



## A DNA sequence-based identification checklist for Taiwanese chondrichthyans

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### Abstract

In an effort to establish a DNA sequence based checklist of the highly diverse chondrichthyan fauna of Taiwan, we sequenced the mitochondrial NADH2 gene of 257 freshly sampled specimens of Taiwanese chondrichthyans, which were identified to species level by experts in the field. The newly generated sequences were analysed in the context of an already published phylogeny based on NADH2 sequences of chondrichthyans to identify taxonomically interesting lineages as well as enhancing the already published phylogeny with species hitherto not included. Results show a number of noteworthy findings indicating distinct population differences or cryptic diversity and enhance published data with 22 new species not analyzed so far. Our study may be helpful for further taxonomic work on the Taiwanese chondrichthyan fauna.

**Key words:** biodiversity, Chondrichthyes, cryptic species, NADH2, Taiwan

### Introduction

The island of Taiwan lies within a triangle of three major water bodies: the East China Sea to the north and west, the South China Sea to the south, and the open waters of the Philippine Sea to the east. Furthermore, Taiwan is situated at the edge of the shelf of continental Asia, neighbouring the steep and deep slopes of branches of the Philippine and Ryukyu Trenches. These biogeographic features, together with nutrient enriched upwelling regions mainly influenced by the Kuroshio Current, are assumed to attract numerous predatory species including cartilaginous fishes (sharks, skates and rays) as Taiwan lies within a hotspot of marine biodiversity (Caldecott *et al.* 1996; Tittensor *et al.* 2010).

In an effort to establish a current and accurate checklist of the Taiwanese chondrichthyan fauna, an international workshop focusing on the taxonomy of Taiwanese chondrichthyans was held in Taiwan in March 2012. Previously, during, and after this workshop, fresh chondrichthyan specimens were collected from local fish markets, e.g. Da-xi, Keelung, Taichong, and Nan-fang-ao in northern Taiwan as well as Cheng-gong and Dong-gang in southern Taiwan. The specimens collected were identified by chondrichthyan taxonomists and retained as voucher specimens representing Taiwanese cartilaginous fish diversity. Tissue samples were taken from the vast majority of the voucher specimens for subsequent DNA analysis.

The opportunity to analyse voucher specimens from a molecular perspective can be extremely useful for clarifying potential taxonomic issues between morphologically similar specimens, and for identifying potential cryptic species that may not have been identified using classical taxonomic approaches alone. In this paper, we present an analysis of the molecular data of the Taiwanese chondrichthyan tissue samples collected during the workshop, all of which are linked to expertly identified voucher specimens that have been deposited in various ichthyological collections worldwide.

The mitochondrial NADH dehydrogenase subunit 2 (NADH2) was used as the discriminating molecular marker in this study due to its desirable properties of being both fast evolving and protein-coding. Not only has NADH2 been shown to reliably distinguish closely related species (Naylor *et al.*, 2012a), but it also gives a reasonable estimate of deeper levels of divergence (Naylor *et al.*, 2012b). In order to place the Taiwanese diversity in context, sequences generated from this study were combined with a previously published data set of NADH2 sequences from 611 different chondrichthyan species (Naylor *et al.*, 2012b) for an integrated phylogenetic analysis. This approach allows for the characterization of the diversity of Taiwanese cartilaginous fishes in a global context, and investigation of whether any endemic and/or cryptic lineages are present.

## Material and methods

All specimens were opportunistically sampled from various local fish markets whose catches are known to come from Taiwanese waters. Most specimens collected were a component of the bycatch of demersal shrimp trawl fisheries. In a single case, only a tissue sample was retained as the actual specimen was muscle flesh for sale on Keelung fish market. Specimens were retained on ice until processing in the ichthyological laboratories of either the Academia Sinica (ASIZP) in Taipei or the Pisces Collection, National Museum of Marine Biology and Aquarium (NMMB-P) in Pingtung. All specimens were identified by a panel of chondrichthyan taxonomists based on external morphology and coloration. Each specimen was photographed, measured for total and standard length, and a small sample of muscle tissue was removed for the subsequent DNA analysis. While voucher material was preserved in 10% formaldehyde solution for fixation, tissue samples were stored in 100% laboratory-grade ethanol. Voucher specimens were subsequently deposited in one of the following ichthyological collections: Academia Sinica (ASIZP) and Pisces Collection, National Museum of Marine Biology and Aquarium (NMMB-P) in Taiwan; Australian National Fish Collection (CSIRO) in Hobart; Muséum National d'Histoire naturelle (MNHN) in Paris; Hokkaido University (HUMZ) in Hakodate; California Academy of Sciences (CAS) in San Francisco; and the American Museum of Natural History (AMNH) in New York. All tissue samples were assigned a unique identification number and deposited in the chondrichthyan tissue collection of GN.

Genomic DNA of 257 Taiwanese specimens was extracted following the manufacturer's protocol provided along with the Promega Wizard® DNA Purification System. Two microliters of genomic DNA extract were used to amplify the mitochondrial NADH dehydrogenase subunit 2 (NADH2) gene. Universal primer sequences were: forward primer ILEM (5' AAG-GAG-CAG-TTT-GAT-AGA-GT 3') and reverse primer ASNM (5' AAC-GCT-TAG-CTG-TTA-ATT-AA 3') (Naylor *et al.* 2005). PCR amplification included denaturing of DNA at 94° C for 2 minutes, subsequent 30 cycles of another denaturing step at 94° C for 30 seconds, annealing of primers at 50° to 48° C for 30 seconds followed by an extension step at 72° C for 1 minute. After PCR cycling, a final extension step of 72° C for 2 minutes was applied, followed by a cooling step down to 4° C for 15 minutes. All PCR reactions were conducted on Eppendorf Mastercycler® pro S machines. PCR efficacy was evaluated on 1% agarose gels. PCR products were cleaned using Exonuclease I—shrimp alkaline phosphatase cleanup, followed by sequencing on an ABI 3730 sequencer at the Retrogen Inc. Custom DNA Sequencing Facility, San Diego, CA, USA.

Sequences were edited and aligned using Geneious® Pro v5.6.2 by implementing MUSCLE (Edgar, 2004) as an alignment tool. All sequences were translated into amino acids to check for sequencing errors and stop codons. The sequences from the Taiwanese specimens were aligned to a previously aligned reference set of 611 NADH2 sequences used for an earlier phylogenetic study (Naylor *et al.*, 2012b) of chondrichthyans, which itself represents a subset of a larger alignment of 4283 NADH2 sequences (Naylor *et al.*, 2012a) used to explore species level differences in mitochondrial sequences. The total number of sequences used in the current study was 868, of which 611 are from the Naylor *et al.* (2012b) dataset and 257 are sequences from the newly collected Taiwanese material. A constrained neighbour-joining analysis of the complete alignment was carried out using PAUP\* v. 4.0a126 (Swofford, 2003) in which topology was constrained to conform to the backbone resulting from the likelihood analysis presented in the Naylor *et al.* (2012b) phylogenetic study (Supplementary Material 1).

The resultant tree topology was examined for noteworthy patterns associated with the Taiwanese taxa, including any implications of taxonomic uncertainties or cryptic diversity. Any groups of interest were further investigated by increasing haplotype representation using available sequence data (Naylor *et al.*, 2012a, 2012b and unpublished sequences). For that, a neighbour-joining tree analysis was performed using a taxonomically close

species as outgroup and applying a bootstrap analysis using 150 replicates. Phylogenetic analyses were conducted in PAUP\* v. 4.0a126 (Swofford, 2003). An overview of specimens included in this study but not previously analysed in Naylor *et al.* (2012b) is provided in Supplementary Material 2. All newly generated sequences were submitted to Genbank. Type locality information for the various species was obtained from Eschmeyer (2013).

## Results and discussion

A neighbour-joining analysis was used to explore sequence differences at the species level within the context of the backbone tree derived from Naylor *et al.* (2012b). The samples collected in Taiwan included representatives of 31 families, 43 genera, and 63 species, which represents about a third of the known chondrichthyan diversity found in Taiwanese waters (Ebert *et al.* this issue). Focusing on species level interrelationships, we considered several cases to be noteworthy, including instances where novel taxa were identified that were not present in Naylor *et al.* (2012b), or where taxonomic issues are indicated and, in some cases, able to be resolved. Here, we will discuss these findings within the context of the currently recognized diversity of cartilaginous fishes. In this way we are adding to the information presented in Naylor *et al.* (2012a, 2012b), while also specifically characterising Taiwanese chondrichthyan diversity from a molecular perspective. The following list summarizes and interprets results of the initial neighbour-joining analysis that includes the new Taiwanese samples and the data set of Naylor *et al.* (2012b) (Supplementary Material 1). To better account for intraspecific diversity and therefore allowing further detailed interpretation, a number of additional independent neighbour-joining analyses were performed subsequently for any taxonomically noteworthy cases. For that, we increased the haplotype diversity by either adding sequences from Naylor *et al.* (2012a) or newly generated sequences from more recent field collections, expanding available sampling locations. This allowed us to assign Taiwanese specimens to specific subclades found in Naylor *et al.* (2012a) and/or validate newly indicated subclades with an enhanced haplotype diversity (Figures 1–9).

## SELACHII

### Order Hexanchiformes

#### Family Chlamydoselachidae

##### *Chlamydoselachus anguineus* Garman, 1884

Type locality: Japan seas.

The two Taiwanese samples of *C. anguineus* included in this analysis formed a single cluster with a sample from the Mid-Atlantic Ridge (GN1403). They represent the western North Pacific component of the nearly circumglobal distribution of this species (Supplementary Material 1).

#### Family Hexanchidae

##### *Heptanchias perlo* (Bonnaterre, 1788)

Type locality: France, northwestern Mediterranean Sea.

The three Taiwanese samples included in this analysis formed a single cluster with another sample (GN978) also from Taiwan (Supplementary Material 1). They represent a very small component of the circumglobal distribution of this species (Supplementary Material 1).

## Order Squaliformes

### Family Centrophoridae

#### ***Centrophorus granulosus* (Bloch & Schneider, 1801)**

Type locality: unknown.

The 13 Taiwanese samples of *C. granulosus* included in this analysis formed a single cluster that are nearly identical to a sample from Japan (GN7425) and one from Portugal (GN6615) (Supplementary Material 1, Figure 1). These samples were previously identified as either *C. niukang* or *C. acus* but a revision of this species revealed these names are both junior synonyms of *C. granulosus* (White *et al.*, 2013). The cluster of samples for this species was sister to the *C. squamosus* cluster (see below).

#### ***Centrophorus cf. lusitanicus***

Type locality: possibly undescribed.

The two Taiwanese samples *C. cf. lusitanicus* included in this analysis (GN10189 and GN10190) formed a single cluster with another sample identified as *Centrophorus* sp. 2 (GN974; see Naylor *et al.*, 2012a, b) also from Taiwan (Supplementary Material 1, Figure 1). These three samples are similar to a sample identified as *C. sp. 3* (GN4348) from the Philippines. They are distinct from a morphologically similar species from off Madagascar, which has also been informally identified as *C. cf. lusitanicus* (GN2011). This species is closest morphologically to *C. lusitanicus* described from off Portugal but is considered to be distinct from this species and probably undescribed. A comprehensive taxonomic revision of this genus, including this species, is in progress.

#### ***Centrophorus moluccensis* Bleeker, 1860**

Type locality: Ambon Island, Molucca Islands, Indonesia.

The two samples of *C. moluccensis* included in this study formed a single cluster that included a sample from off Western Australia (GN4922) (Supplementary Material 1, Figure 1).

#### ***Centrophorus squamosus* (Bonnaterre, 1788)**

Type locality: probably eastern North Atlantic.

The four samples of *C. squamosus* included in this analysis formed a single cluster that includes a sample (GN6514) from Portugal (Supplementary Material 1, Figure 1), which is sister to *C. granulosus*. These represent a western North Pacific and eastern North Atlantic component of the circumglobal range of this species.

#### ***Deania cf. profundorum***

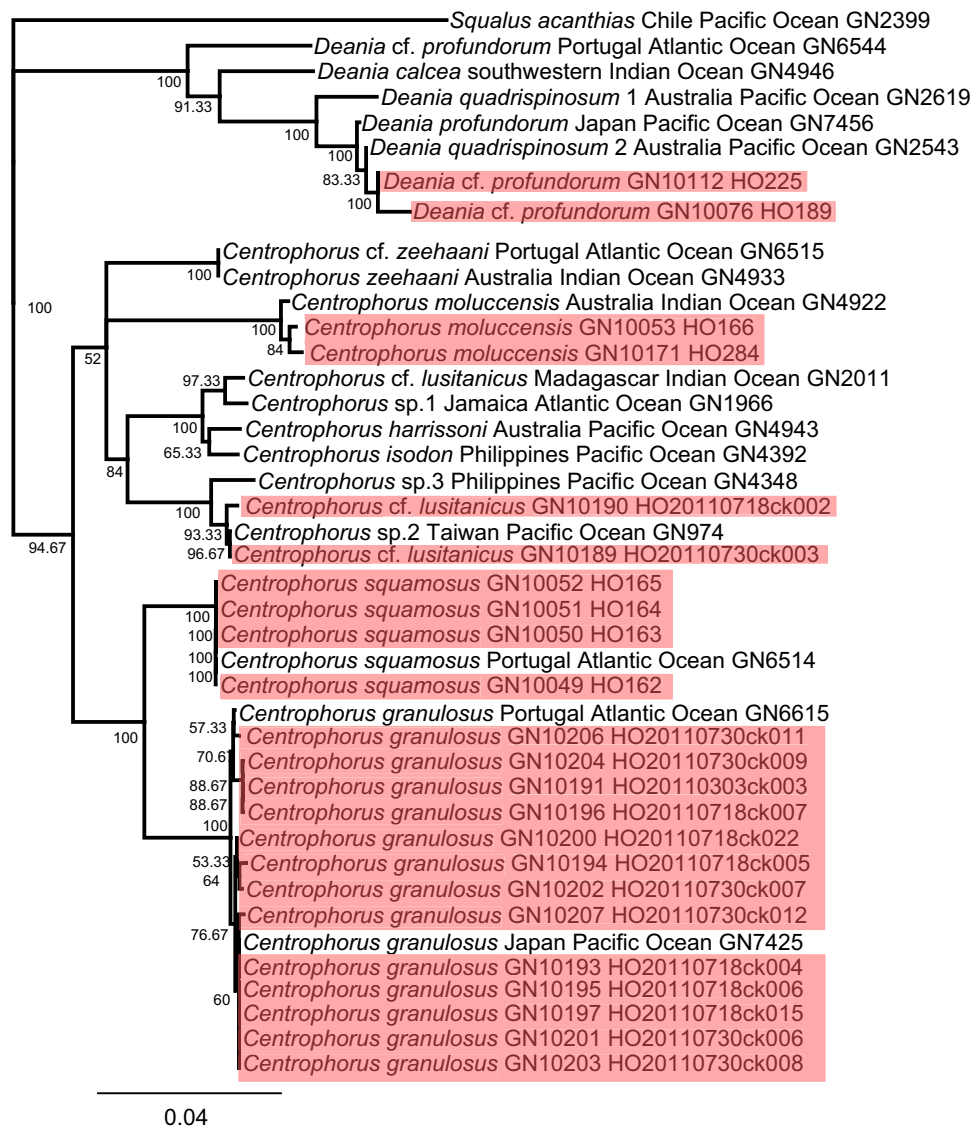
The two *Deania* samples collected in Taiwan were initially identified as *D. cf. quadrispinosa*. They formed a single cluster that included a sample from off Japan (GN7456) labeled as *D. profundorum* and a sample from off New South Wales, Australia (GN2543) identified as *D. quadrispinosum* 2 (Supplementary Material 1, Figure 1). This cluster grouped closest to, but distinct from, a *D. quadrispinosum* 1 sample (GN2619) from New South Wales, Australia. A taxonomic revision of this group is currently underway. This species is provisionally identified as *D. cf. profundorum*, but is distinct from the *D. cf. profundorum* sample from Portugal (Figure 1), which is certainly a distinct species.

## Family Dalatiidae

### *Squaliolus aliae* Teng, 1959

Type locality: Tongkang, Taiwan.

The four Taiwanese samples included in this analysis formed two very similar sub-clusters (Supplementary Material 1). The difference between these two sub-clusters is very small and likely due to sub-population differences or haplotype polymorphism (Supplementary Material 1).



**FIGURE 1.** Focal point neighbour-joining analysis of Centrophoridae. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Squalus acanthias* was defined as outgroup.

## Family Etmopteridae

### *Etmopterus brachyurus* Smith & Radcliffe, 1912

Type locality: off Jolo Island, Philippines, 6°02'00" N, 120°44'40" E.

The two Taiwanese samples of *E. brachyurus* formed a single cluster together with a sample from Japan (GN7423), and were sister to the *E. cf. molleri* clade from Taiwan (Figure 2, Supplementary Material 1).

### ***Etmopterus burgessi* Schaaf da Silva & Ebert, 2006**

Type locality: Da-xi fish market, northeastern Taiwan.

The six Taiwanese samples of *E. burgessi* included in this analysis formed a single cluster, distinct from its congeners and sister to a clade comprising *E. lucifer* samples from New Zealand (Figure 2).

### ***Etmopterus joungi* Knuckey, Ebert & Burgess, 2011**

Type locality: Da-xi, Taiwan.

The three Taiwanese samples of *E. joungi* formed a single cluster together with *E. pusillus* samples from Australia and Portugal. Specimens of *E. pusillus* included in Naylor *et al.* (2012a, 2012b) indicate haplotypes that are geographically widespread across the world's oceans. A revision of this species is required to ascertain whether *E. joungi* is a western North Pacific sub-population of *E. pusillus*, and thus a junior synonym of *E. pusillus*, or whether it is a distinct species (Figure 2, Supplementary Material 1).

### ***Etmopterus cf. molleri***

The 12 Taiwanese samples of *E. cf. molleri* included in this analysis formed a single cluster together with a Taiwan sample of *E. molleri* (GN997), which was sister to an *E. brachyurus* clade (Figure 2, Supplementary Material 1). Naylor *et al.* (2012a) commented that *E. molleri* samples from Taiwan need to be compared with the recently described *E. burgessi* to ensure they are not conspecific. This analysis includes the first sequenced samples of *E. burgessi* and shows they are quite distinct from this species. Samples of *E. molleri* from the type location of Australia are required to ascertain whether the Taiwan species represents a distinct species or whether they are conspecific.

### ***Etmopterus sheikoi* (Dolganov, 1986)**

Type locality: Kyushu Palau Ridge, western North Pacific.

The two Taiwanese *E. sheikoi* samples included in this analysis formed a single cluster that included a sample (GN7440) from Taiwan. As discussed in Straube *et al.* (2010), the species is inferred to be the sister group to the *E. lucifer* clade. Potential misidentification can be ruled out as the morphology is highly distinctive and specimens analysed herein are vouchered. The phylogenetic affinities of *E. sheikoi* are especially interesting. Straube *et al.* (2010) showed that flank markings in *Etmopterus* mirror the relationships inferred from molecular data for all species, except *E. sheikoi*. From a morphological perspective, the species would be expected to cluster within the *E. pusillus* clade rather than the *E. lucifer* clade as suggested by the molecular data (Figure 2). This is an unusual case in which morphology and genetics are inconsistent and need further data collection and analysis from both approaches. This species was previously allocated to the genus *Miroscyllium*, but molecular data suggest this species belongs to the genus *Etmopterus*.

### ***Etmopterus splendidus* Yano, 1988**

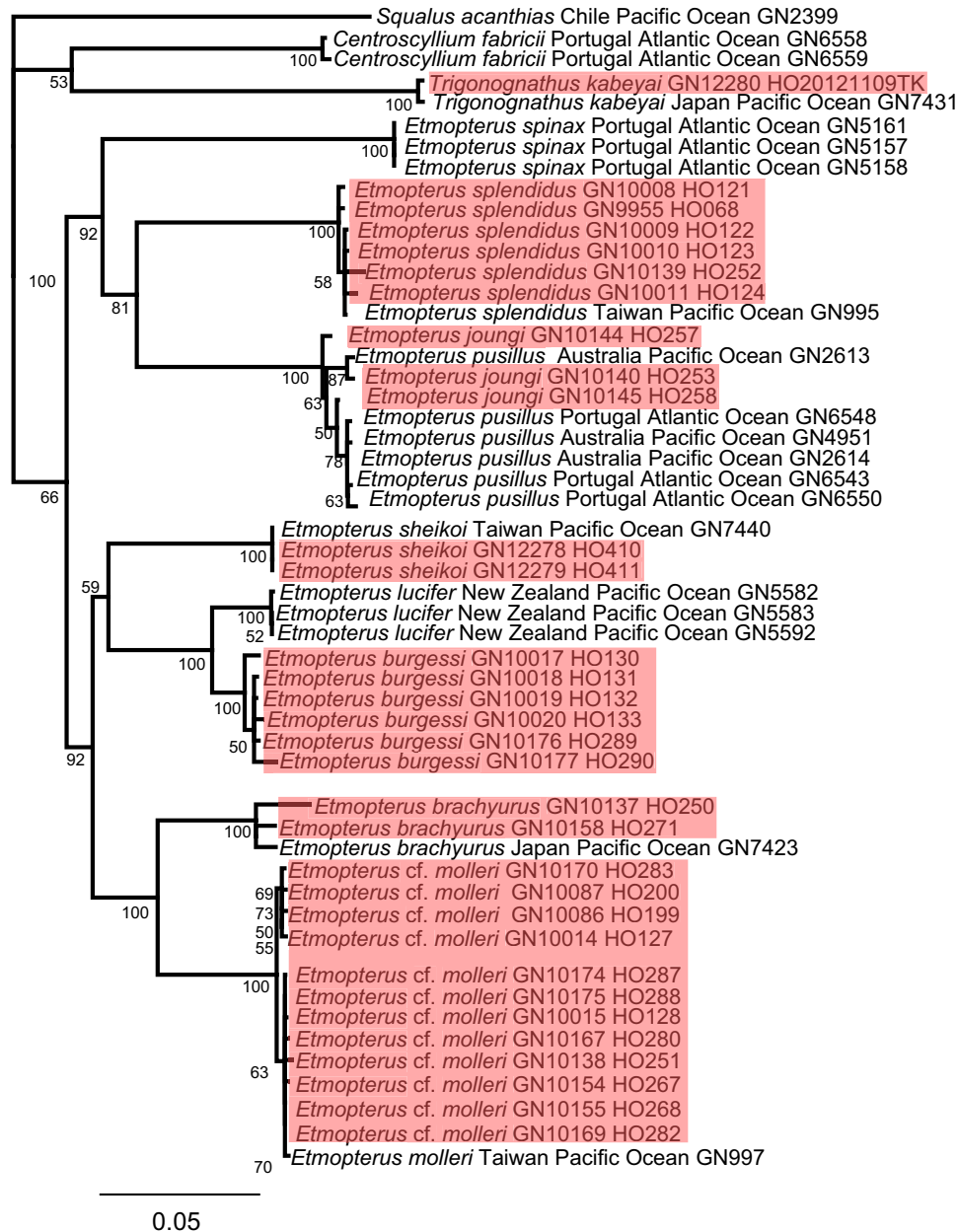
Type locality: off Shimo-Koshiki Island, 31°46.0' N, 129°43.8' E, Japan.

The six Taiwanese samples of *E. splendidus* formed a single cluster together with an additional sample (GN995) also from Taiwan (Figure 2, Supplementary Material 1). This cluster is sister to a clade comprising one sample of *E. sentosus* (GN7402) from the Indian Ocean off Mozambique.

## *Trigonognathus kabeyai* Mochizuki & Ohe, 1990

Type locality: Off Shionomisaki, Wakayama Prefecture, Japan

The single *T. kabeyai* sample from Taiwan included in this analysis formed a single cluster that included a sample from off Japan (GN7431) (Figure 2). This represents the first sequence record for this species from Taiwan. The species is very distinct from, but sister to, the four *Centroscyllum* species (Supplementary Material 1).



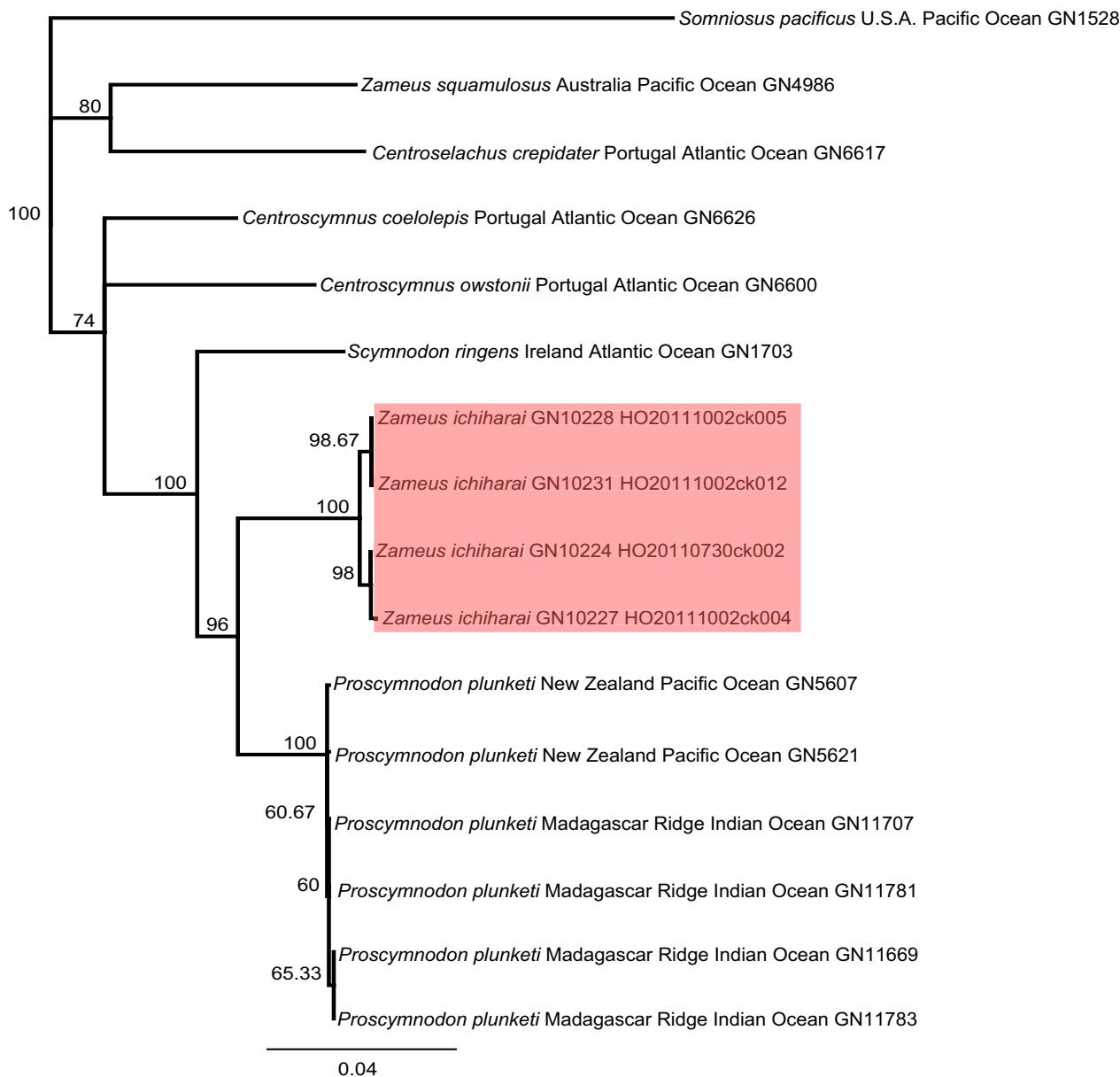
**FIGURE 2.** Focal point neighbour-joining analysis of Etmopteridae. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Squalus acanthias* was defined as outgroup.

## Family Somniosidae

### *Zameus ichiharai* (Yano & Tanaka, 1984)

Type locality: Suruga Bay off Okitsu, 35°02.9'N, 138°34.6'E, Japan

The four samples formed a single cluster closely related to *Proscymnodon plunketi* (GN5607) (Supplementary Material 1). Analysis of additional sequences of *P. plunketi* from New Zealand (the type locality) and the southern Madagascar Ridge further support that *Z. ichiharai* samples from Taiwan are more closely related to *Proscymnodon* than *Zameus squamulosus* (Figure 3). Taxonomic investigation of this species is currently underway to clarify the generic relationships of the genera *Zameus* and *Proscymnodon*. This species had not been previously recorded from Taiwanese waters.



**FIGURE 3.** Focal point neighbour-joining analysis of *Zameus* and *Proscymnodon*. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Somniosus pacificus* was defined as outgroup.

## Family Squalidae

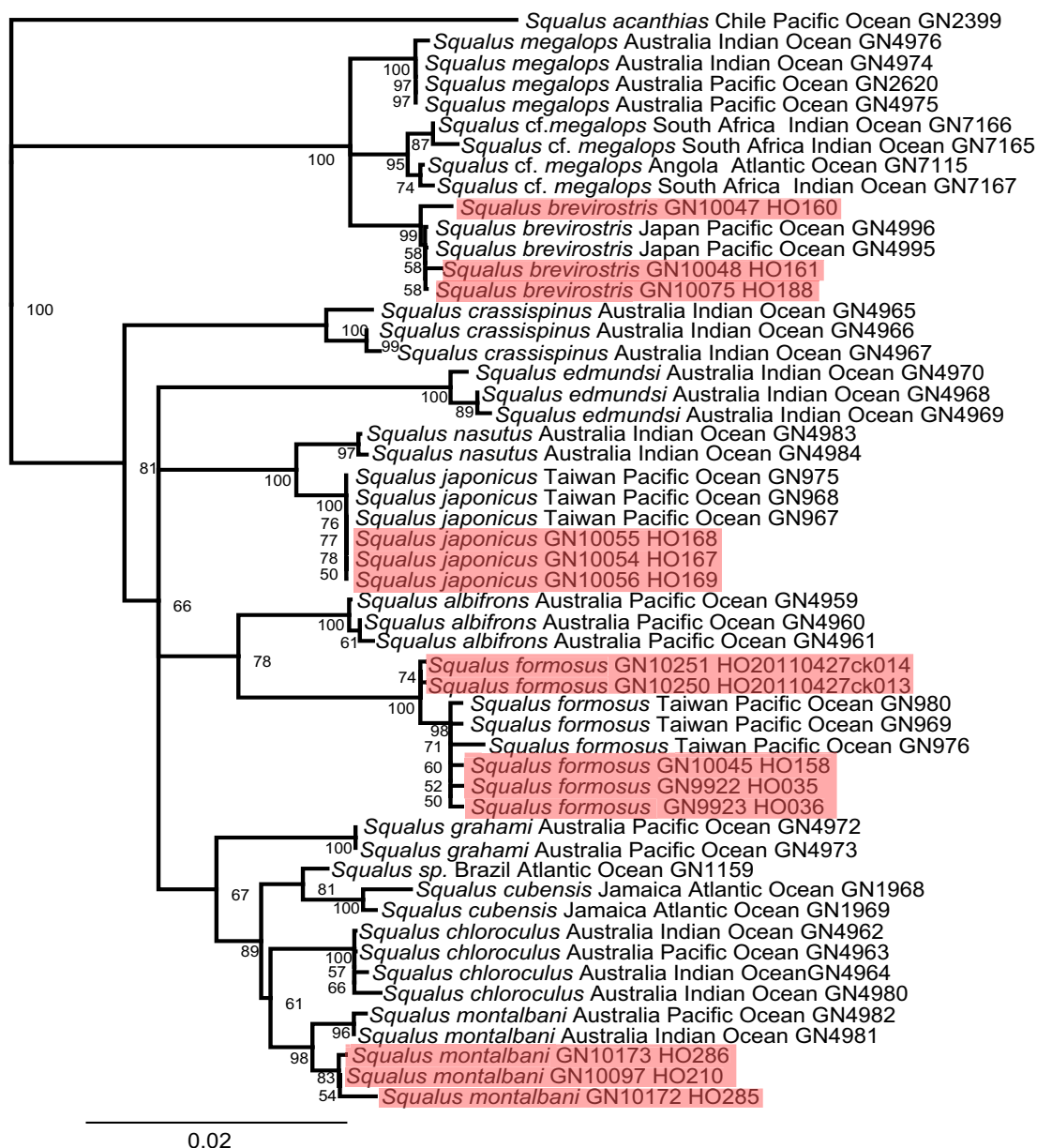
### *Squalus brevirostris* Tanaka, 1917

Type locality: Tokyo fish market, probably from Shimonoseki, Japan.

The three Taiwanese samples of *S. brevirostris* included in this analysis formed a single cluster that included two samples of *S. brevirostris* from Japan (Figure 4). Until recently, this species was considered to be a junior synonym of *S. megalops* but molecular and morphological studies have shown this is a species complex. The *S.*



*brevirostris* clade is sister to the *S. cf. megalops* clade that includes samples from off South Africa and Angola (Figure 4).



**FIGURE 4.** Focal point neighbour-joining analysis of Taiwanese *Squalus* samples. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Squalus acanthias* was defined as outgroup.

### *Squalus formosus* White & Iglésias, 2011

Type locality: Da-xi fish market, northeastern Taiwan.

The five Taiwanese samples of *S. formosus* included in this analysis formed a single cluster along with three other Taiwanese samples (Figure 4). This clade is distinct from, but sister to, the *S. albifrons* clade from off eastern Australia.

### *Squalus japonicus* Ishikawa, 1908

Type locality: Sagami Bay via Tokyo market; Kagoshima, Japan.

The three Taiwanese samples of *S. japonicus* included in this analysis form a single cluster together with three other Taiwan samples of this species (Figure 4). This clade was sister to the *S. nasutus* clade comprising samples from off Western Australia.

### ***Squalus montalbani* Whitley, 1931**

Type locality: Off Sombrero Island, Luzon Island, 13°45'15"N, 120°46'30"E, Philippines

The three Taiwanese samples of *Squalus montalbani* included in this analysis formed a single cluster along with two further *S. montalbani* samples from Australia (Figure 4).

## **Order Squatiniformes**

### **Family Squatinidae**

#### ***Squatina nebulosa* Regan, 1906**

Type locality: Japan.

The three Taiwanese samples of *S. nebulosa* included in this analysis formed a single cluster that is distinct from, but sister to, the *S. formosa* clade which consists of a single sample (GN986) from Taiwan (Supplementary Material 1).

## **Order Heterodontiformes**

### **Family Heterodontidae**

#### ***Heterodontus zebra* (Gray, 1831)**

The single *H. zebra* sample from Taiwan included in this analysis formed a single cluster that included samples of this species from from Malaysian Borneo and Japan (Supplementary Material 1; Naylor *et al.*, 2012a). These specimens represent the majority of the range of this species. This cluster is distinct from *H. cf. zebra* (GN4844) from northern Australia, which is possibly an undescribed species.

## **Order Orectolobiformes**

### **Family Hemiscylliidae**

#### ***Chiloscyllium plagiosum* (Anonymous [Bennett], 1830)**

Type locality: Java Sea, Sumatra, Indonesia, 5°58' S, 106°48' E.

The two Taiwanese samples of this species included in this analysis formed a single cluster that included samples from off Singapore and Malaysian and Indonesian Borneo (Supplementary Material 1, Naylor *et al.*, 2012a).

#### ***Chiloscyllium punctatum* Müller & Henle, 1838**

Type locality: off Jakarta, Java, Indonesia.

The four Taiwanese samples of *C. punctatum* included in this analysis form a single cluster, distinct from a *C. punctatum* clade containing one sample from Indonesian Borneo (GN4446) and from a *C. cf. punctatum* clade containing one sample from northeastern Australia (GN2590) (Supplementary Material 1). This supports the findings of Naylor *et al.* (2012a) who suggested that this species represents a species complex, with two distinct

clades present. The findings of this study introduce a third clade from off Taiwan. Taxonomic revision of this species is required to determine whether these different clades represent distinct species or just population structuring.

### **Family Parascylliidae**

#### ***Cirrhoscyllium formosanum* Teng, 1959**

Type locality: off Kaohsiung, Taiwan.

The five Taiwanese samples of *C. formosanum* included in this analysis formed a single, discrete cluster that is most closely related to *Parascyllium collare* from off southern Australia (GN1927) (Supplementary Material 1).

### **Family Orectolobidae**

#### ***Orectolobus* cf. *leptolineatus***

The single *Orectolobus* cf. *leptolineatus* sample from Taiwan included in this analysis cluster with three other Taiwanese samples (GN7838 to GN7840) (Figure 5). This cluster of Taiwanese samples is very closely related to the *O. leptolineatus* clade containing five samples from Lombok, Indonesia (GN7829, GN7831 to GN7834) (Fig. 5). Given the close species interrelationships within the genus (Corrigan & Beheregaray, 2009; Naylor *et al.*, 2012a, 2012b), and recognised taxonomic uncertainty among species (Last *et al.*, 2010) we tentatively designate the Taiwanese samples as *O. cf. leptolineatus*. Samples of *O. japonicus* are required in the future to determine its relationship with *O. cf. leptolineatus* and *O. leptolineatus*.

### **Order Lamniformes**

#### **Family Pseudocarchariidae**

#### ***Pseudocarcharias kamoharai* (Matsubara, 1936)**

The single Taiwanese sample of *P. kamoharai* included in this analysis grouped with the Taiwan sub-cluster highlighted in Naylor *et al.* (2012a). This sub-cluster was found to be slightly divergent from a Hawaiian sub-cluster. These sub-clusters represent only the western North Pacific and Central Pacific populations of this species (Figure 6).

#### **Family Alopiidae**

#### ***Alopias pelagicus* Nakamura, 1935**

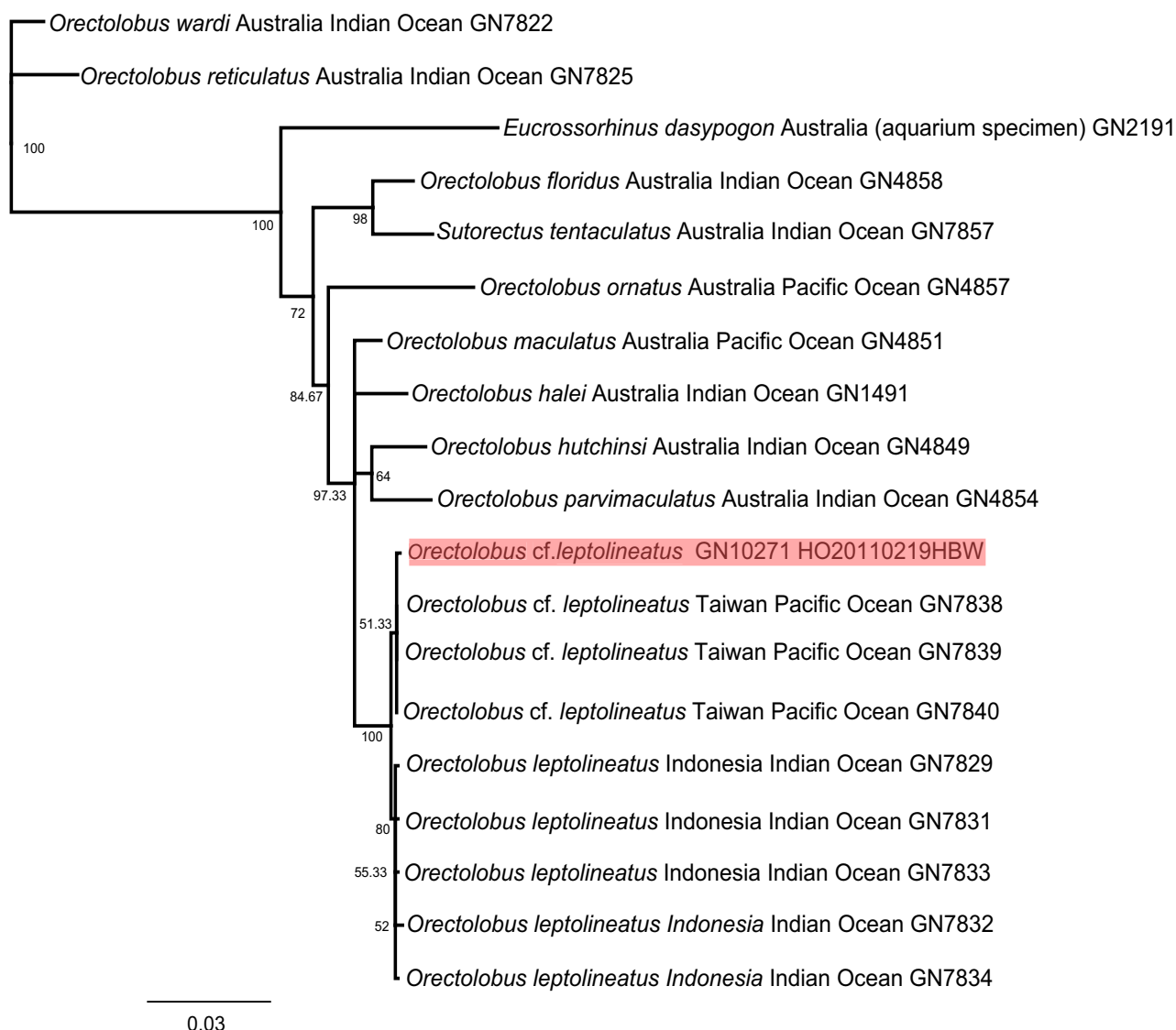
The single Taiwanese *A. pelagicus* sample included in this analysis formed a single cluster with a sample from off Baja California (GN5402) (Supplementary Material 1). When included in a subsequent analysis comprising further samples analysed in Naylor *et al.* (2012a), the specimen clustered with the subcluster discussed in Naylor *et al.* (2012a) including predominantly Pacific and Indian Ocean samples (Figure 6).

#### **Family Lamnidae**

#### ***Isurus oxyrinchus* Rafinesque, 1810**

The identification of the single Taiwanese sample of *I. oxyrinchus* included in this analysis is based on molecular

analysis only as the sample was taken from a large piece of meat at Keelung fishmarket. *I. oxyrinchus* samples in Naylor *et al.* (2012a) formed two location independent subclusters. The sample analyzed herein falls into a subcluster of haplotypes common in the Atlantic, Indian, and western Pacific Ocean (Figure 6).



**FIGURE 5.** Focal point neighbour-joining analysis of *Orectolobus cf. leptolineatus*. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Orectolobus wardi* was defined as outgroup.

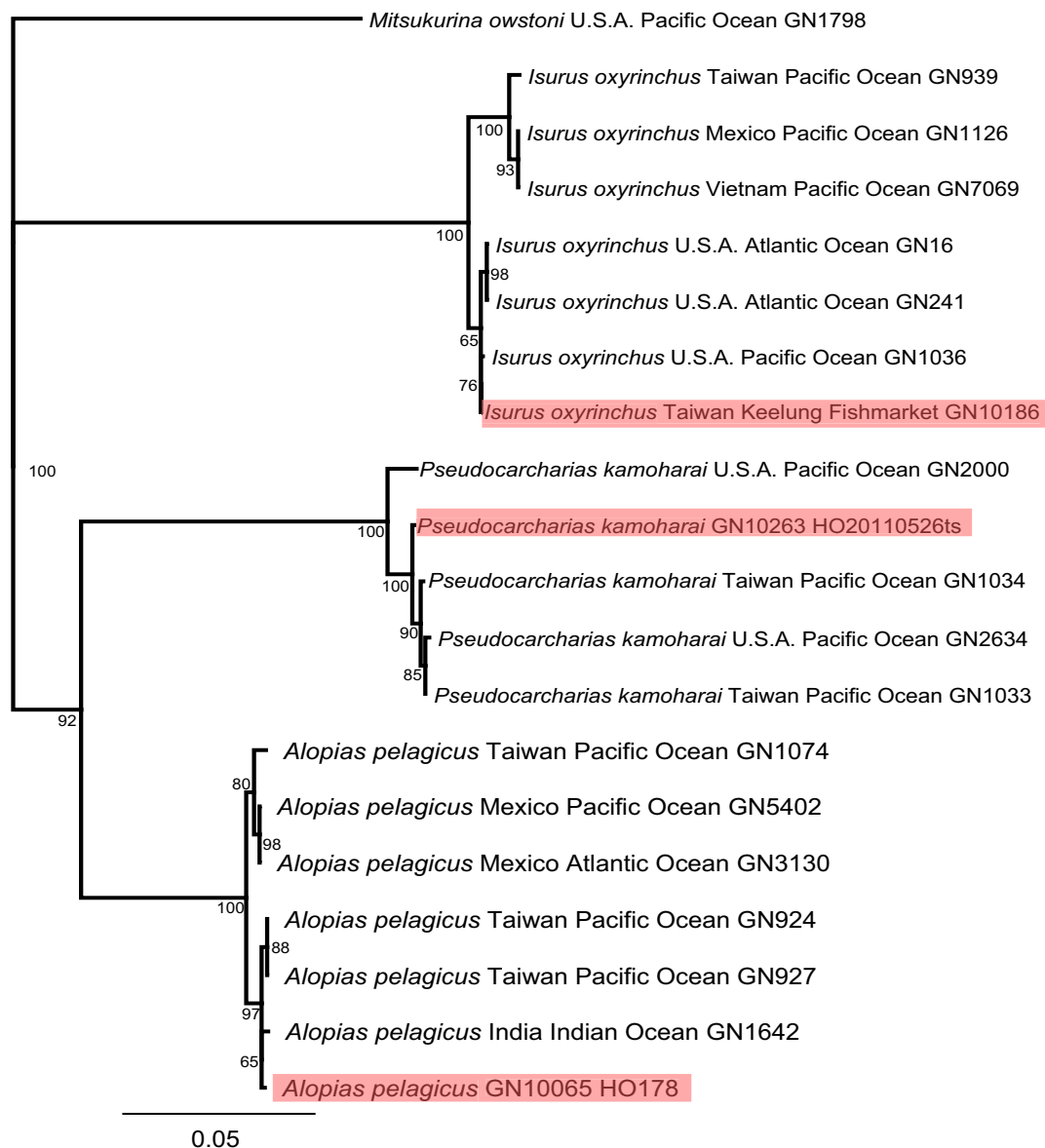
## Order Carcharhiniformes

### Family Scyliorhinidae

#### *Apristurus herklotsi* (Fowler, 1934)

Type locality: Cagayan Island, Jolo Sea, Philippines

The single Taiwanese sample of *A. herklotsi* included in this analysis formed a distinct lineage which is close, and sister to, the morphologically similar *A. australis* (GN4877) from Western Australia (Supplementary Material 1).



**FIGURE 6.** Focal point neighbour-joining analysis of selected lamniform species. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Mitsukurina owstoni* was defined as outgroup.

### *Apristurus macrostomus* Chu, Meng & Li, 1985

Type locality: off Zhujiang, South China Sea.

The 13 Taiwanese samples of *A. macrostomus* formed a single cluster that includes other previously collected samples from Taiwan (Supplementary Material 1). Sample GN1013 is labeled as *A. macrorhynchus* in Naylor *et al.* (2012a, 2012b). Based on results of this study, the sample may have to be transferred to *A. macrostomus*. This clade is closest to, but very distinct from, an *A. exsanguis* clade from New Zealand.

### *Cephaloscyllium umbratile* Jordan & Fowler, 1903

Type locality: Nagasaki, Japan, 32°43' N, 129°50' E.

The six Taiwanese samples of *C. umbratile* included in this analysis formed a single cluster that included

samples previously collected from Taiwan, and is sister to a potentially undescribed species from the Philippines, *C. sp. 1* (Supplementary Material 1; Naylor *et al.*, 2012a).

### ***Galeus nipponensis* Nakaya, 1975**

Type locality: Mimase, off Kochi Prefecture, Honshu, Japan.

The single *G. nipponensis* sample included in this analysis is the first representative sample for this species and it formed a distinct lineage, sister to *Parmaturus xaniurus* from off California (GN1536) (Supplementary Material 1). As indicated in Naylor *et al.* (2012b), the genus *Galeus* may be paraphyletic and thus the generic placement of this species needs to be examined in the future.

### ***Galeus sauteri* (Jordan & Richardson, 1909)**

Type locality: Kaohsiung, southwestern Taiwan.

The 14 Taiwanese samples of *G. sauteri* included in this analysis formed a single cluster that includes previously collected samples of this species from Taiwan (Supplementary Material 1). This clade is distinct from, but most closely related to, the *Galeus nipponensis* and *Parmaturus xaniurus* lineages.

## **Family Proscyllidae**

### ***Eridacnis radcliffei* Smith, 1913**

Type locality: off Jolo Light, Sulu Islands, Philippines, 6°11'50" N, 121°08'20" E.

The two Taiwanese samples of *E. radcliffei* included in this analysis formed a single cluster that was slightly divergent from the *Eridacnis* sp. 1 sample from the Philippines (GN2212) (Supplementary Material 1). A revision of this group is required to determine whether these two lineages reflect different species or population structuring within *E. radcliffei*.

### ***Proscyllium habereri* Hilgendorf, 1904**

Type locality: Kaohsiung, southwestern Taiwan.

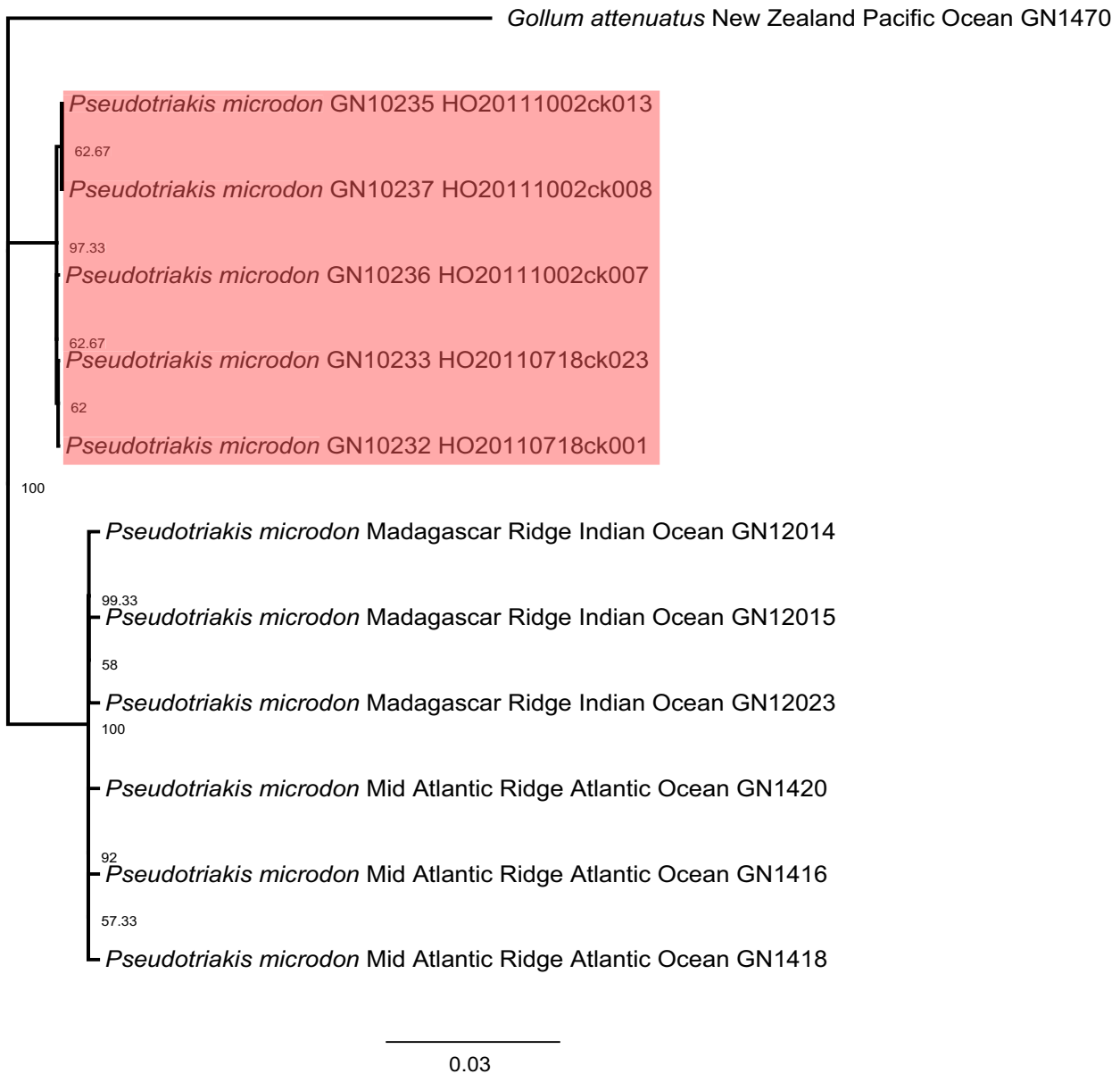
The six samples of *P. habereri* included in this analysis formed a single cluster (Supplementary Material 1). This cluster was very similar, but slightly divergent from a single *P. habereri* sample from the Okinawa Islands in Japan (GN2601). This likely represents population structuring within this species, but a taxonomic investigation of these populations should be carried out to confirm this.

## **Family Pseudotriakidae**

### ***Pseudotriakis microdon* de Brito Capello, 1868**

Type locality: Setubal, Portugal, Northeastern Atlantic.

The five Taiwanese samples of *P. microdon* included in this analysis formed a single cluster, distinct from, but sister to, the *P. microdon* samples from the southern Madagascar Ridge and the Mid-Atlantic Ridge (Figure 7). These results challenge the currently accepted view that this species is circumglobal in distribution (e.g. Compagno *et al.*, 2005) and suggest that the western North Pacific population is distinct from the Northern Atlantic/southwestern Indian populations. A taxonomic revision of this genus is required to determine whether these two clades represent distinct species and if so, determine whether *Pseudotriakis acrales* Jordan & Snyder 1904 described from Suruga Bay in Japan should be resurrected as a valid species.



**FIGURE 7.** Focal point neighbour-joining analysis of cryptic diversity within *Pseudotriakis microdon*. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Gollum attenuatus* was defined as outgroup.

## Family Triakidae

### *Hemitriakis japonica* (Müller & Henle, 1839)

Type locality: Japan.

The five Taiwanese samples of *H. japonica* included in this analysis formed a single cluster that included previously collected samples of this species from Taiwan, Japan and Vietnam (Supplementary Material 1; Naylor *et al.*, 2012a). This cluster is closely related and sister to the *H. leucoperiptera* clade consisting of samples from the Philippines (Supplementary Material 1).

### ***Mustelus griseus* Pietschmann, 1908**

Type locality: Japan.

The three *M. griseus* samples included in this analysis formed a single cluster amongst a polytomy of other closely-related *Mustelus* species (Supplementary Material 1). As discussed in Naylor *et al.* (2012b), the genus *Mustelus* may be paraphyletic and the current results further support this hypothesis (Supplementary Material 1).

### ***Mustelus manazo* Bleeker, 1855**

Type locality: Nagasaki, Japan; northwestern Indian Ocean.

The four Taiwanese samples of *M. manazo* included in this analysis formed a single cluster that includes samples previously collected from Taiwan, Vietnam, Borneo and Japan (Supplementary Material 1; Naylor *et al.*, 2012a). This clade falls within a group of seven closely-related *Mustelus* species, which form a distinct grouping separate to the other group of *Mustelus* which includes *M. griseus* (Supplementary Material 1).

## **Family Carcharhinidae**

### ***Carcharhinus tjujot* (Bleeker, 1852)**

Type locality: Jakarta, Java, Indonesia.

The two Taiwanese samples of *C. tjujot* included in this analysis formed a single cluster that includes a sample of this species from Indonesian Borneo (GN4597), and is sister to the *C. dussumieri* clade from the northwestern Indian Ocean (Supplementary Material 1). A recent revision of the *C. dussumieri*-*C. sealei* complex informed by both molecular and morphological data resurrected *Carcharhinus tjujot* as a valid nominal species (White, 2012).

### ***Scoliodon macrorhynchos* (Bleeker, 1852)**

Type locality: Jakarta, Java, Indonesia.

The four Taiwanese samples of *S. macrorhynchos* included in this analysis formed a single cluster that included previously collected samples from Vietnam, Malaysia and Taiwan (Supplementary Material 1; Naylor *et al.*, 2012a). This clade was sister to the *S. laticaudus* clade consisting of individuals from off India (Supplementary Material 1; Naylor *et al.*, 2012a). This species was recently resurrected as a valid nominal species following a taxonomic revision (White *et al.*, 2010) and the results of this study further support these findings.

## **BATOIDEA**

### **Order Rhinobatiformes**

#### **Family Plathyrhinidae**

### ***Platyrhina tangi* Iwatsuki, Zhang & Nakaya, 2011**

Type locality: Meitsu, Miyazaki, Japan, 31°32'12" N, 131°24'16" E.

The six Taiwanese samples formed a single cluster that was distinct from, but sister to, *Platyrhinoides triseriata* (GN1043) from off California (Supplementary Material 1). These six samples represent the centre of the known distribution of this species, which extends from Japan to Vietnam.



## Family Rhinobatidae

### *Rhinobatos hynnicephalus* Richardson, 1846

Type locality: probably China Seas, Canton, China (no types known).

The three Taiwanese samples included in this study formed a single cluster, sister to *R. schlegelii* from Taiwan (Supplementary Material 1). The samples were preliminary identified as *R. schlegelii* but later assigned to *R. hynnicephalus* (B. Séret, pers. comm.).

### *Rhinobatos schlegelii* Müller & Henle, 1841

Type locality: Japan

Four samples were analysed in total, two of which were preliminary identified as *R. formosensis* Norman 1926, two as *R. schlegelii*. After further morphological investigation, all samples were referred to as *R. schlegelii*. All samples cluster together (Supplementary Material 1). The status of *R. formosensis* and *R. schlegelii* are currently under revision, it is likely that *R. formosensis* is a junior synonym of *Rhinobatos schlegelii*. The clade forms a distinct lineage sister to *R. hynnicephalus* (Supplementary Material 1). It is worth noting that specimens in this cluster were also clearly distinct from the *R. cf. schlegelii* (GN4326) specimen from the Philippines, and thus is not conspecific with this currently unresolved species.

## Family Rhynchobatidae

### *Rhynchobatus immaculatus* Last, Ho & Chen, 2013 [this issue]

Type locality: Keelung, Taiwan

The two samples of *Rhynchobatus immaculatus* n. sp. included in our analysis represent a distinct lineage, sister to a clade comprising several *Rhynchobatus* species from Malaysia and Australia, i.e. *R. australiae* (GN2996), *R. cf. laevis* (GN2065), *R. laevis* (GN3004) and *R. palpebratus* (GN2044) (Supplementary Material 1). This species was determined as a new and previously undescribed species during this study based on both, the molecular results and morphological information.

## Order Torpediniformes

### Family Narcinidae

#### *Benthobatis yangi* Carvalho, Compagno & Ebert, 2003

Type locality: off Tongkang, southwestern Taiwan.

The two Taiwanese samples included in our analysis formed a single cluster, sister to but very distinct from a *Typhlonarke aysoni* sample from off New Zealand (GN6759) (Supplementary Material 1). This species appears endemic to Taiwan and thus these two samples are representative of the range of this species.

## Order Rajiformes

### Family Rajidae

#### *Dipturus gigas* (Ishiyama, 1958)

Type locality: off Aichi Prefecture, Japan.

The single Taiwan sample of *D. gigas* was distinct from the other rajid species and clustered closest to a *D. springeri* sample (GN7382) from off South Africa (Supplementary Material 1).

### ***Dipturus kwangtungensis* (Chu, 1960)**

Type locality: Jia-Bo, China.

The eight Taiwanese samples of *D. kwangtungensis* formed a single cluster that also included a sample from Naylor *et al.* (2012b) labeled as *Raja* sp. 1 (GN6181), also from Taiwan (Supplementary Material 1). Due to the nearly identical sequences of this specimen and the eight new Taiwanese samples of *D. kwangtungensis*, it is most likely this specimen is also *D. kwangtungensis*.

### ***Dipturus tengu* (Jordan & Fowler, 1903)**

Type locality: Matsushima Bay, Sendai, Japan.

The seven Taiwanese samples of *D. tengu* sequenced in this study formed a single cluster, sister to a specimen identified as *Dipturus* sp. 4 from the Philippines (GN4353) in Naylor *et al.* (2005b) (Supplementary Material 1). Interestingly they did not cluster with a specimen identified as *D. tengu* (GN1025), also from Taiwan in Naylor *et al.* (2005b). This could represent cryptic speciation within this species, but more samples of skates from Taiwanese waters will be required to resolve this issue.

### ***Okamejei acutispina* (Ishiyama, 1958)**

Type locality: Sea of Japan, off Shimane Prefecture, Japan

The seven Taiwanese samples of this species formed a single cluster, sister to *Okamejei cairae* (GN4735) from Indonesia (Supplementary Material 1).

### ***Okamejei hollandi* (Jordan & Richardson, 1909)**

Type locality: Kaohsiung, Taiwan.

The two Taiwanese samples of *O. hollandi* formed a single cluster with a sample (GN4738) from Indonesian Borneo (Supplementary Material 1). Results of this analysis indicate that the Indonesian and Taiwanese specimens are conspecific.

## **Order Myliobatiformes**

### **Family Dasyatidae**

#### ***Dasyatis* cf. *akajei***

Type locality: southwestern coast of Japan.

The five Taiwanese specimens of *D.* cf. *akajei* form a clearly distinct lineage within the diverse clade of Dasyatidae (Supplementary Material 1). The conspecificity of Taiwanese and Japanese forms is questionable and is presently being evaluated.

#### ***Dasyatis zugei* (Müller & Henle, 1841)**

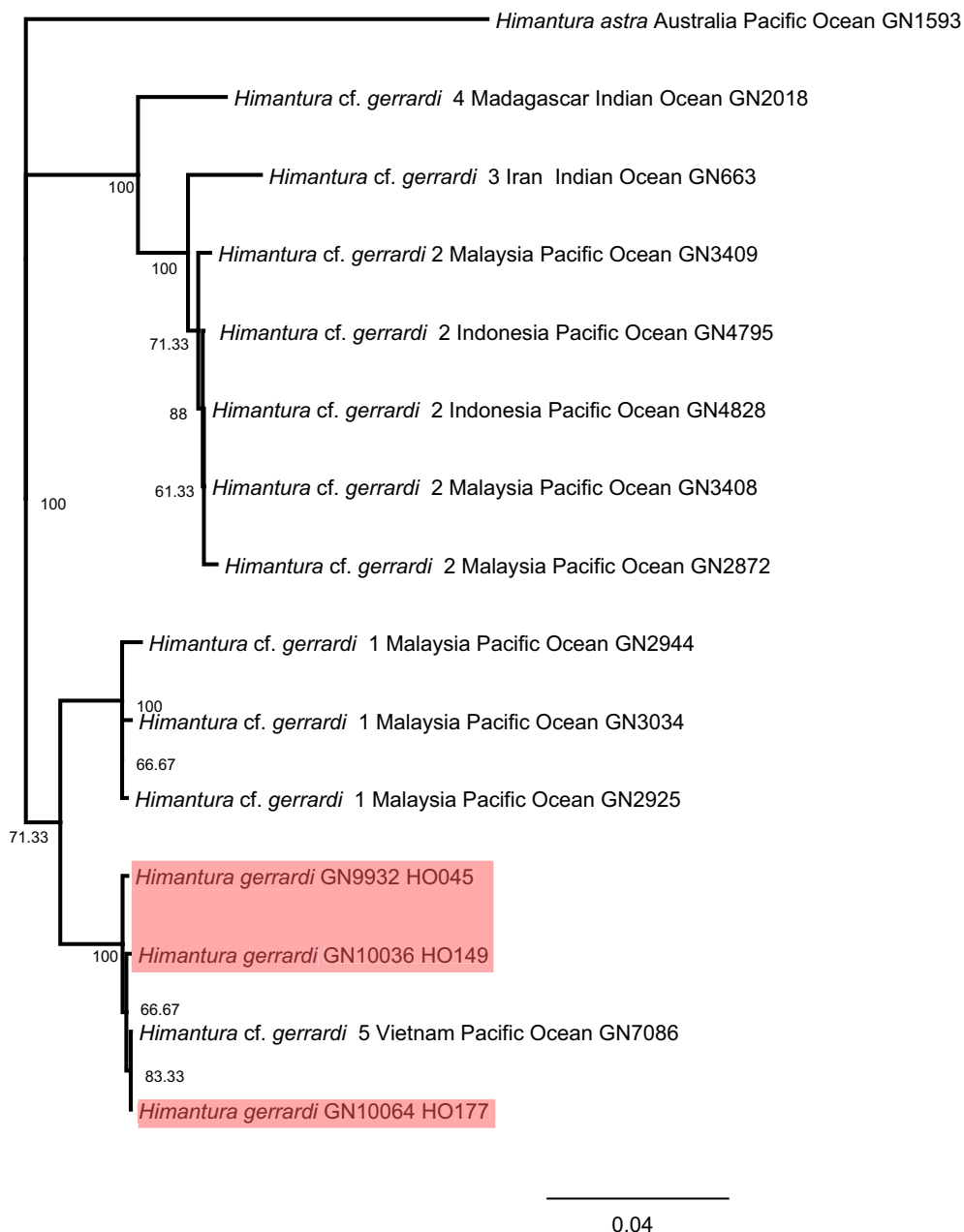
Type locality: Macao.

The seven Taiwanese samples of *D. zugei* included in this analysis formed a single cluster that included previously sequenced samples from Vietnam (Supplementary Material 1; Naylor *et al.*, 2012a). This clade was sister to, but distinct from, a *D.* cf. *zugei* clade comprising samples from Indonesian and Malaysian Borneo and supports the findings of Naylor *et al.* (2012a).

## *Himantura gerrardi* (Gray, 1851)

Type locality: India.

As in *Neotrygon* cf. *kuhlii*, samples analysed in previous studies (Naylor *et al.*, 2012a, 2012b) indicate cryptic diversity within this species. In those studies, the authors defined five sub-clusters within a species complex designated as *Himantura* cf. *gerrardi* 1 to 5. The three additional Taiwanese specimens analysed herein cluster with *H. cf. gerrardi* 5 (GN7086) from Vietnam (Figure 8). Since vouchered specimens are available from each of these sub-clusters, a detailed morphological taxonomic study is required to identify potential species-specific characters that may distinguish these sub-clusters from each other and help determine whether multiple species are involved.



**FIGURE 8.** Focal point neighbour-joining analysis of cryptic diversity in the *Himantura gerrardi* complex. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red. *Himantura astra* was defined as outgroup.

### ***Neotrygon cf. kuhlii***

Type locality: India, Vanicoro (Solomon Islands), New Guinea

Naylor *et al.* (2012a, 2012b) previously highlighted taxonomic complexity within this species, identifying 4 separate clades (GN3698 = *N. kuhlii* 1, GN3621 = *N. kuhlii* 2, GN2016 = *N. kuhlii* 3, and GN2093 = *N. kuhlii* 4, Naylor *et al.*, 2012b). The six Taiwanese samples included in this analysis cluster with *N. kuhlii* 2 from Malaysia and Thailand (Figure 9), further supporting the idea of cryptic diversity. Puckridge *et al.* (2013) also highlighted potential cryptic diversity within this species and included Taiwan samples (Clade 1 in this study). Clade 1 of Puckridge *et al.* (2013) included samples from Taiwan, Thailand, Malaysia and Vietnam. The species complex is currently under revision.

### **Family Urolophidae**

#### ***Urolophus aurantiacus* Müller & Henle, 1841**

Type locality: Gotto Island, Nagasaki, Japan (the additional type locations of Westernport Victoria, and Tasmania in Australia are incorrect and refer to *Urolophus cruciatus*).

The two samples of *U. aurantiacus* from Taiwan formed a single cluster, sister to *U. paucimaculatus* (GN2554) from southern Australia (Supplementary Material 1). This represents the Taiwan range of this species, which was described from southern Japan.

### **Family Plesiobatidae**

#### ***Plesiobatis daviesi* (Wallace, 1967)**

Type locality: Mozambique Channel, off mouth of Limpopo River, about 25°25' S, 33°35' E, South Africa.

The three Taiwanese samples of *P. daviesi* included in this analysis formed a single cluster that includes previously collected samples from Malaysian Borneo and the Philippines (Supplementary Material 1; Naylor *et al.*, 2012a). These represent only the western North Pacific component of the Indo-West and Central Pacific distribution of this species.

### **Family Gymnuridae**

#### ***Gymnura zonura* (Bleeker, 1852)**

Type locality: Jakarta, Java, Indonesia.

The three Taiwanese samples of this species included in this analysis formed a single cluster that included samples from Malaysian and Indonesian Borneo and Singapore (Supplementary Material 1; Naylor *et al.*, 2012a). This clade is sister to *Gymnura* sp. 1 from the western North Atlantic.

### **Family Myliobatidae**

#### ***Aetomylaeus nichofii* (Bloch & Schneider, 1801)**

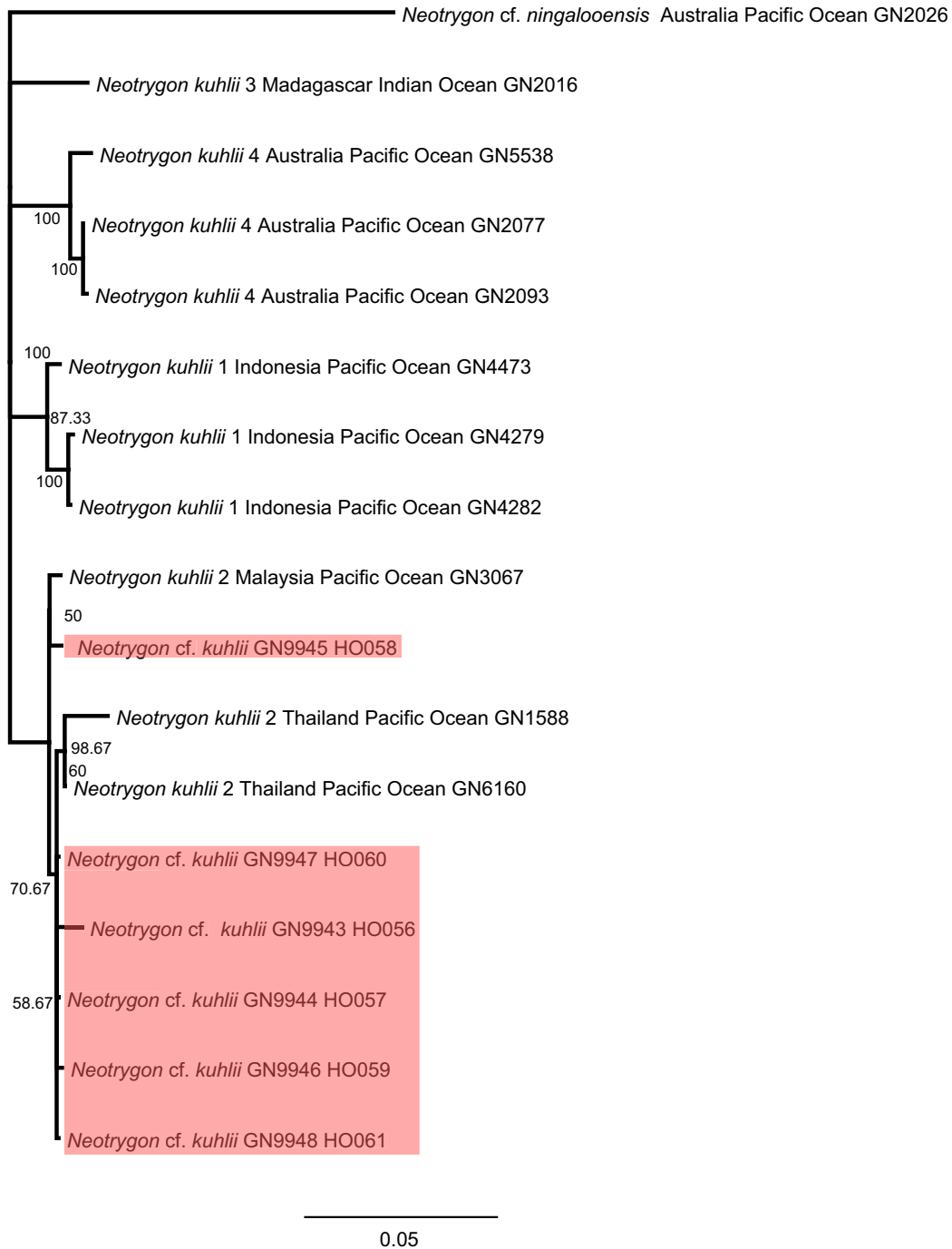
Type locality: East Indies (Indonesia).

The three Taiwan samples of *A. nichofii* included in this analysis formed a single cluster that includes previously collected samples of this species from Indonesian and Malaysian Borneo (Supplementary Material 1; Naylor *et al.*, 2012a). This clade was sister to the *A. cf. nichofii* 1 (*sensu* Naylor *et al.*, 2012a) clade, which comprises a single sample from Iran in the northwestern Indian Ocean. Taxonomic revision of this species complex is currently underway to resolve how many distinct species comprise this complex.

***Myliobatis tobijei* Bleeker, 1854**

Type locality: Nagasaki, Japan.

The two Taiwanese samples of this species included in this analysis formed a single cluster that includes previously collected samples of this species from Japan and the Philippines (Supplementary Material 1; Naylor *et al.*, 2012a). This clade was sister to the *Myliobatis aquila* clade from South Africa.



**FIGURE 9.** Focal point neighbour-joining analysis of cryptic diversity of *Neotrygon cf. kuhlii*. Analysis based on NADH2 sequence data applying the Jukes Cantor model. Numbers above branches indicate bootstrap support values applying 150 bootstrap replicates. Taiwanese samples collected for this analysis highlighted red *Neotrygon cf. ningalooensis* was defined as outgroup.

## HOLOCEPHALA

### Order Chimaeriformes

#### Family Chimaeridae

##### *Chimaera phantasma* Jordan & Snyder, 1900

Type locality: Tokyo Bay, Japan.

The ten Taiwanese samples included in this analysis represent the centre of the known distribution of this species, which extends from Japan to the Philippines. The analysis yielded a single cluster (Supplementary Material 1).

##### *Hydrolagus mitsukurii* (Jordan & Snyder, 1904)

Type locality: Japan.

The four specimens identified as *Hydrolagus mitsukurii* formed a distinct clade sister to the only other chimaeroid species included in this analysis, *Chimaera phantasma* (Supplementary Material 1). These four Taiwanese samples represent the centre of the known distribution of this species, which extends from Japan to the Philippines.

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## Supplementary Material

**Supplementary Material 1** (available online at <http://www.mapress.com/zootaxa/list/2013/3752%281%29.html>)  
 Constrained tree neighbour-joining analysis of combined Naylor *et al.* 2012b and newly generated sequence dataset attained from recent sampling efforts in Taiwan. Analysis based on NADH2 sequence data using the Jukes Cantor model, backbone constrained using the phylogenetic tree from Naylor *et al.* (2012b). Taiwanese samples colored red. *Rhinochimaera pacifica* was defined as outgroup.

**Supplementary Material 2** (available online at <http://www.mapress.com/zootaxa/list/2013/3752%281%29.html>)  
 Overview of specimens included in this study, which are new to the dataset analysed in Naylor *et al.* (2012a, 2012b) and associated Genbank accession numbers.