspond to *T. truncatus*-type and *T. aduncus*-type dolphins, respectively. Bootstrap values from a neighbor-joining analysis are shown in circles and bootstrap consensus values (100 minimum length trees of 134 steps) from a maximum parsimony analysis are shown in boxes. Haplotypes T1 and T4 are from Brazil and North Africa, respectively; T15-T22 are Genbank sequences from U.S. waters; A5 and A6 are from Indonesia; and all other haplotypes are from Chinese waters (see Table 1 for more details). *S.a.* = pantropical spotted dolphin (*Stenella attenuata*); *D.d.* = short-beaked common dolphin (*Delphinus delphis*); *D.c.* = long-beaked common dolphin (*D. capensis*).


T6
T3
T12
T16
T15
T8
T20
T10
T7
T17
T9
T18
T19
T5
T11
T. truncatus-type
(morphotype)
T14
T1
T13
T2
T22
T21
T
T4
76
100
0.01
D.c.
90
S.a.
100
99
A6
A5
100
A8
D.d.
A2
T. aduncus-type
(morphotype)
A4
A10
A7
A11
A1
A3
A9
A

trees were also in agreement. The molecular and morphological classifications for all specimens were completely congruent The highest bootstrap value (99%) in the neighbor-joining tree supported the monophyly of the *aduncus*-type while the bootstrap value for monophyly of the *truncatus*-type was lower at 76%. This may be partially due to the large number of divergent operational taxonomic units (haplotypes) within the *truncatus*-type (see Zharkikh & Li 1995). There were 100 equally parsimonious trees requiring 134 steps. Bootstrap consensus values of the maximum parsimony analysis fully supported (100%) the *truncatus*- and *aduncus*-types as monophyletic groups.

Diversity and divergence

There were 12 haplotypes among the 17 samples of *truncatus*-type dolphins from Chinese waters (T2, T3, and T5 to T14) and 11 haplotypes (A1 to A11) among the 19 *aduncus*-type dolphins. Each of the eight *Tursiops* sequences from Genbank was a unique haplotype (T15 to T22). There were two haplotypes (T1 and T4) among the three Atlantic individuals examined. The most common haplotype (T2) was from the *truncatus*-type group and was represented by 5 individuals from Chinese waters (Table 1 and Fig. 2). Estimates of haplotypic diversity for Chinese *truncatus*-type and *aduncus*-type dolphins were very similar at 0.92 and 0.93, respectively. The estimate of nucleotide divergence between *aduncus*-type dolphins and Chinese *truncatus*-type dolphins at 4.4% ± 1.09 (SE) was greater than the estimates of nucleotide diversity for Chinese *truncatus*-type and *aduncus*-type dolphins (1.9% ± 0.43 (SE) and 1.6% ± 0.47(SE), respectively). The nucleotide divergence between the sympatric morphotypes of *Tursiops* was greater than the divergence between the outgroups and each of the morphotypes. However, the standard errors of these divergence estimates overlapped substantially (see Table 2).

Discussion

The congruence of the mtDNA data and morphology (the phenotypic expression of nuclear genes) is strong evidence that reproductive isolation exists between the sympatric morphotypes of *Tursiops* in Chinese

Table 2. Estimates of percent nucleotide diversity (along diagonal) and divergence (above diagonal) for bottlenose dolphin groups and outgroup species (pantropical spotted, long- and short-beaked common dolphins). Standard errors are indicated in parentheses.

	Chinese *T. truncatus*	*T. aduncus*	Outgroup Species
Chinese *T. truncatus*	1.9	4.4	3.5
	(0.43)	(1.09)	(0.96)
T. aduncus	--	1.6	3.1
		(0.47)	(0.88)
Outgroup Species	--	--	--

waters. Furthermore, the closer relationship of *truncatus*-type individuals from two discontinuous oceanic basins than between Chinese *truncatus*-type and the sympatric *aduncus*-type provides further evidence that this genus is not monotypic. Additional support for, though not the basis of, separate *Tursiops* species comes from comparisons with the studies of *Delphinus* classification (Heyning & Perrin 1994; Rosel *et al.* 1994). The wide acceptance of separate species within the genus *Delphinus* was also based on congruent morphological and molecular separation and provided a precedent for the present recommendations for *Tursiops*. Moreover, seven fixed diagnostic site differences separates the two *Tursiops* species, while only one was found between the two species of *Delphinus* and the average nucleotide divergence between sympatric *Tursiops* morphotypes (4.4%) was about four times greater than between *Delphinus* species (1.09%, Rosel *et al.* 1994). Assuming the mtDNA control regions of these two genera evolved at similar rates, the mtDNA lineages within *Tursiops* diverged much earlier than within *Delphinus*. These comparisons strengthen the conclusion that the sympatric morphotypes in Chinese waters represent distinct species with diagnostic morphological and molecular characters. From here on, the provisional names *T. truncatus* and *T. aduncus*, suggested in Chapter 2 will be used to refer to these species in the study.

The relationship of Tursiops *and other delphinids*

The close relationship of each *Tursiops* species to the delphinid outgroups relative to the divergence between *Tursiops* species and the topology of the outgroups (i.e., *Delphinus* species were not sister groups) were unexpected. The simplest explanation is that the results were an artifact of analysing a region of DNA that evolves too rapidly and is thus inappropriate for assessing relationships beyond the intraspecific level. Rosel *et al.* (1995a) found that transitions in the control region of the true porpoises (Phocoenidae) saturated rapidly and cautioned against the use of control region sequences for inferring relationships among species.

An alternate hypothesis is that *Tursiops* is paraphyletic. However, the osteological data indicated clearly a greater affinity between the *Tursiops* species than between *T. aduncus* and the genus *Stenella* which contains a species, the Atlantic spotted dolphin (*Stenella frontalis*), that is superficially very similar to *T.*

aduncus (Chapter 2). Distinguishing between the two *Tursiops* species using cranial characters without detailed measurements is difficult whereas differentiating between *Tursiops* and *Stenella* can be achieved easily (e.g., using total number of teeth).

The present study is inadequate to address these, and possibly other hypotheses. Further molecular and morphological studies with more delphinid species are needed to better understand the relationship of these *Tursiops* and other delphinid species.

Speciation and zoogeography

The substitution rate for cetacean mtDNA control region was estimated to be 0.5% per million years (Hoelzel *et al.* 1991). Using this rate, the mtDNA lineages of the sympatric *Tursiops* would have diverged about 6.6 to 11 million years ago (mya) or during the late Miocene. Since the earliest fossils identifiable as *Tursiops* were found in formations that dated to only 4-7 mya (Barnes 1990), the palaeontological and molecular estimates of *Tursiops* divergence do not agree completely. Some studies have suggested that this rate may be too slow for some species of small cetaceans (see Rosel *et al.* 1995b; Wang *et al.* 1996) and it may also be too slow for *Tursiops*. If we assume that the nucleotide substitution rate of the mtDNA control region for *Tursiops* is similar to that for humans (14% per million years: Vigilant *et al.* 1991) or birds (21% per million years: Quinn 1992), the sequence divergence between the two sympatric species of *Tursiops* (4.4%) suggests that the divergence of *Tursiops* mtDNA lineages (about 160,000 to 390,000 years ago) occurred during the Pleistocene epoch. This estimate would not contest the palaeontological estimate and would support the speculation by Ross & Cockcroft (1990) that Pleistocene glaciation events in the Indian Ocean were responsible for the evolution of the tropical, "spotted" bottlenose dolphin (*T. aduncus*). These events have also been implicated in the speciation of other species pairs of closely-related delphinids (Perrin *et al.* 1978, as cited by Gaskin (1985)) and Rosel *et al.* 1994.

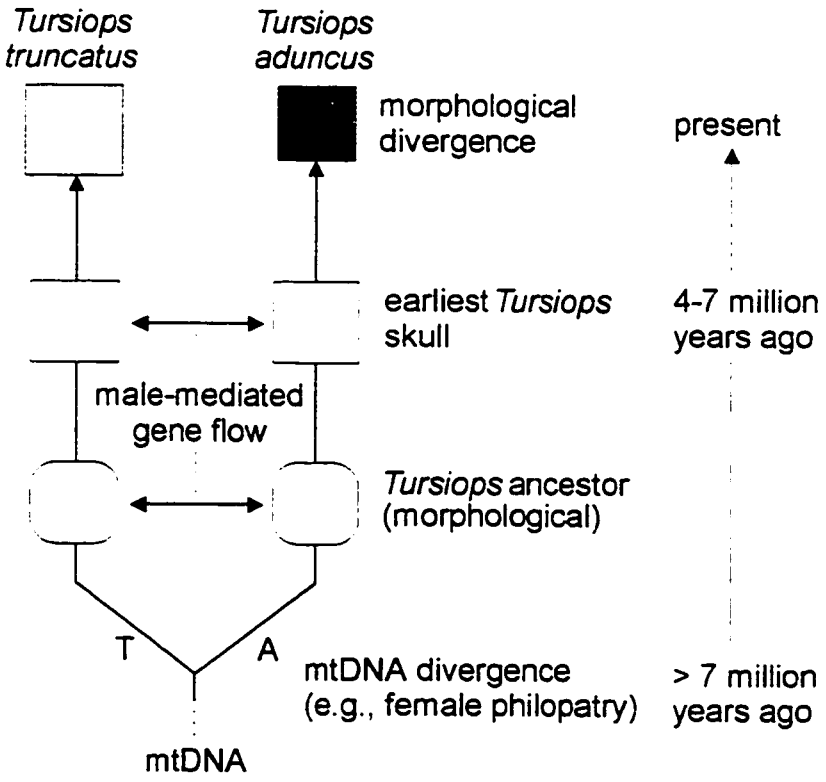
Another explanation for the discrepancy in the divergence times may be that the mtDNA lineages diverged before morphology. For example, female philopatry may have been the mechanism for isolating

maternal lineages, while male-mediated genetic exchange maintained morphological similarity between groups of the ancestor to *Tursiops* and *Tursiops*. Later morphological modifications for ecological specialization may have completed the genetic isolation between *Tursiops* species resulting in the present observed congruence between morphology and molecular data (Fig. 4). Of course, it may also be that Pleistocene events were not a factor in *Tursiops* speciation. Clearly, refinements in both the palaeontological and molecular dating methods are needed before these hypotheses can be tested.

Conservation implications of separate species

Our finding of more than one species of *Tursiops* in Chinese waters has important conservation implications. Since all current regional and international conservation legislation (e.g., the Wildlife Conservation Law of Taiwan) recognize only *T. truncatus*, adequate protection is not afforded to bottlenose dolphins, particularly in regions where more than one species are present. *T. aduncus* is especially vulnerable to exploitation due to: 1) its coastal distribution and thus close proximity to human threats; 2) its distribution being limited to the Indo-Pacific, where many developing nations have minimal conservation legislation or enforcement; and 3) the overwhelming preference for this species by aquaria and zoos since it survives better in captivity (Reeves *et al.* 1994), is less aggressive, easier to train, smaller, and also less expensive to maintain than *T. truncatus* (training staff at Ocean Park of Hong Kong personal communication). With increasing interest in constructing new dolphinaria in Asian nations, the pressure to collect this species may increase. There are concerns about the level of dolphin mortality during the capture, holding and transportation of animals and the low survivorship of dolphins in most dolphinaria in Southeast Asia (UNEP 1996). Since dolphin survivorship, even in one of the most affluent and advanced facilities in Southeast Asia (Ocean Park, Hong Kong), was below the "industry average" (see Reeves *et al.* 1994), it is unlikely that new institutions in poorer nations of this region will have better success. And since legislation protecting marine mammals may be lax or poorly enforced in many Asian countries, removals from wild populations may be more economical than improving care and husbandry knowledge or facilities for maintaining or stocking the captive populations in these institutions. If unregulated or poorly regulated, this industry can pose a serious threat to this species.

Figure 4. A hypothesis of the order of events that could lead to a discrepancy between the divergence times of two bottlenose dolphin (*Tursiops truncatus* and *T. aduncus*) species estimated using morphological and mitochondrial DNA data.


Tursiops truncatus
Tursiops aduncus
morphological divergence
present
earliest Tursiops skull
4-7 million years ago
male-mediated gene flow
Tursiops ancestor (morphological)
T
A
mtDNA divergence (e.g., female philopatry)
> 7 million years ago
mtDNA

In contrast, *T. truncatus* are harvested predominantly for human consumption markets throughout Taiwan waters, especially along the east coast where *T. aduncus* are not found (Chen 1990; J.Y. Wang unpublished data). In order to afford proper protection to both species, existing conservation legislation must be amended to include *T. aduncus*, and the impact of exploitation or other threats to each species must be considered independently.

Understanding population divisions within each species is also critical for effective conservation programs. Presently, little is known about the boundaries of *Tursiops* populations, especially outside U.S. waters (see Curry & Smith 1997). However, there is some evidence that suggests the presence of different populations within *T. aduncus*. The two Indonesian haplotypes (A5 and A6) grouped together consistently in all phylogenetic analyses (i.e., maximum likelihood, neighbor-joining and maximum parsimony). These dolphins may also be different morphologically from those of Chinese waters. Like South African specimens (Ross 1977, 1984), Indonesian *T. aduncus* appears to be smaller, has a shorter beak and develops ventral spotting at a shorter body length. The ventral spots on Indonesian dolphins at Ocean Park (Hong Kong) apparently begin to appear at lengths less than 200 cm and the density of the spotting becomes moderate to extreme at body lengths of about 220 cm. Similar development in ventral spotting was also reported for *T. aduncus* from Monkey Mia, Western Australia (P. Berggren, Department of Zoology, University of Stockholm, Sweden personal communication). In contrast, spotting on the Penghu Island *T. aduncus* only begin to appear at about 210 to 230 cm in body length, and these specimens are also some of the largest reported for this species (see Chapter 2 and 4). Further genetic studies using both mtDNA and nuclear markers (e.g., microsatellites loci) with more sampling throughout the distribution of *Tursiops* is needed, and non-molecular information such as morphology, seasonal migration, behaviour, etc. should be used to guide the design of these studies. Finally, even though the two species can be identified with osteological features and molecular methods, characters are still needed for field identifications. Until field identifications can be performed with certainty, studies dependent on identification of free-ranging dolphins (e.g., estimating abundance of each species using traditional survey techniques) must be treated as suspect and unreliable. A detailed analysis of external morphology is underway to find characters that may provide confident field identifications.

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References

ABI (1992) *SeqEd™: DNA Seqeunce Editor, Version 1.0.3.* ABI, Foster City.

Anderson S, Bankier AT, Barrell BG *et al.* (1981) Sequence and organization of the human mitochondrial genome. *Nature*, **29**, 457-465.

Barnes LG (1990) The fossil record and evolutionary relationships of the genus *Tursiops*. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 3-26. Academic Press, San Diego.

Chen CT (1994) Dolphin catches in Taiwan. In: *Gillnets and cetaceans* (eds. Perrin WF, Donovan GP, Barlow J), *Report of the International Whaling Commission*, Special Issue **15**, pp. 623-624 (abstract). The International Whaling Commission, Cambridge.

Curry BE, Smith J (1997) Phylogeographic structure of the bottlenose dolphin *(Tursiops truncatus)*: stock identification and implications for management. In: *Molecular genetics of marine mammals* (eds. Dizon AE, Chivers SJ, Perrin WF), *The Society for Marine Mammalogy*, Special Publication **3**, pp. 227-247 Allen Press, Lawrence.

Duffield DA, Ridgeway SH, Cornell LH (1983) Hematology distinguishes coastal and offshore forms of dolphins (*Tursiops*). *Canadian Journal of Zoology*, **61**, 930-933.

Felsenstein J (1995) *PHYLIP (Phylogenetic Inference Package), Version 3.5c*. University of Washington, Seattle.

Gao A, Zhou K, Wang Y (1995) Geographical variation in morphology of bottlenose dolphins (*Tursiops sp.*) in Chinese waters. *Aquatic Mammals*, **21**, 121-135.

Gaskin DE (1985) *The ecology of whales and dolphins*. Heinemann, London.

Hasegawa M, Kishino H, Saitou N (1991) On the maximum likelihood method in molecular phylogenetics. *Journal of Molecular Evolution*, **32**, 443-445.

Hersh SL, Duffield DA (1990) Distinction between Northwest Atlantic offshore bottlenose dolphins based on hemoglobin profile and morphometry. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 129-139. Academic Press, San Diego.

Hershkovitz P (1966) Catalog of living whales. *US National Museum Bulletin*, **246**, Smithsonian Institute, Washington, DC.

Heyning JE, Perrin WF (1994) Evidence for two species of common dolphins (genus *Delphinus*) from the eastern North Pacific. *Contribution in Science*, **442**, 1-35.

Hillis DM (1987) Molecular and morphological approaches to systematics. *Ann. Rev. Ecol. Syst.* **18**: 23-42.

Hoelzel RA, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial DNA D-loop region. *Molecular Biology and Evolution*, **8**, 475-493.

Huelsenbeck JP (1995) The robustness of two phylogenetic methods: four taxon simulations reveal a slight superiority of maximum likelihood over neighbor joining. *Molecular Biology and Evolution*, **12**, 843-849.

Kenney RD (1990) Bottlenose dolphins off the northeastern United States. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 369-386. Academic Press, San Diego.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196-6200.

Kumar S, Tamura K, Nei M (1993) *MEGA: Molecular Evolutionary Genetics Analysis, Version 1.0.* The Pennsylvania State University, University Park.

Malik S, Wilson PJ, Smith RJ, Lavigne DM, White BN (1997) Pinniped penises in trade: a molecular-genetic investigation. *Conservation Biology*, **11**, 1365-1374

Mead JG, Potter CW (1990) Natural history of bottlenose dolphins along the central Atlantic coast of the United States. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 165-195. Academic Press, San Diego.

Mead JG, Potter CW (1995) Recognizing two populations of the bottlenose dolphin (*Tursiops truncatus*) off the Atlantic coast of North America: morphologic and ecologic considerations. *IBI Report*, **5**, 31-44.

Mitchell ED [ed.] (1975) Report on the meeting on smaller cetaceans. *Journal of the Fisheries Research Board of Canada*, **32**, 889-983.

Murray BW, McClymont RA, Strobeck C (1995) Forensic identification of ungulate species using restriction digests of PCR-amplified mitochondrial DNA. *Journal of Forensic Sciences*, **40**, 943-951.

Nei M, Jin, L (1989) Variances of the average numbers of nucleotide substitutions within and between populations. *Molecular Biology and Evolution*, **6**, 290-300.

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. *Genetics*, **97**, 145-163.

Quinn TW (1992) The legacy of mother goose - phylogenetic patterns of lesser snow geese, *Chen caerulescens caerulescens*, maternal lineages. *Molecular Ecology*, **1**, 105-117.

Reeves RR, DeMaster DP, Hill CL, Leatherwood S (1994) Survivorship of odontocete cetaceans at Ocean Park, Hong Kong, 1974-1994. *Asian Marine Biology*, **11**, 107-124.

Rosel PE, Dizon AE, Haygood MG (1995b) Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena* on interoceanic and regional scales. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1210-1219.

Rosel PE, Dizon AE, Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus*). *Marine Biology*, **119**, 159-167.

Rosel PE, Haygood MG, Perrin WF (1995a) Phylogenetic relationships among the true porpoises (Cetacea: Phocoenidae). *Molecular Phylogenetics and Evolution*, **4**, 463-474.

Ross GJB (1977) The taxonomy of bottlenosed dolphins *Tursiops* species in South African waters, with notes on their biology. *Annals of the Cape Provincial Museums (Natural History)*, **11**, 135-194.

Ross GJB (1984) The smaller cetaceans of the southeast coast of southern Africa. *Annals of the Cape Provincial Museums (Natural History)*, **15**, 173-410.

Ross GJB, Cockcroft VG (1990) Comments on Australian bottlenose dolphins and the taxonomic status of *Tursiops aduncus* (Ehrenberg, 1832). In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 101-128. Academic Press, San Diego.

Scott M, Chivers S (1990) Distribution and herd structure of bottlenose dolphins in the eastern tropical Pacific Ocean. In: *The Bottlenose Dolphin* (eds. Leatherwood S, Reeves RR), pp. 387-402. Academic Press, San Diego.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82-90.

Tateno Y, Takezaki N, Nei M (1994) Relative efficiencies of the maximum likelihood, neighbor-joining, and maximum-parsimony methods when substitution rate varies with site. *Molecular Biology and Evolution*, **11**, 261-277.

Tomilin AG (1957) *Mammals of the U.S.S.R. and adjacent countries. Volume IX. Cetacea*. Translated from Russian by the Israel Program for Scientific Translations, Jerusalem.

UNEP (1996) Report of the workshop on the biology and conservation of small cetaceans and dugongs of southeast Asia. UNEP(W)/EAS WG.1/2. United Nations Environmental Programme, Bangkok.

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC (1991) African populations and the evolution of human mitochondrial DNA. *Science*, **253**, 1503-1507.

Walker WA (1981) Geographical variation in morphology and biology of bottlenose dolphins (*Tursiops*) in the eastern North Pacific. *National Oceanic and Atmospheric Administration, US Department of Commerce, Administrative Report* LJ-81-03C. National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, California.

Wang JY, Gaskin DE, White BN (1996) Mitochondrial DNA analysis of harbour porpoise, *Phocoena phocoena*, subpopulations in North American waters. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 1632-1645.

Yang HC (1976) Studies on the whales, porpoises and dolphins of Taiwan. *Annual Report of Science, Taiwan Museum (Taipei)*, **19**, 131-178. [In Chinese with English abstract].

Zharkikh A, Li WH (1995) Estimation of confidence in phylogeny: the complete-and-partial bootstrap technique. *Molecular Phylogenetics and Evolution*, **4**, 44-63.

Zhou K (1987) Notes on two species of dolphins of the genus *Tursiops* in Chinese waters. *Acta Theriologica Sinica*, **7**, 246-254. [in Chinese with English abstract].

Zhou K, Qian W (1985) Distribution of the dolphins of the genus *tursiops* in the China Seas. *Aquatic Mammals*, **1**, 16-19.

Chapter 4

Differences in the external morphology of two sympatric species of bottlenose dolphins (genus *Turisops*) in Chinese waters

(With a few minor exceptions for the purposes of thesis presentation, this manuscript was prepared in the format of the *Journal of Mammalogy* and is currently being reviewed for publication)

DIFFERENCES IN THE EXTERNAL MORPHOLOGY OF TWO SYMPATRIC SPECIES OF BOTTLENOSE DOLPHINS (GENUS TURSIOPS) IN CHINESE WATERS

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Abstract

Clear genetic and osteological characters exist to distinguish two sympatric species of bottlenose dolphins (genus Tursiops) in Chinese waters. However, these characters are not useful for the identification of free-ranging dolphins. To determine if these species could be differentiated by external morphology, a discriminant analysis was performed on eight external morphometric characters of 57 specimens, 40 of which were classified, *a priori*, as T. truncatus and 17 as T. aduncus using genetic, osteological and other information. External morphological separation of the two species was highly significant and the discriminant scores were non-overlapping. In addition, the classification functions were completely successful in classifying several new specimens from both Chinese and Indonesian waters. Furthermore, rostrum length (as an absolute measure and as a proportion of total body length or snout-to-eye length) revealed non-overlapping distributions for the two species in Chinese waters, which offered a useful field character for classifying fresh carcasses, stranded or captive specimens, photographs of dolphins, and in some situations, free-ranging individuals.

Introduction

Although considerable variation exists in the bottlenose dolphin, most researchers have taken the conservative view of recognising only one species, Tursiops truncatus (Montagu, 1821) (e.g., Tomilin, 1957; Ross and Cockcroft, 1990). Recent genetic and detailed osteological studies of sympatric morphotypes of bottlenose dolphins in Chinese waters showed complete congruence between molecular and osteological data with no intermediates (Chapter 2 and 3). These results provided strong evidence that reproductive isolation (i.e., no genetic exchange) between the forms exists and are not consistent with the monotypic hypothesis. Comparisons with South African and British specimens from other regions revealed that the osteology of the two forms in Chinese waters resembled closely the descriptions of T. truncatus and T. aduncus (see Ross, 1977, 1984; Chapter 2), but a formal taxonomic revision of this genus is still required to validate these names. In the meantime, for consistency across studies, we will refer to the two species by these provisional names.

Diagnostic differences in the mitochondrial DNA (mtDNA) control region and skeletal characters allow confident identification of specimens (Chapter 2 and 3). However, genetic and osteological data are impractical or impossible to obtain during most field studies, especially those that are non- or minimally-intrusive. Therefore, external characters that can allow differentiation of the two species are needed. Some studies have suggested that the extremities (i.e., snout or beak, flippers and dorsal fin) of T. aduncus are proportionately larger than in T. truncatus (Ross, 1977; Zhou, 1987). However, Gao et al. (1995) only found differences in adult body size between the two species in Chinese waters. Since these species are sympatric and frequently in mixed schools in this region (Zhou and Qian, 1985; J.Y. Wang, unpublished data), adult body size has very limited use for field identification. Therefore, the aim of this study was to determine if the two sympatric species in Chinese waters were also distinct in external morphology that is consistent with the molecular and osteological information and whether any characters would be useful for field identification.

Materials and Methods

Up to 31 external characters were measured for each of 111 specimens collected from Chinese waters. Due to the state of some carcasses and the working conditions in some field situations (e.g., dim light,

severe weather, unlevel terrain, etc.), the complete set of measurements could not be recorded for all specimen. Twenty-one of these measurements were taken following Norris (1961) while 10 measurements were either additional or slight modifications to those described by Norris (Fig. 1). Specimens were measured using a fiberglass or cloth tape to the precision of 0.5 cm (0.25 cm for measurements that were < ~30 cm) by J.Y.W. and several assistants who were instructed by J.Y.W. Morphometric data for 20 additional T. aduncus specimens from the Penghu Islands were provided by Ocean Park, Hong Kong. With the exception of captive display dolphins, measurements were obtained from carcasses that were collected opportunistically from stranding events or mortality due to interactions with fisheries and predominantly from late autumn to early spring between 1994 and 1998.

Since multivariate statistical methods are sensitive to missing data, most measurements and many specimens had to be omitted from the main analyses. Most girths (12 and 14-17) and measurement 5 had to be omitted because many stranded specimens were distended by bloating from decomposition or, in a few cases, pregnancy. Other girth measurements (13, 18 and 19) were excluded because they could not be replicated consistently (especially among measurers) due to the rapid tapering of the dolphins in these regions. Desiccation, difficulty in measuring and missing pieces precluded the measurements of the dorsal fin (2, 30 and 31) and flukes (23-25) from the analysis, whereas the flipper characters (26-29) of only a few specimens were affected due to the presence of supporting bones. However, measurement 26 was highly dependent on the position of the flipper and therefore was deemed unreliable across measurers and omitted. Other measurements that were excluded due to difficulties in measuring or the condition of the animals included: 3, 7, 9-11 and 21. Since measurements 4 and 20 are highly correlated, the former measurement was omitted from further analyses because a comparison of repeated measures of several specimens by the same and different measurers showed that the latter was less variable between and within measurers (J.Y. Wang, unpublished data).

Unfortunately, after all the omissions, only 8 reliable characters (1, 6, 8, 20, 22, 27, 28 and 29) and 57 specimens (28 measured by J.Y.W.; 24 by assistants; three by Ocean Park staff; and two by Hong Kong researchers) could be subjected to multivariate analyses. The characters were: 1, total body length; 6, snout-to-

FIGURE 1. External morphological characters that were measured for bottlenose dolphins from Chinese waters. Most characters were measured following Norris (1961). *, denotes other characters or those that were slight modifications to Norris (1961). Circled measurements indicate characters analysed with multivariate methods.

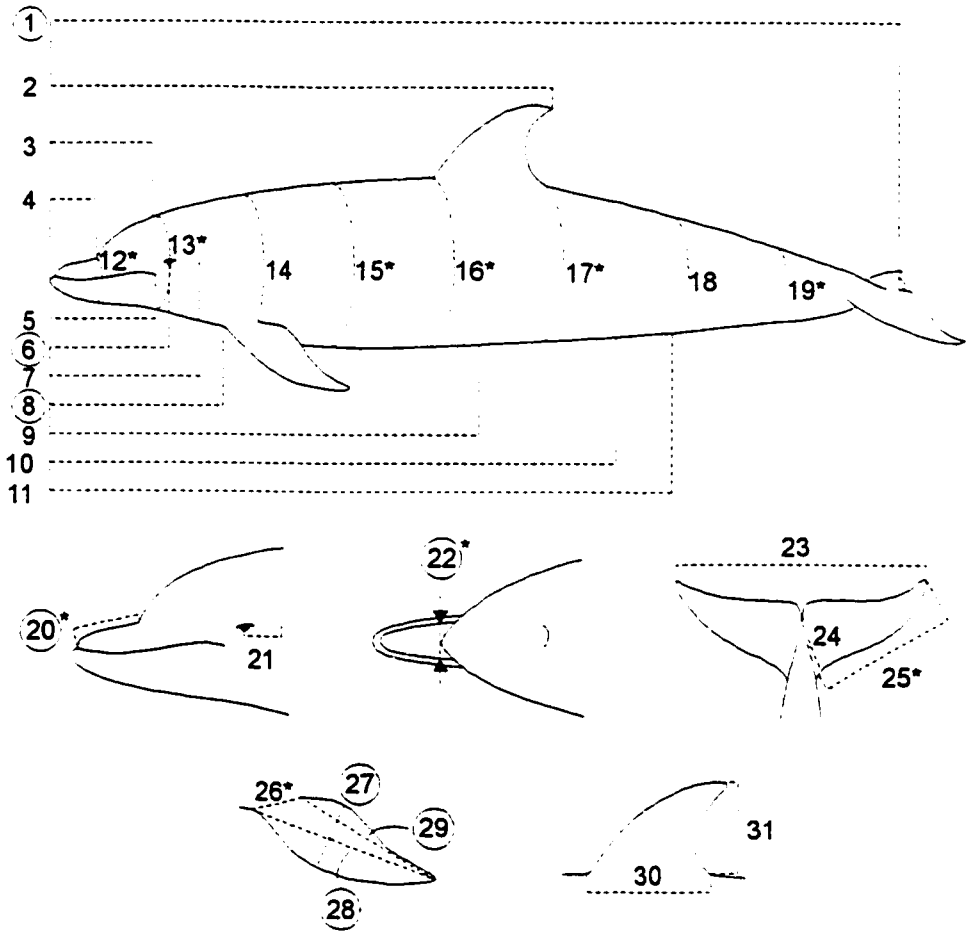
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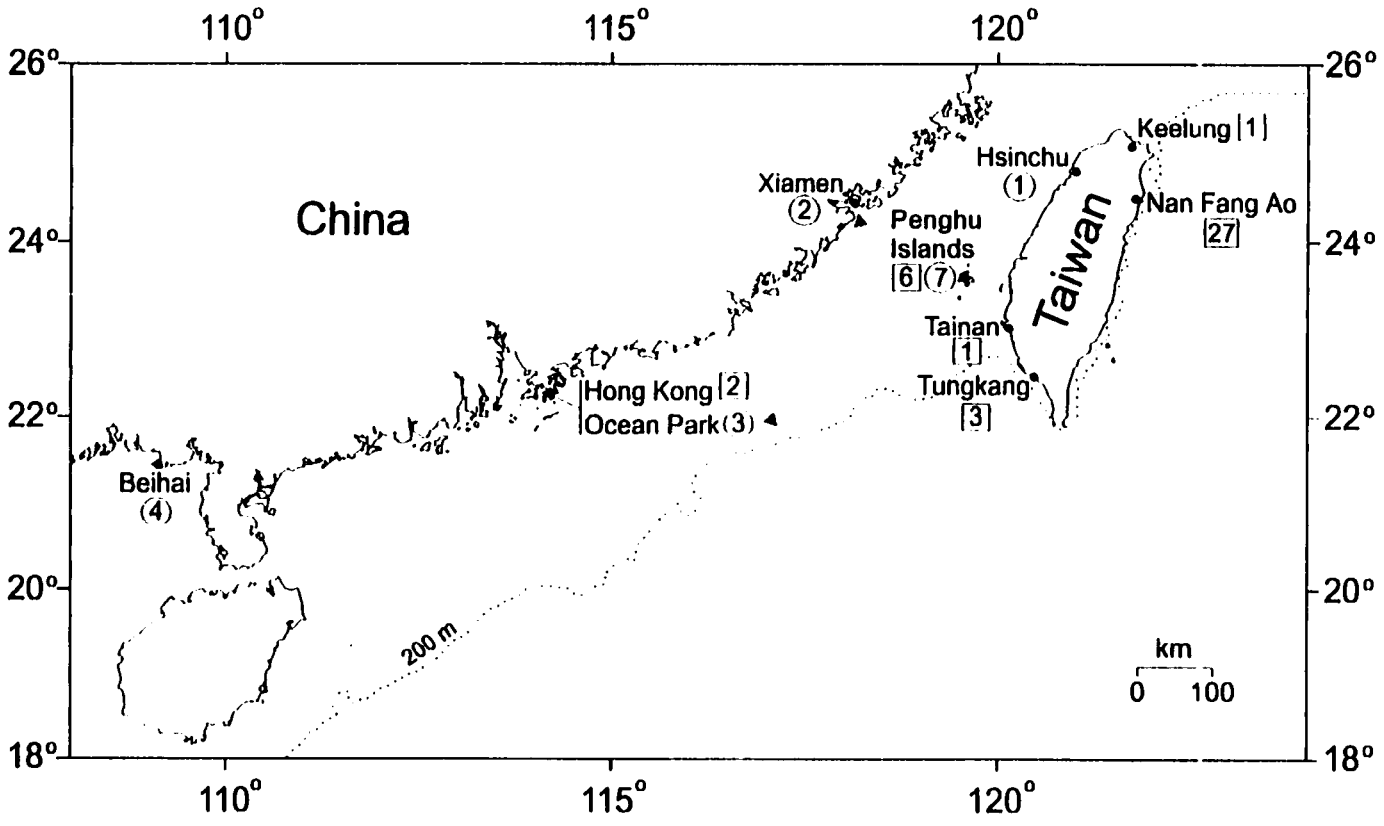
FIGURE 2. Collection locations of bottlenose dolphin specimens used in the discriminant analysis. Numbers in circles and squares represent sample sizes of Tursiops aduncus and T. truncatus, respectively. The arrows indicate that both Ocean Park and Xiamen specimens were captured from the Penghu Islands. The collection location for Tungkang specimens is unknown but most likely in Taiwan waters.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Taiwan
Xiamen
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Penghu
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Hsinchu
(1)
Keelung [1]
Nan Fang Ao
[27]
Tainan
[1]
Tungkang
[3]
Hong Kong [2]
Ocean Park (3)
Beihai
(4)
200 m
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TABLE 1. Classification of 57 specimens that were used in the main analysis based on rostrum length (RL), rostrum length proportions and the discriminant analysis. Classifications are shown in parentheses: A = Tursiops aduncus; T = T. truncatus. TBL = total body length; SEY = snout-to-eye length. See figure 2 for locations.

Specimens[a]	Location	RL (cm)	RL/TBL (%)	RL/SEY (%)	Discriminant Scores	Measurer
BH-95-02 (A)[b]	Gulf of Tonkin, China	8.8 (T)	6.3 (A)	38.9 (A)	2.11 (A)	J.Y.W.
BH-95-03 (A)	Gulf of Tonkin, China	12.8 (A)	6.3 (A)	41.1 (A)	3.75 (A)	J.Y.W.
BH-95-04 (A)	Gulf of Tonkin, China	12.8 (A)	6.0 (A)	38.6 (A)	4.18 (A)	J.Y.W.
BH-95-05 (A)	Gulf of Tonkin, China	13.8 (A)	6.0 (A)	42.3 (A)	4.38 (A)	J.Y.W.
HC-96-01 (A)	Hsinchu, Taiwan	14.0 (A)	6.1 (A)	41.8 (A)	3.56 (A)	J.Y.W.
HK-94-03 (T)	Hong Kong	7.5 (T)	2.6 (T)	19.9 (T)	-3.36 (T)	HK researchers
HK-94-04 (T)	Hong Kong	12.0 (T)	4.9 (T)	32.7 (T)	-1.52 (T)	HK researchers
KL-97-02 (T)	Keelung, Taiwan	11.5 (T)	4.6 (T)	34.0 (T)	-1.92 (T)	Assistants
PE-94-12 (T)	Penghu Isl., Taiwan	9.5 (T)	5.0 (T)	30.2 (T)	0.55 (T)	Assistants
PE-94-13 (A)	Penghu Isl., Taiwan	15.0 (A)	5.6 (A)	41.7 (A)	3.92 (A)	Assistants
PE-94-14 (A)	Penghu Isl., Taiwan	13.5 (A)	5.9 (A)	42.2 (A)	5.25 (A)	Assistants
PE-94-15 (A)	Penghu Isl., Taiwan	13.5 (A)	6.7 (A)	41.5 (A)	4.04 (A)	Assistants
PE-95-02 (A)	Penghu Isl., Taiwan	12.8 (A)	5.3 (A)	37.5 (A)	3.19 (A)	J.Y.W.
PE-95-03 (A)	Penghu Isl., Taiwan	13.0 (A)	5.6 (A)	37.1 (A)	3.95 (A)	J.Y.W.
PE-95-05 (T)[b]	Penghu Isl., Taiwan	8.0 (T)	3.7 (T)	27.1 (T)	-2.38 (T)	J.Y.W.
PE-97-01 (T)	Penghu Isl., Taiwan	11.0 (T)	4.7 (T)	33.8 (T)	0.41 (T)	J.Y.W.
PE-97-02 (T)	Penghu Isl., Taiwan	11.0 (T)	4.1 (T)	31.0 (T)	-1.73 (T)	J.Y.W.
PE-97-03 (T)	Penghu Isl., Taiwan	8.0 (T)	2.8 (T)	24.2 (T)	-3.11 (T)	J.Y.W.
PE-97-04 (T)	Penghu Isl., Taiwan	10.5 (T)	3.7 (T)	32.8 (T)	-1.43 (T)	J.Y.W.

PE-97-05 (A)	Penghu Isl., Taiwan	14.0 (A)	5.8 (A)	39.4 (A)	3.90 (A)	Assistants
PE-97-06 (A)	Penghu Isl., Taiwan	15.5 (A)	6.3 (A)	43.3 (A)	4.80 (A)	Assistants
SU-94-05 (T)	Nan Fang Ao, Taiwan	7.0 (T)	3.2 (T)	24.1 (T)	-2.49 (T)	J.Y.W.
SU-94-07 (T)	Nan Fang Ao, Taiwan	10.5 (T)	4.3 (T)	31.8 (T)	-1.66 (T)	J.Y.W.
SU-94-08 (T)	Nan Fang Ao, Taiwan	8.0 (T)	3.5 (T)	25.8 (T)	-2.75 (T)	J.Y.W.
SU-94-11 (T)	Nan Fang Ao, Taiwan	10.5 (T)	3.8 (T)	27.3 (T)	-2.44 (T)	J.Y.W.
SU-94-12 (T)	Nan Fang Ao, Taiwan	9.5 (T)	4.1 (T)	32.8 (T)	-0.50 (T)	J.Y.W.
SU-94-13 (T)	Nan Fang Ao, Taiwan	8.5 (T)	3.8 (T)	27.4 (T)	-2.11 (T)	J.Y.W.
SU-94-14 (T)	Nan Fang Ao, Taiwan	9.5 (T)	4.2 (T)	29.7 (T)	-1.19 (T)	J.Y.W.
SU-94-16 (T)	Nan Fang Ao, Taiwan	9.0 (T)	3.4 (T)	29.0 (T)	-1.94 (T)	J.Y.W.
SU-94-17 (T)	Nan Fang Ao, Taiwan	10.5 (T)	4.6 (T)	36.2 (T)	0.44 (T)	J.Y.W.
SU-94-49 (T)	Nan Fang Ao, Taiwan	11.5 (T)	4.3 (T)	29.5 (T)	-1.51 (T)	Assistants
SU-94-61 (T)	Nan Fang Ao, Taiwan	8.0 (T)	4.1 (T)	27.6 (T)	-1.28 (T)	Assistants
SU-94-96 (T)	Nan Fang Ao, Taiwan	9.0 (T)	3.7 (T)	25.4 (T)	-1.18 (T)	Assistants
SU-94-105 (T)	Nan Fang Ao, Taiwan	10.3 (T)	4.5 (T)	29.3 (T)	-1.60 (T)	Assistants
SU-94-113 (T)	Nan Fang Ao, Taiwan	9.5 (T)	4.1 (T)	29.7 (T)	-1.28 (T)	Assistants
SU-94-116 (T)	Nan Fang Ao, Taiwan	10.3 (T)	4.1 (T)	29.3 (T)	-1.19 (T)	Assistants
SU-95-07 (T)	Nan Fang Ao, Taiwan	9.5 (T)	3.4 (T)	27.1 (T)	-2.72 (T)	Assistants
SU-95-10 (T)	Nan Fang Ao, Taiwan	9.5 (T)	4.1 (T)	29.2 (T)	-0.62 (T)	Assistants
SU-95-14 (T)	Nan Fang Ao, Taiwan	12.0 (T)	4.7 (T)	31.6 (T)	0.55 (T)	Assistants
SU-95-21 (T)	Nan Fang Ao, Taiwan	10.5 (T)	3.8 (T)	28.0 (T)	-1.80 (T)	Assistants
SU-95-22 (T)	Nan Fang Ao, Taiwan	10.0 (T)	4.1 (T)	26.3 (T)	-1.60 (T)	Assistants
SU-95-39 (T)	Nan Fang Ao, Taiwan	10.5 (T)	4.1 (T)	29.2 (T)	-1.27 (T)	Assistants
SU-95-43 (T)	Nan Fang Ao, Taiwan	9.0 (T)	3.9 (T)	28.1 (T)	-1.02 (T)	Assistants
SU-95-65 (T)	Nan Fang Ao, Taiwan	9.0 (T)	3.0 (T)	25.4 (T)	-3.78 (T)	J.Y.W.
SU-95-66 (T)	Nan Fang Ao, Taiwan	7.8 (T)	3.7 (T)	25.8 (T)	-2.42 (T)	J.Y.W.

SU-95-98 (T)	Nan Fang Ao, Taiwan	10.5 (T)	4.2 (T)	32.8 (T)	-0.75 (T)	Assistants
SU-95-101 (T)	Nan Fang Ao, Taiwan	10.5 (T)	4.0 (T)	27.6 (T)	-2.54 (T)	Assistants
SU-95-121 (T)	Nan Fang Ao, Taiwan	9.3 (T)	3.9 (T)	30.3 (T)	-2.21 (T)	Assistants
TA9401 (A)[c]	Penghu Isl., Taiwan	14.0 (A)	6.1 (A)	38.9 (A)	4.16 (A)	Ocean Park Staff
TA7901 (A)[d]	Penghu Isl., Taiwan	14.0 (A)	5.7 (A)	38.9 (A)	2.03 (A)	Ocean Park Staff
TA8403 (A)[d]	Penghu Isl., Taiwan	14.5 (A)	5.8 (A)	40.8 (A)	5.43 (A)	Ocean Park Staff
TG-94-01 (T)	Unknown location, Taiwan [e]	9.0 (T)	3.9 (T)	27.3 (T)	-2.98 (T)	J.Y.W.
TG-94-02 (T)	Unknown location, Taiwan [e]	9.0 (T)	3.5 (T)	25.0 (T)	-2.07 (T)	J.Y.W.
TG-94-06 (T)	Unknown location, Taiwan [e]	8.0 (T)	3.4 (T)	24.2 (T)	-2.73 (T)	J.Y.W.
TN-97-03 (T)	Tainan, Taiwan	10.0 (T)	3.5 (T)	33.3 (T)	-1.14 (T)	Assistants
XM-97-03 (A)	~Penghu Isl., Taiwan	13.3 (A)	6.2 (A)	40.8 (A)	3.83 (A)	J.Y.W.
XM-95-07 (A)	~Penghu Isl., Taiwan	12.8 (A)	6.0 (A)	40.5 (A)	3.79 (A)	J.Y.W.

[a], *a priori* classifications are shown in parentheses.

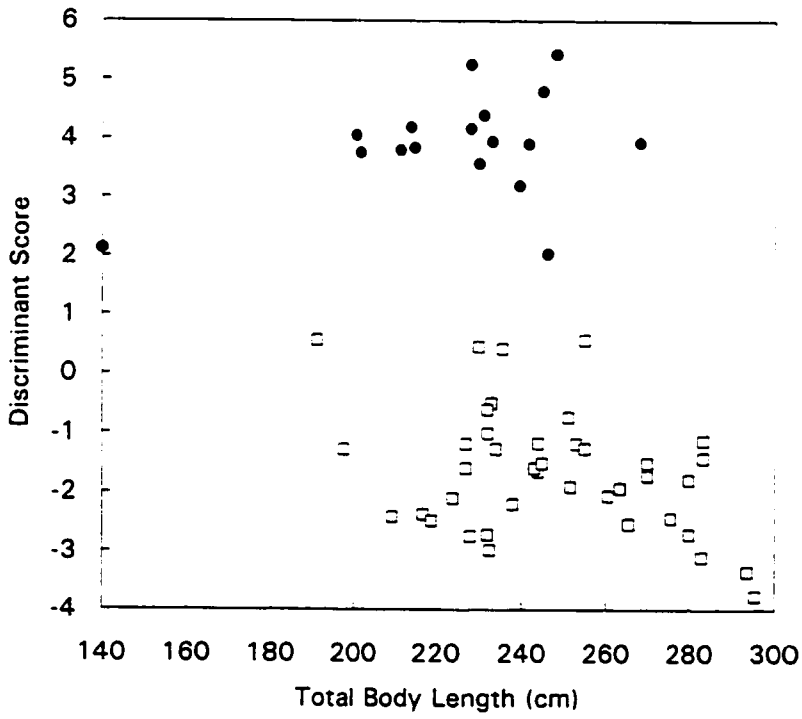
[b], nursing calves.

[c], nursing calf of TA7804 (see Table 3); living display specimen of Ocean Park (Hong Kong).

[d], living display specimen of Ocean Park (Hong Kong).

[e], collection location unknown but likely in Taiwan waters.

FIGURE 3. Scatterplot of discriminant scores against total body length of 57 bottlenose dolphin specimens from Chinese waters. Solid circles and open squares represent specimens of Tursiops aduncus and T. truncatus, respectively.


6
5
4
3
2
1
0
-1
-2
-3
-4
Discriminant Score
140
160
180
200
220
240
260
280
300
Total Body Length (cm)

multivariate analysis of variance (MANOVA) was performed for each species (T. truncatus: 19 males and 21 females; T. aduncus: 8 males and 9 females). No sex effects were detected within either species (for T. truncatus, Wilks' $\Lambda = 0.770$; $df_1 = 8$; $df_2 = 31$; $p = 0.355$; for T. aduncus, Wilks' $\Lambda = 0.740$; $df_1 = 8$; $df_2 = 8$, $p = 0.920$). Therefore, pooling male and female specimens within each species for the main analysis was acceptable.

To assess the classification functions of the discriminant analysis, these functions were applied to data from new specimens (i.e., not included in the original analysis) and their classifications compared to those based on information other than external morphometric characters (e.g., mtDNA, osteology, pigmentation, etc.). Data from five specimens collected recently from the waters of the Penghu Islands and five from Indonesian waters provided by Ocean Park, Hong Kong were obtained. However, only eight of these specimens (four from each location) had sufficient data to be classified using the functions. Based on field observations and a key of osteological characters (see Chapter 2), two of the Penghu Islands specimens were identified (by J.Y.W.) as T. aduncus and two as T. truncatus, while all the Indonesian specimens were classified as T. aduncus based on mtDNA sequences or ventral spotting pigmentation. Subjecting new specimens to the classification functions required their data to be standardized. The information required for standardization of new data and the coefficients for the discriminant and classification functions are shown in Table 2. The discriminant analysis classifications of these specimens were completely consistent with their original classifications. Discriminant scores for these specimens are shown in Table 3.

The eight characters in the main analyses were also examined for proportions that could be used for field identification. Rostrum length (measurement 20) as a proportion of total body length (measurement 1) and as a proportion of the snout-to-eye length (measurement 6) showed diagnostic (non-overlapping) differences between the species in Chinese waters (Table 4). Furthermore, if nursing calves (inferred from finding milk in their stomachs or the majority of their teeth being unerupted) were excluded, the distributions of rostrum length were also non-overlapping. To test the distributional limits of rostrum length and rostrum length proportions, the five new specimens from the Penghu Islands, the five Indonesian specimens and 33 Chinese specimens omitted from the main analyses due to incomplete data were used. No conflicting

TABLE 2. Specimen sums for each external morphological character of bottlenose dolphins and the equation coefficients of the discriminant and classification functions. The character numbers correspond to the characters in figure 1.

Characters	Specimen Sums	Standardized Discriminant Function Coefficients	Classification Function Coefficients for T. aduncus	Classification Function Coefficients for T. truncatus
1	13704.75	-0.301	-1.250	0.531
6	1903.10	-0.515	-1.990	0.846
8	3060.90	-0.058	-0.225	0.096
20	612.25	1.488	9.635	-4.095
22	486.90	-0.990	-3.941	1.675
27	798.85	1.347	5.264	-2.237
28	2173.20	-0.235	-0.911	0.387
29	1598.70	-0.574	-2.221	0.944
Constants			-8.806	-1.726

*To classify new specimens, standardize the new measurements for each character by using the specimen sums (based on n = 57 specimens) (i.e., new value - [specimen sum + new value]/n'; where n' = 57+1). Then scores for the functions of each species are calculated using the new (standardized) values in the equations: $A = -8.806 + \Sigma c_i x_i$ and $T = -1.726 + \Sigma k_i x_i$, where A and T = *T. aduncus* and *T. truncatus* function scores, respectively; c_i and k_i = coefficients of the ith character for *T. aduncus* and *T. truncatus* functions, respectively; and x_i = the standardized value of the ith character for the new specimen. The new specimen is classified as the species whose function produces the highest score (e.g., A > T, new specimen = *T. aduncus*). Discriminant scores for new specimens are calculated using the standardized discriminant function coefficients.

TABLE 3. Classification of specimens that were not included in the main analysis using rostrum length (RL), rostrum length proportions and the discriminant analysis. Classifications are shown in parentheses: A = Tursiops aduncus; T = T. truncatus; I = intermediate. TBL = total body length; SEY = snout-to-eye length. See figure 2 for locations.

Specimens[a]	Location	RL (cm)	RL/TBL (%)	RL/SEY (%)	Discriminant Scores
HK-95-02 (T)	Hong Kong	11.5 (T)	4.89 (T)	33.8 (T)	---
J1 (A)[b]	Indonesia	11.5 (T)	5.20 (I)	35.9 (T)	9.06 (A)
J2 (A)[b]	Indonesia	12.0 (T)	5.38 (A)	34.3 (T)	1.36 (A)
J3 (A)[b]	Indonesia	10.5 (T)	5.00 (I)	35.0 (T)	---
J8 (A)[b]	Indonesia	11.0 (T)	4.78 (T)	34.4 (T)	3.84 (A)
J13 (A)[b]	Indonesia	12.0 (T)	5.08 (I)	37.5 (A)	7.60 (A)
TA7804 (A)[b]	Penghu Isl., Taiwan	14.0 (A)	5.58 (A)	37.8 (A)	---
PE-96-01 (T)	Penghu Isl., Taiwan	10.5 (T)	3.98 (T)	---	---
PE-97-07 (T)	Penghu Isl., Taiwan	11.0 (T)	4.20 (T)	32.4 (T)	---
PE-97-17 (A)	Penghu Isl., Taiwan	12.5 (A?)	5.54 (A)	39.1 (A)	9.41 (A)
PE-98-01 (T)	Penghu Isl., Taiwan	9.5 (T)	3.75 (T)	29.7 (T)	-3.48 (T)
PE-98-02 (T?)	Penghu Isl., Taiwan	10.0 (T)	3.48 (T)	---	---
PE-98-05 (T)	Penghu Isl., Taiwan	10.5 (T)	4.15 (T)	28.8 (T)	-4.92 (T)
PE-98-09 (A)	Penghu Isl., Taiwan	14.0 (A)	6.57 (A)	41.2 (A)	12.82 (A)
SU-94-03 (T)	Nan Fang Ao, Taiwan	9.0 (T)	3.47 (T)	---	---
SU-94-04 (T)	Nan Fang Ao, Taiwan	8.5 (T)	---	---	---
SU-94-34 (T)	Nan Fang Ao, Taiwan	11.5 (T)	---	---	---
SU-94-35 (T)	Nan Fang Ao, Taiwan	11.5 (T)	---	---	---
SU-94-43 (T)	Nan Fang Ao, Taiwan	9.5 (T)	---	---	---
SU-94-110 (T)	Nan Fang Ao, Taiwan	11.5 (T)	---	---	---
SU-95-33 (T)	Nan Fang Ao, Taiwan	8.5 (T)	3.95 (T)	---	---
SU-95-49 (T)	Nan Fang Ao, Taiwan	11.5 (T)	4.60 (T)	---	---

SU-95-61 (T)	Nan Fang Ao, Taiwan	11.0 (T)	4.30 (T)	---	---
SU-95-62 (T)	Nan Fang Ao, Taiwan	9.5 (T)	3.56 (T)	---	---
SU-95-89 (T)	Nan Fang Ao, Taiwan	10.0 (T)	3.69 (T)	27.8 (T)	---
TG-94-03 (T)	Unknown location, Taiwan [c]	9.5 (T)	3.74 (T)	28.8 (T)	---
TP-96-03 (T)	Nan Fang Ao, Taiwan	8.5 (T)	---	25.4 (T)	---
YL-94-20 (T)	Nan Fang Ao, Taiwan[d]	9.5 (T)	3.83 (T)	---	---
YL-96-07 (T)	Nan Fang Ao, Taiwan[d]	10.0 (T)	---	---	---
YL-96-08 (T)	Nan Fang Ao, Taiwan[d]	10.8 (T)	---	---	---
YL-96-09 (T)	Nan Fang Ao, Taiwan[d]	10.5 (T)	---	---	---
YL-96-10 (T)	Nan Fang Ao, Taiwan[d]	10.5 (T)	---	---	---
YL-96-11 (T)	Nan Fang Ao, Taiwan[d]	11.5 (T)	---	---	---
YL-96-12 (T)	Nan Fang Ao, Taiwan[d]	10.5 (T)	---	---	---
YL-96-13 (T)	Nan Fang Ao, Taiwan[d]	10.0 (T)	---	---	---
YL-96-14 (T)	Nan Fang Ao, Taiwan[d]	10.5 (T)	---	---	---
YL-96-15 (T)	Nan Fang Ao, Taiwan[d]	10.5 (T)	---	---	---
YL-96-31 (T)	Nan Fang Ao, Taiwan[d]	11.5 (T)	---	---	---
YL-96-35 (T)	Nan Fang Ao, Taiwan[d]	9.8 (T)	---	---	---
YL-96-45 (T)	Nan Fang Ao, Taiwan[d]	11.0 (T)	---	---	---
YL-96-65 (T)	Nan Fang Ao, Taiwan[d]	9.0 (T)	---	---	---
YL-96-66 (T)	Nan Fang Ao, Taiwan[d]	11.8 (T)	---	---	---
YL-96-67 (T)	Nan Fang Ao, Taiwan[d]	11.0 (T)	---	---	---

[a], original classifications are shown in parentheses.

[b], living display specimen of Ocean Park (Hong Kong).

[c], collection location unknown but most likely in Taiwan waters.

[d], most likely collection location.

TABLE 4. Comparison of basic statistics for rostrum length (RL) and rostrum length proportions of each species of bottlenose dolphin from Chinese waters (Tursiops aduncus: n = 17; T. truncatus: n = 40). TBL = total body length; SEY = snout-to-eye length.

	Tursiops aduncus			Tursiops truncatus		
Character	Mean	SD	Min.-Max.	Mean	SD	Min.-Max.
RL (cm)	13.4	1.44	12.8-15.5 (8.8*)	9.6	1.25	7.0-12.0
RL/TBL (%)	6.0	0.34	5.3-6.7	3.9	0.53	2.6-5.0
RL/SEY (%)	40.3	1.78	37.1-43.3	28.8	3.36	19.9-36.2

* recorded for one very young specimen that was nursing and had few erupted teeth; no other T. aduncus specimens had RL measurements < 12.8 cm.

classifications were found among the new Penghu Islands specimens nor among the 33 incomplete Chinese specimens. The rostrum length of one new Penghu Islands specimen (PE-97-17: 12.5 cm) was slightly shorter than the lower limit for T. aduncus (12.8 cm), to which it was classified in the field, by the classification functions and by the rostrum length proportions (Table 3). In contrast, classification of Indonesian T. aduncus specimens using rostrum length alone grouped them with the T. truncatus of Chinese waters, and the proportions classified them inconsistently. The rostrum length to snout-to-eye length proportion classified all Indonesian specimens except one as T. truncatus, while the rostrum length to total body length proportion classified one as T. aduncus, one as T. truncatus and three as intermediates (Table 3).

Basic statistics for the specimens and other external characters examined in the main analysis for each species are shown in Table 5.

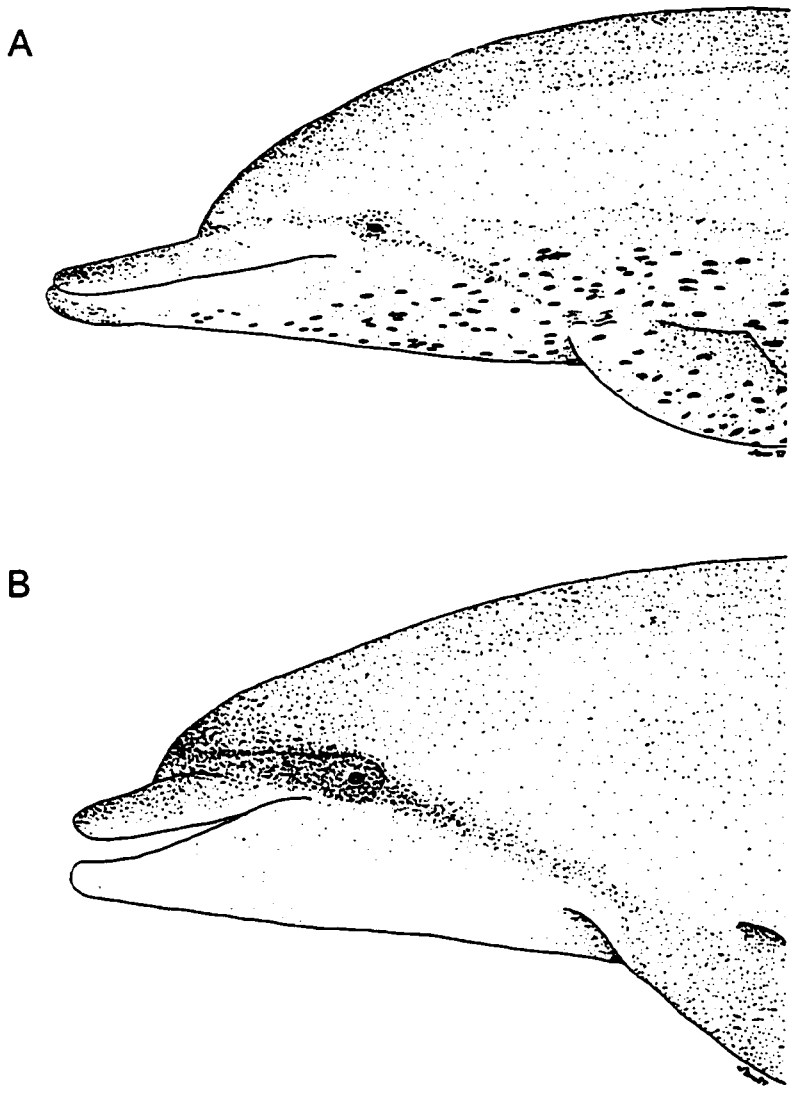
Discussion

The results demonstrate clearly that T. truncatus and T. aduncus in Chinese waters are not only distinct in molecular and osteological characters but also in external morphology with no intermediates. The classification functions of the discriminant analysis classified the new specimens from Chinese waters perfectly, which offers more rapid identification of fresh carcasses than either molecular or osteological characters. Furthermore, like osteology and mtDNA control region sequences, diagnostic differences in external morphology were also found between the two species. The distributions of rostrum length, for all but nursing calves, and rostrum length proportions were non-overlapping for specimens from Chinese waters and should allow even faster identification of fresh carcasses than the classification functions. These characters should also be useful for identifying living specimens that are stranded, in captivity or free-ranging provided they can be observed closely enough to obtain good photographs and observations of the head region (see figure 4 a and b for illustrations of each species). Rostrum length as a proportion of snout-to-eye length can be obtained from photographs of the head region that are taken perpendicular to the longitudinal axis of the body and used for identification. However, for many studies of free-ranging dolphins (e.g., line-transect surveys of abundance), identification requiring rostrum length may be difficult since this character is not always visible for all

TABLE 5. Basic statistics of the external morphological characters analysed (except rostrum length or character 20) for each species of bottlenose dolphin from Chinese waters (Tursiops aduncus: n = 17; T. truncatus: n = 40). The character numbers correspond to the circled characters in figure 1.

	Tursiops aduncus			Tursiops truncatus		
Character	Mean	SD	Min.-Max.	Mean	SD	Min.-Max.
1	224.7	28.08	140.0-268.0	247.1	25.67	191.0-295.5
6	33.2	3.26	22.5-36.0	33.5	3.04	29.0-39.0
8	52.7	4.87	36.0-58.0	54.1	4.57	45.0-62 8
22	8.2	0.98	6.0-10.0	8.7	0.92	7.0-11.4
27	14.4	2.00	9.3-18.0	13.9	1.36	10.8-17.0
28	37.6	4.13	25.3-45.0	38.3	3.52	30.0-44.0
29	28.3	3.49	18.8-34.5	27.9	2.77	20.5-33.0

FIGURE 4. Examples of the head region of Tursiops aduncus (A) and T. truncatus (B) illustrating the longer snout and the ventral spotting pigmentation of T. aduncus.


A
B

individuals. Furthermore, if the rostra of dolphins are not clearly different (i.e., at the adjacent tails of the distributions) and the dolphins are in mixed schools or at some distance, then identifications may be dubious even by experienced researchers. Further studies are needed to determine whether other more visible characters can differentiate the two species.

As with many cetaceans, differences in pigmentation and behaviour may also be useful for discriminating the two Tursiops species in the wild. Ventral spotting appears to be common for T. aduncus throughout its distribution (Yang, 1976; Ross, 1977; Zhou, 1987; Ross and Cockcroft, 1990; Miyashita, 1993) while ventral spotting has only been reported for a few old female T. truncatus from the North Atlantic (Leatherwood et al., 1976). In this study, no specimens belonging to T. truncatus had ventral spotting while for T. aduncus, nine of the 17 specimens were spotted, six were unspotted and data for the other two were unavailable. The unspotted T. aduncus specimens tended to be the smaller individuals, but the total body length between spotted and unspotted individuals overlapped (140 to 230 cm and 214 to 268 cm , respectively). A detailed study of the relationships between age, sexual maturity and the development and intensity of ventral spotting pigmentation is needed. Since the two species occupy different niches (Ross, 1977; J.Y. Wang, unpublished data), behavioural differences (e.g., swimming or surfacing patterns) may exist, and behavioural observations of free-ranging dolphins should be conducted as well. At present, any study claiming that these Tursiops species can be distinguished at distances at which most other dolphin species can be identified confidently must be examined critically.

The classification functions of the discriminant analysis were also completely successful in classifying T. aduncus specimens from Indonesian waters. However, rostrum length and its associated proportions were not successful. The rostrum of T. aduncus from Indonesia were shorter in absolute size than those of Chinese waters and about the same length as for the T. truncatus in this study. It is highly unlikely that they belong to T. truncatus because pigmentation, mtDNA (Chapter 3) and the discriminant analysis of the present study all classified them with T. aduncus. Analysis of a limited number of mtDNA control region sequences revealed that haplotypes of Indonesian T. aduncus were embedded within the T. aduncus lineage and that they shared with the Chinese T. aduncus haplotypes the same seven fixed nucleotide site differences from T. truncatus

(Chapter 3). Preliminary findings of an independent mtDNA analysis of T. aduncus specimens from South Africa, Timor Sea (situated between southern Indonesia and northern Australia) and the western Pacific Ocean also supported a monophyletic group (see Curry and Smith, 1997). The Indonesian dolphins may represent a different population of T. aduncus. They are smaller than those in Chinese waters, begin developing ventral spotting pigmentation (which may coincide with the onset of sexual maturity) at a much shorter length (J.Y. Wang, unpublished data) and the spotting is extensive by the time they reach a length of about 210 to 220 cm. Similar observations were also made for T. aduncus in the waters of western Australia (P. Berggren, Department of Zoology, University of Stockholm, Sweden, personal communication). In contrast, the ventral spotting of T. aduncus in Chinese waters only begin to appear at about 210 to 230 cm in length (J.Y. Wang, unpublished data) and individuals from the Penghu Islands are the largest reported (up to a total body length of 268 cm; see Table 5). Molecular and morphological analyses of samples from a wider distribution is needed to better understand the population divisions within both species in this region.

Conservation implications - In Chinese waters, bottlenose dolphins are heavily exploited. Mortality due to interactions with fisheries is the most obvious and immediate problem (J.Y. Wang, unpublished data). Even though the Wildlife Conservation Law of Taiwan was amended in 1990 to include all cetaceans, it affords the bottlenose dolphin with the lowest legal protection status of all cetacean species found in Taiwan waters. Since very little biological informatior exists for cetaceans in this region, the basis for the status assigned to bottlenose dolphins can not be scientific. Ironically, even cetacean species for which there are no confirmed records in Taiwan waters are given higher levels of protection than bottlenose dolphins. The recent finding of two Tursiops species co-existing in Chinese waters exacerbates the consequences of this serious legislative flaw and clearly indicates that further amendments are needed. To be effective, conservation legislation and programs must be based on basic biological and demographic information (e.g., taxonomy, population structure, abundance, mortality, recruitment, etc.).

Bottlenose dolphins are the most popular cetaceans for living displays at oceanaria, and T. aduncus is preferred overwhelmingly in this region. Interest in dolphinaria is increasing in Taiwan, China and other nations in southeast Asia. These institutions will almost certainly be stocked with wild dolphins. Concerns

about the activities of this industry have been raised regarding the mortality of dolphins during capture, holding and transportation and the low survivorship of dolphins in oceanaria of Southeast Asia (Reeves et al., 1994; UNEP, 1996). If unchecked, removals from wild populations to stock and maintain captive populations can pose a substantial threat to the already heavily-exploited bottlenose dolphins of this region. Regulations, minimum facility standards and expertise in husbandry for this industry must be established to reduce the need to replace captive dolphins and the burden on wild populations. At the minimum, nations with oceanaria or planning to build oceanaria should satisfy the recommendations made by scientists during a workshop on the conservation of small cetaceans in Southeast Asia (UNEP, 1996).

Our understanding of Tursiops in Chinese waters is just beginning, and more studies are needed to guide the conservation of this popular species. Recent genetic and osteological studies and the present study provide overwhelming evidence that the sympatric forms of bottlenose dolphins in Chinese waters represent two species. Therefore, as a first step in the conservation of these species, it is essential that both species of Tursiops be recognised by the Wildlife Conservation Law of Taiwan. Furthermore, it is prudent to give these exploited species the same high level of legislative protection as other cetaceans while we better our understanding of the boundaries and demographic health of the populations in this region. Similar amendments to the conservation legislations of other nations and the protected species lists of international organizations (e.g., Convention on the International Trade of Endangered Species of Fauna and Flora, International Union for Conservation of Nature and Natural Resources, etc.) are also urged. Finally, legislation, although necessary, is not sufficient for effective conservation. Enforcement, research, education, stewardship and the willingness to conserve are also integral for ensuring that natural populations of these species are maintained in a healthy and sustainable state.

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Literature Cited

Curry, B. E., and Smith, J. 1997. Phylogeographic structure of the bottlenose dolphin (Tursiops truncatus): stock identification and implications for management. Pp. 227-247, in Molecular genetics of marine mammals: the Society for Marine Mammalogy, Special Publication 3 (A.E. Dizon, S.J. Chivers and W.F. Perrin, eds.). Allen Press, Lawrence, Kansas, 388 pp.

Gao, A., K. Zhou, and Y. Wang. 1995. Geographical variation in morphology of bottlenose dolphins (Tursiops sp.) in Chinese waters. Aquatic Mammals, 21(2): 121-135.

Hersh, S. L., Odell, D. K., and Asper, E. D. 1990. Sexual dimorphism in bottlenose dolphins from the east coast of Florida. Marine Mammal Science, 6: 305-315.

Leatherwood, S., Caldwell, D. K., and Winn, H. E. 1976. Whales, dolphins and porpoises of the western North Atlantic. A guide to their identification. NOAA Technical Report, National Marine Fisheries Service, Circulation 396, Seattle, Washington, 176 pp.

Miyashita, T. 1993. Abundance of dolphin stocks in the western North Pacific taken by the Japanese drive fishery. Report of the International Whaling Commission, 43: 417-437.

Norris, K. S. (ed.). 1961. Standardized methods for measuring and recording data on smaller cetaceans. Journal of Mammalogy, 42: 471-476.

REEVES, R. R., D. P. DEMASTER, C. L. HILL, and S. LEATHERWOOD. 1994. Survivorship of odontocete cetaceans at Ocean Park, Hong Kong, 1974-1994. Asian Marine Biology, 11: 107-124.

ROSS, G. J. B. 1977. The taxonomy of bottlenosed dolphins Tursiops in South African waters, with notes on their biology. Annals of the Cape Provincial Museums (Natural History), 11(9): 135-194.

ROSS, G. J. B. 1984. The smaller cetaceans of the south east coast of southern Africa. Annals of the Cape Provincial Museums (Natural History), 15(2): 173-410.

ROSS, G. J. B., and V. G. COCKCROFT. 1990. Comments on Australian bottlenose dolphins and the taxonomic status of Tursiops aduncus (Ehrenberg, 1832). Pp. 101-128, in The bottlenose dolphin (S. Leatherwood and R.R. Reeves, eds). Academic Press, San Diego, California, 653 pp.

TOLLEY, K. A., A. J. READ, R. S. WELLS, K. W. URIAN, M. D. SCOTT, A. B. IRVINE, and A. A. HOHN. 1995. Sexual dimorphism in wild bottlenose dolphins (Tursiops truncatus) from Sarasota, Florida. Journal of Mammalogy, 76: 1190-1198.

TOMILIN, A. G. 1957. Mammals of the U.S.S.R. and adjacent countries. Volume IX. Cetacea. Translated from Russian by the Israel Program for Scientific Translations, Jerusalem, 1967, 717 pp.

UNEP 1996. Report of the workshop on the biology and conservation of small cetaceans and dugongs of southeast Asia. UNEP(W)/EAS WG.1/2. United Nations Environment Programme, Bangkok, 101 pp.

YANG, H. C. 1976. Studies on the whales, porpoises and dolphins of Taiwan. Annual Report of Science, Taiwan Museum (Taipei), 19: 131-178 (in Chinese with English abstract).

ZHOU, K. 1987. Notes on two species of dolphins of the genus Tursiops in Chinese waters. Acta Theriologica Sinica, 7: 246-254 (in Chinese with English abstract).

ZHOU, K., and W. QIAN. 1985. Distribution of the dolphins of the genus tursiops in the China Seas. Aquatic Mammals, 1: 16-19.

Chapter 5

General Discussion

This thesis has resolved one of the most enduring controversies in cetacean taxonomy. This was achieved objectively, with a clear hypothesis and under the theoretical framework of the most widely accepted species definition, the Biological Species Concept. The congruent results of the present osteological, genetic and external morphological studies demonstrated very convincingly (Ryder, 1986; Hillis, 1987; Knowlton, 1993) that the sympatric forms of *Tursiops* in Chinese waters are reproductively isolated from each other and thus represent separate biological species (for a list of the specimens examined in the thesis and their classifications, see Appendix). In addition, the morphological and molecular evidence is consistent with the criteria of the other species concepts (i.e., ESC, PSC, CPC and CSC). Given these arguments, there is little doubt that *Tursiops* is not monotypic. Therefore, immediate amendments to both regional and international wildlife conservation laws are urged. Presently, the evidence indicates that both *T. truncatus* and *T. aduncus* should be given species status; whether other species of *Tursiops* exist remains to be determined.

The results of the studies of this thesis were very clear. However, most taxonomic treatments are not so straightforward and may require other objective methods for assessing the available information and classification. Dizon et al. (1992) proposed a hierarchical system for determining the likelihood of populations being Evolutionary Significant Units (ESUs). Even though their focus was at the intraspecific level, this system may be applied to the species level with different criteria. Based on a variety of information (genetic, distribution, ecology, morphology), the likelihood of populations being ESUs are determined by considering evidence for and against ESU status. The highest likelihood of being an ESU is the Category I population, which was described as genetically divergent and geographically separated from other populations. This strategy provided some advances in the ranking of the worthiness of populations for conservation resources by

being objective and independent of political issues. However, two major points need to be re-examined. First, Dizon et al. (1992) appraised "negative" results (or the lack of evidence for separation) as evidence for "lumping" populations. Non-significant results should not be interpreted as evidence for "lumping" but as there being no evidence for "splitting" (based on the information, scope and limitations of the particular study being sourced). This distinction is important in science and the proper interpretation should be emphasized to policy-makers.

The second problem with this approach is that a population defined under Category I (genetically divergent and with strong geographic separation) is given a higher probability of being an ESU than one that is classified under Category II (genetically divergent but with weak geographic partitioning). Under this scheme, the sympatric species of *Delphinus* (and *Tursiops*) would be classified under Category II while genetically distinct and allopatric populations within a species would be classified under Category I. As a result, some populations would have greater importance than some species. Therefore, the implications of the categories under this system need to be re-evaluated.

Future research

Since little is known of the biology of the *Tursiops* species in Chinese waters, more research is essential for identifying conservation needs. In particular, understanding population divisions, abundance, reproductive biology and the level of exploitation of each species should be research priorities. Detailed examination of mtDNA control region sequences and microsatellite loci of many more samples from throughout the range of each species would help greatly for elucidating population boundaries. This is a large and ambitious undertaking that would benefit greatly from collaborators from throughout the distribution of bottlenose dolphins. Once populations have been defined, abundance estimates can be determined using ship-board survey or photo-identification (mark-recapture) techniques, depending on a rough approximation of the population size and range. Non-natural mortality information can be collected and analysed concurrently with other studies.

Understanding migratory movements and distributions is also very important for conservation decisions affecting these animals. The most direct method for determining migratory behaviour and home range is to use radio- or satellite-telemetry. However, such studies can be prohibitively costly and concern for the welfare of the animals may limit data collection to only a few individuals. Observing identifiable individuals in different regions and seasons, documenting changes in seasonal abundances, and analysis of stable isotope ratios in dolphin tissues may all contribute to the understanding of the movement patterns for each species.

Concluding remarks

Regardless of the units of conservation (i.e., species, populations, ESUs, etc.) or the approach we take in conservation (i.e., taxa-by-taxa or ecosystem), the process of determining the boundaries of taxa needs to be objective. Although most agree that the population (or some other intraspecific taxon) is the unit of evolution and therefore the unit to conserve, the reality is that conservation is still focused at the species level. Species are recognized in legislation, formally named (although some trinomials also exist), given higher importance than populations and used in determining biodiversity "hotspots" for conservation at the ecosystem level. However, it is clear that each concept and approach to defining species has limitations. Therefore, any position that exalts religiously the virtues of one approach or concept to the exclusion of all others can only be harmful to conservation and should be received cautiously. For the sake of wildlife conservation, it may be prudent for legislation to recognize any group of organisms as a "conservation species" if it satisfies the criteria of any one of the most widely-accepted concepts rather than adhering to a single definition. In addition, this recommendation may help in preventing esoteric debates (i.e., phenetics versus cladistics; the best species concept, etc.) and maintaining the focus on conservation. However, this recommendation should not be viewed as automatic recognition of all potential groups of organisms as species. Rigorous testing of clearly stated hypotheses of status using sound scientific methods is still required.

Even with well-designed studies, taxonomists are still faced with a dilemma in conservation. The role of the taxonomist in conservation is to delimit taxon boundaries, identify distinguishing features of the taxa, and

occasionally, participate in specimen identification when expert knowledge is required. As scientists, taxonomists are conditioned to be conservative in the interpretation of biological data. In other words, taxonomists are conditioned to avoid making the false conclusion of separate species (i.e., a Type I error - rejecting a true null hypothesis) which is considered more serious than not detecting separate species when they actually exist (i.e., Type II error - failing to reject a false null hypothesis). However, in conservation, the consequences of making a Type II error in classification can be devastating. By maintaining a low probability of making Type I errors at the expense of making Type II errors, the future of some organisms may be endangered. Presently, species status for most organisms are determined with little to no participation from conservation biologists (Rojas, 1992). As a result, the precautionary principle of conservation does not have adequate representation in classification decisions. If classification continues to play a key role in conservation decisions, adequate consideration must be given for the consequences of making Type II errors in taxonomic studies. Whether an acceptable compromise between the precautionary principles of science and conservation can be obtained remains to be seen.

Literature Cited

Avise, J.C., and R.M. Ball, Jr. 1990. Principles of genealogical concordance in species concepts an biological taxonomy. *Oxford Surveys in Evol. Biol.* **7:** 45-67.

Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornith.* **1:** 159-187.

Davis, J.I., and K.C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. *Syst. Biol.* **41:** 421-435.

Dizon, A.E., C. Lockyer, W.F. Perrin, D.P. DeMaster, and J. Sisson. 1992. Rethinking the stock concept: a phylogeographic approach. *Conserv. Biol.* **6:** 24-36.

Donoghue, M., and A. Wheeler. 1990. *Save the dolphins*. Toronto: Key Porter Books.

Gao, A., K. Zhou, and Y. Wang. 1995. Geographical variation in morphology of bottlenose dolphins (*Tursiops sp.*) in Chinese waters. *Aquat. Mammals* **21:** 121-135.

Harrison, R.J., and S.H. Ridgway. 1971. Gonadal activity in some bottlenose dolphins (*Tursiops truncatus*). *J. Zool. Lond.* **165:** 355-366.

Hersh, S.L., and D.A. Duffield. 1990. Distinction between Northwest Atlantic offshore bottlenose dolphins based on hemoglobin profile and morphometry. In *The bottlenose dolphin*: 129-139. Leatherwood, S., and R.R. Reeves (Eds). San Diego. Academic Press.

Hershkovitz, P. 1966. Catalog of living whales. *U.S. National Museum Bulletin* **246:** 1-259.

Hillis, D.M. 1987. Molecular and morphological approaches to systematics. *Annu. Rev. Ecol. Syst.* **18:** 23-42.

Hohn, A.A. 1980. Age determination and age related factors in the teeth of western North Atlantic bottlenose dolphins. *Sci. Rep. Whales Res. Inst. Tokyo* **32:** 39-66.

Kasuya, T. 1985. Fishery-dolphin conflict in the Iki Island area of Japan. In *Marine mammals and fisheries*: 253-272. Beddington, J.R., R.J.H. Beverton and D.M. Lavigne (Eds.). London: George Allen and Unwin.

King, M. 1993. *Species evolution: the role of chromosome change*. Cambridge: Cambridge University Press.

Knowlton, N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* **24:** 189-216.

Leatherwood, S., and R.R. Reeves. 1983. *The Sierra Club handbook of whales and dolphins*. San Francisco: Sierra Club Books.

Mayr, E. 1942. *Systematics and the origin of species*. New York: Columbia University Press.

Mead, J.G. 1975. Preliminary report on the former net fisheries for *Tursiops truncatus* in the western North Atlantic. *J. Fish. Res. Borad Can.* **32:** 1155-1162.

Mead, J.G., and C.W. Potter. 1990. Natural history of bottlenose dolphins along the central Atlantic coast of the United States. In *The bottlenose dolphin*: 165-195. Leatherwood, S., and R.R. Reeves (Eds). San Diego: Academic Press.

Mitchell, E. (Ed.). 1975. Report of the meeting on smaller cetaceans, Montreal April 1-11, 1974. *J. Fish. Res. Board Can.* **32:** 889-983.

Miyashita, T. 1993. Abundance of dolphin stocks in the western North Pacific taken by the Japanese drive fishery. *Rep. Int. Whal. Comm.* **43:** 417-437.

Morton, B. 1978. Osteomyelitis (Pyogenic Spondylitis) of the spine in a dolphin. *J. Am. Veterin. Medical Assoc.* **173:** 1119-1120.

O'Brien, S.J. 1994. Genetic and phylogenetic analyses of endangered species. *Annu. Rev. Genet.* **28:** 467-489

O'Brien, S.J., and E. Mayr. 1991a. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* **251:** 1187-1188.

O'Brien, S.J., and E. Mayr. 1991b. Species hybridization and protection of endangered animals. *Science* **253:** 251-252.

Odell, D.K. 1975. Status of aspects of the life history of the bottlenose dolphin, *Tursiops truncatus*, in Florida. *J. Fish. Res. Board Can.* **32:** 1055-1058.

Perrin, W.F., and S.B. Reilly. 1984. Reproductive parameters of dolphins and small whales of the Family Delphinidae. *Rep. Int. Whal. Comm.* **Special Issue 6:** 97-133.

Perrin, W.F., G.P. Donovan, and J. Barlow (Eds.). 1994. *Gillnets and cetaceans. Report of the International Whaling Commission (Special Issue 15).* Cambridge: The International Whaling Commission.

Rojas, M. 1992. The species problem and conservation: what are we protecting? *Conserv. Biol.* **6:** 170-178.

Ross, G.J.B. 1977. The taxonomy of bottlenosed dolphins *Tursiops* in South African waters, with notes on their biology. *Ann. Cape Prov. Mus. (nat. Hist.)* **11:** 135-194.

Ross, G.J.B. 1984. The smaller cetaceans of the south east coast of southern Africa. *Ann. Cape Prov. Mus. (nat. Hist.)* **15:** 173-410.

Ross, G.J.B., and V.G. Cockcroft. 1990. Comments on Australian bottlenose dolphins and the taxonomic status of *Tursiops aduncus* (Ehrenberg, 1832). In *The bottlenose dolphin*: 101-128. Leatherwood, S., and R.R. Reeves (Eds). San Diego: Academic Press.

Ryder, O.A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends in Ecol. and Evol.* 1: 9-10.

Schroeder, J.P. 1990. Breeding bottlenose dolphins in captivity. In *The bottlenose dolphin*: 435-446. Leatherwood, S., and R.R. Reeves (Eds). San Diego: Academic Press.

Scott, M.D., R.S. Wells, and A.B. Irvine. 1990. A long-term study of bottlenose dolphins on the west coast of Florida. In *The bottlenose dolphin*: 235-244. Leatherwood, S., and R.R. Reeves (Eds). San Diego: Academic Press.

Simpson, G.G. 1961. *Principles of animal taxonomy: the species and lower categories.* New York: Columbia University Press.

Sites, Jr., J.W., and K.A. Crandall. 1997. Testing species boundaries in biodiversity studies. *Conserv. Biol.* **11**: 1289-1297.

Sylvestre, J., and S. Tasaka. 1985. On the intergeneric hybrids in cetaceans. *Aquat. Mammals* **11.3:** 101-108.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. In *Speciation and its consequences*: 3-27. Otte, D., and J.A. Endler (Eds.). Sunderland: Sinauer Associates.

Templeton, A.R. 1994. The role of molecular genetics in speciation studies. In *Molecular ecology and evolution: approaches and applications*. Basel, Switzerland: Birkhauser Verlag.

Tomilin, A.G. 1957. *Mammals of the U.S.S.R. and adjacent countries.* Jerusalem: translated from Russian by the Israel Program for Scientific Translations.

UNEP. 1996. Report of the workshop on the biology and conservation of small cetaceans and dugongs of Southeast Asia. UNEP(W)/EAS WG.1/2. United Nations Environmental Programme, Bangkok.

Vogler, A.P., and R. DeSalle. 1993. Diagnosing units of conservation management. *Conserv. Biol.* **8:** 354-363.

Walker, W.A. 1981. *Geographical variation in morphology and biology of bottlenose dolphins (Tursiops) in the eastern North Pacific.* U.S. National Marine Fisheries Service Administrative Rep. LJ-81-03C.

Wiley, E.O. 1978. The evolutionary species concpet reconsidered. *Syst. Zool.* **27:** 17-26.

Yang, H.C. 1976. Studies on the whales, porpoises and dolphins of Taiwan. *Annu. Rep Science, Taiwan Museum (Taipei)* **19:** 131-178.

Zhou, K. 1987. Notes on two species of dolphins of the genus *Tursiops* in Chinese waters. *Acta Theriologica Sinica* **7:** 246-254. (in Chinese with English abstract).

Zhou, K., and W. Qian. 1985. Distribution of the dolphins of the genus *tursiops* in the China Seas. *Aquat. Mammals* **1:** 16-19.

Zhou, K., and X. Wang. 1994. Brief review of passive fishing gear and incidental catches of small cetaceans in Chinese waters. *Rep. Int. Whal. Comm.* **Special Issue 15:** 347-354.

Zhou, K., S. Leatherwood, and T.A. Jeffeson. 1995. Records of small cetaceans in Chinese waters: a review. *Asian Mar. Biol.* **12:** 119-139.

APPENDIX

Specimens examined and their classifications based on different types of information.

Original	NMNS[a]			Classification				
Code	Code	Sex	Location	Field	Cranium[b]	Vertebrae[b]	mtDNA[c]	Morph.[d]
BH-95-02		M	Gulf of Tonkin, China	A		A		A
BH-95-03		M	Gulf of Tonkin, China	A	A	A	A	A
BH-95-04		M	Gulf of Tonkin, China	A	A	A	A	A
BH-95-05		F	Gulf of Tonkin, China	A	A	A	A	A
BRZ-92-03[e]		F	Rio Grande, Brazil	T			T	
BRZ-92-05[e]		M	Rio Grande, Brazil	T			T	
DO-94-01		?	~Penghu Isl., Taiwan	A			A	
HC-96-01	1297	M	Hsinchu, Taiwan	A	A	A	A	A
HK-94-03[f]		M	Hong Kong	T			T	T
HK-94-04[f]		F	Hong Kong	T			T	T
HK-95-02[f]		F	Hong Kong	T			T	T
(J1)[g]			Indonesia	A				A
(J2)[g]			Indonesia	A				A
(J3)[g]			Indonesia	A				A
INDO-01 (J8)[g]		M	Indonesia	A			A	A
INDO-02 (J13)[g]		M	Indonesia	A			A	A
KL-97-02		M	Keelung, Taiwan	T				T
ML-97-01	1393	M	Miaoli, Taiwan	A	A	A		
NAF-93-04[h]		?	Mauritania	T			T	
	3	?	Penghu Isl., Taiwan		A	A		
	4	?	Penghu Isl., Taiwan		T	T		
NMNS-334[i]	1316	?	Penghu Isl., Taiwan		T	T		

	863	?	Penghu Isl., Taiwan		T			
OP-95-05[g]		?	Captive born	A		A		
PE-01 (TA7804)[g]		F	Penghu Isl., Taiwan	A			A	A
PE-03 (TA7805)[g]		F	Penghu Isl., Taiwan	A			A	
PE-04 (TA7901)[g]		M	Penghu Isl., Taiwan	A			A	A
PE-05 (TA8403)[g]		F	Penghu Isl., Taiwan	A			A	A
PE-94-01	1135	?	Penghu Isl., Taiwan		A			
PE-94-02	629	?	Penghu Isl., Taiwan	T	T			
PE-94-11	591	?	Penghu Isl., Taiwan		A	A		
PE-94-12	738	F	Penghu Isl., Taiwan	T	T	T	T	T
PE-94-13	736	F	Penghu Isl., Taiwan	A	A	A	A	A
PE-94-14	740	M	Penghu Isl., Taiwan	A	A	A	A	A
PE-94-15	731	F	Penghu Isl., Taiwan	A	A	A	A	A
PE-95-01	730	?	Penghu Isl., Taiwan	A	T	T		
PE-95-02	739	F	Penghu Isl., Taiwan	A	A	A	A	A
PE-95-03	815	F	Penghu Isl., Taiwan	A	A	A	A	A
PE-95-04	737	?	Penghu Isl., Taiwan	T	T	T	T	
PE-95-05	932	M	Penghu Isl., Taiwan	T	T	T	T	T
PE-95-08	1051	?	Penghu Isl., Taiwan		A	A		
PE-96-01	1298	F	Penghu Isl., Taiwan	T	T	T	T	T
PE-96-02	1287	M	Penghu Isl., Taiwan		T	T		
PE-96-03	1289	?	Penghu Isl., Taiwan	T	T	T		
PE-97-01	1374	F	Penghu Isl., Taiwan	T	T	T		T
PE-97-02	1375	F	Penghu Isl., Taiwan	T	T	T		T
PE-97-03	1376	M	Penghu Isl., Taiwan	T	T	T		T
PE-97-04	1379	M	Penghu Isl., Taiwan	T	T	T		T

PE-97-05	1395	F	Penghu Isl., Taiwan	A	A	A		A
PE-97-06	1389	F	Penghu Isl., Taiwan	A	A	A		A
PE-97-07	1392	F	Penghu Isl., Taiwan	T	T	T		T
PE-97-08	1394	?	Penghu Isl., Taiwan	T	T			
PE-97-09	1391	F	Penghu Isl., Taiwan	T	T	T		
PE-97-10	2037	M	Penghu Isl., Taiwan	T	T	T		
PE-97-13	2052	?	Penghu Isl., Taiwan	T	T			
PE-97-17	2380	F	Penghu Isl., Taiwan	A	A			A
PE-98-01	2369	M	Penghu Isl., Taiwan	T	T			T
PE-98-02		M	Penghu Isl., Taiwan	T				T
PE-98-03	2376	M	Penghu Isl., Taiwan	T	T			
PE-98-05	2370	M	Penghu Isl., Taiwan	T	T			T
PE-98-07	2379	?	Penghu Isl., Taiwan		T			
PE-98-08	2378	?	Penghu Isl., Taiwan		T			
PE-98-09	2377	M	Penghu Isl., Taiwan	A	A			A
SU-94-03		F	Nan Fang Ao, Taiwan	T			T	T
SU-94-04		F	Nan Fang Ao, Taiwan	T				T
SU-94-05		M	Nan Fang Ao, Taiwan	T				T
SU-94-07	532	F	Nan Fang Ao, Taiwan	T	T		T	T
SU-94-08		M	Nan Fang Ao, Taiwan	T				T
SU-94-11		F	Nan Fang Ao, Taiwan	T				T
SU-94-12		M	Nan Fang Ao, Taiwan	T				T
SU-94-13		M	Nan Fang Ao, Taiwan	T			T	T
SU-94-14		F	Nan Fang Ao, Taiwan	T			T	T
SU-94-16		F	Nan Fang Ao, Taiwan	T				T
SU-94-17		F	Nan Fang Ao, Taiwan	T			T	T

SU-94-34		?	Nan Fang Ao, Taiwan	T		T
SU-94-35		?	Nan Fang Ao, Taiwan	T		T
SU-94-43		M	Nan Fang Ao, Taiwan	T		T
SU-94-49		M	Nan Fang Ao, Taiwan	T		T
SU-94-61		M	Nan Fang Ao, Taiwan	T		T
SU-94-85	735	F	Nan Fang Ao, Taiwan	T	T	
SU-94-96		F	Nan Fang Ao, Taiwan	T		T
SU-94-105		F	Nan Fang Ao, Taiwan	T		T
SU-94-110		M	Nan Fang Ao, Taiwan	T		T
SU-94-113		F	Nan Fang Ao, Taiwan	T		T
SU-94-116		F	Nan Fang Ao, Taiwan	T		T
SU-95-07		F	Nan Fang Ao, Taiwan	T		T
SU-95-10		F	Nan Fang Ao, Taiwan	T		T
SU-95-14		F	Nan Fang Ao, Taiwan	T		T
SU-95-21		M	Nan Fang Ao, Taiwan	T		T
SU-95-22		F	Nan Fang Ao, Taiwan	T		T
SU-95-33		F	Nan Fang Ao, Taiwan	T		T
SU-95-39		M	Nan Fang Ao, Taiwan	T		T
SU-95-43		F	Nan Fang Ao, Taiwan	T		T
SU-95-49		M	Nan Fang Ao, Taiwan	T		T
SU-95-61		M	Nan Fang Ao, Taiwan	T		T
SU-95-62		M	Nan Fang Ao, Taiwan	T		T
SU-95-65		F	Nan Fang Ao, Taiwan	T		T
SU-95-66		F	Nan Fang Ao, Taiwan	T		T
SU-95-89		F	Nan Fang Ao, Taiwan	T		T
SU-95-98		M	Nan Fang Ao, Taiwan	T		T

SU-95-101		M	Nan Fang Ao, Taiwan	T				T
SU-95-121		F	Nan Fang Ao, Taiwan	T				T
SU-96-03	1377	?	Nan Fang Ao, Taiwan	T	T			
SU-96-05	1370	F	Nan Fang Ao, Taiwan	T	T			
SU-96-06	1369	?	Nan Fang Ao, Taiwan	T	T			
SU-97-02	1381	F	Nan Fang Ao, Taiwan	T	T			
SU-97-03	1382	F	Nan Fang Ao, Taiwan	T	T			
SU-97-12	1385	M	Nan Fang Ao, Taiwan	T	T			
SU-97-13	1386	F	Nan Fang Ao, Taiwan	T	T			
SW-95-01[j]		F	Southern Japan	T		T		
(TA9401)[g]		M	Captive born	A				A
TG-94-01		M	Unk. location, Taiwan[k]	T			T	T
TG-94-02		M	Unk. location, Taiwan[k]	T			T	T
TG-94-03		F	Unk. location, Taiwan[k]	T			T	T
TG-94-06		M	Unk. location, Taiwan[k]	T			T	T
TG-94-34		M	Unk. location, Taiwan[k]	T			T	T
TN-97-03		M	Tainan, Taiwan	T	T	T		T
TN-97-05		F	Tainan, Taiwan	T	T	T		
TN-97-08	1387	M	Tainan, Taiwan	T	T	T		
TN-97-09	1388	?	Tainan, Taiwan	T	T			
TP-96-03	1305	?	Nan Fang Ao, Taiwan	T	T			T
XI-95-01		F	~Penghu Isl., Taiwan	A			A	
XM-95-03		F	~Penghu Isl., Taiwan	A	A	A	A	A
XM-95-07		M	~Penghu Isl., Taiwan	A	A	A	A	A
YL-94-20	1355	?	Nan Fang Ao, Taiwan[l]	T	T			T
YL-96-07	1362	F	Nan Fang Ao, Taiwan[l]	T	T			T

YL-96-08	1352	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-09	1366	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-10	1363	F	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-11	1358	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-12	1359	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-13	1360	F	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-14	1364	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-15	1361	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-31	1353	F	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-35	1354	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-45	1365	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-65	1356	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-66	1357	M	Nan Fang Ao, Taiwan[i]	T	T	T
YL-96-67	1367	F	Nan Fang Ao, Taiwan[i]	T	T	T

[a], National Museum of Natural Science (Taichung, Taiwan).

[b], from Chapter 2.

[c], from Chapter 3.

[d], from Chapter 4.

[e], samples and field identification from E. R. Secchi (Museu Oceanográfico, Rio Grande, Brazil).

[f], samples, data and field identification from E. C. M. Parsons (Swire Institute of Marine Science, Hong Kong).

[g], samples and data from Ocean Park, Hong Kong; Ocean Park specimen codes in parentheses. OP-95-05 was the offspring of dolphins captured from the Penghu Islands and Indonesia.

[h], samples, data and field identification from M. A. Vely (presently at Tropical Oceans Mammals Services, Madagascar).

[i], original NMNS code was changed.

[j], skeletal display specimen of the Swire Institute of Marine Science, Hong Kong (original Ocean Park specimen code was TG7510 (for more specimen information, see Morton, 1978).

[k], collection location unknown but most likely in Taiwan waters.

[l], most likely collection location.