Finless porpoises (*Neophocaena asiaeorientalis*) in the East China Sea: Insights into feeding habits using morphological, molecular, and stable isotopic techniques

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Finless porpoises (*Neophocaena asiaeorientalis*) in the East China Sea: Insights into feeding habits using morphological, molecular, and stable isotopic techniques

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Abstract

Describing feeding habits of cetaceans is crucial to understanding their feeding strategies and conservation status. Here, both morphological and molecular techniques were employed to identify the stomach contents of 122 finless porpoises (*Neophocaena* spp.) in the East China Sea for insight into their short-term feeding habits; and stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were used for analyzing prey resource use and trophic position as a manifestation of their long-term feeding habits. In total, 33 prey species comprising of 19 teleosts, 7 crustaceans, 5 cephalopods, and 2 gastropods were identified. In both short and long-term analyses, teleosts represented primary prey, cephalopods and crustaceans were secondary prey, and gastropods were occasional prey; but, the primary prey species composition is different between short and long-term diet. The composition of stomach contents showed sexual and age-related variation. This is supported by stable isotopic analyses which indicated the separation of trophic position of adult male, adult female and young male. Generally, finless porpoises prey on species which are primarily caught by fisheries.

Key words: East China Sea, Feeding habits, Finless porpoise, Species identification, Stable isotopes

Introduction

Describing the diets/feeding habits of apex predators contributes important information for the elaboration of the distribution of small cetaceans (Amir *et al.* 2005), and is important for understanding their prey strategies. The identification and analyses of stomach contents is a common and useful method in many investigations on diets of small cetaceans (Amir *et al.* 2005).

Finless porpoises are distributed throughout the coastal waters of the Indo-Pacific region (Wang *et al.* 2008), and are among the most common species stranded or caught along the Chinese, Japanese, and Korean coasts (Rice 1998; Zhang *et al.* 2004; Zhou 2004). Finless porpoises have been divided into two species: the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*), and the narrow-ridged finless porpoise (*Neophocaena asiaeorientalis*) (Wang *et al.* 2008; Jefferson and Wang 2011). The distinctive difference is the width of the tubercled area on the dorsal surface, the former has a wide area of tubercles (10-18 cm), while the latter shows a narrow tubercled area (0.3-0.7cm) (Wang *et al.* 2008). Based on morphological identification analyses, it is known that finless porpoises feed on a wide variety of fish and cephalopods, and the diet varies among different geographical sites and different species, e. g. *N. asiaeorientalis* in Korean waters (Park *et al.* 2005), Japan (Shirakihara *et al.* 2008), and the Yangtze River (Chen *et al.* 1979); *N. phocaenoides* in Hong Kong (Barros *et al.* 2002), Beibu Gulf (Lu and Fang 2006) and Xiamen (Huang *et al.* 2000). However, very little information is available on the feeding

habits of these species in other parts of their distribution range and, in particular the East China Sea (Fig. 1). The East China Sea has a dense distribution of finless porpoises, and Taiwan strait represents an area of overlap for both species (Gao and Zhou 1995ab; Huang et al. 2000; Zhou 2004; Wang et al. 2008).

But in those researches, only morphological identification analyses on stomach content was applied. Morphological identifications are often compromised, since prey body, including the exoskeleton or bones, can be damaged or digested (Jarman et al. 2002; Deagle 2006). DNA-based method offers a better chance to identify morphologically-unidentifiable fish, shrimp, and squid (Symondson 2002; Jarman and Wilson 2004; Deagle et al. 2005; King et al. 2008; Dunshea 2009). Therefore, DNA-based methods are good supplement to morphological identification. This method is still at a relatively early stage, but in terms of providing quantitative estimates of the composition of diets, results to date are promising (Bowen and Iverson 2013). Although DNA-based methods were rarely been used for cetaceans' diet analysis in the past, there are some successful examples. Jarman et.al (2002, 2004) have identified the krill components of the pygmy blue whale's diet via DNA purified from its faeces and then studied prey diversity and identity in the fin whale by using this method. Dunshea et al. (2009, 2013) have detected diverse prey DNA in fecal and gastric samples to analyze the diet of different species of cetaceans. Méheust et al. (2015) have studied the stomach contents of seven harbour porpoises by DNA-based identification of soft tissues using the BOLD system database. Up to this date, DNA-based methods have not been utilized in the analyses of the

diet of the finless porpoises.

Although the identification of stomach content is the most common method used in diet analyses, the stomach content only represents the last prey ingested by a predator. The stomach content is only indicative of a predator's diet of no more than several days before death, therefore solely representing short-term feeding habits. In fact, the dietary inputs can concentrate over the turnover time of the tissue, which can be presented by stable isotopes (Tieszen et al. 1983; Praca et al. 2011). The stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) can provide information about resource use, e.g. distribution of isotopes amongst all species in the diet and trophic position in the food web (Kelly 2000; Parnell et al. 2010; Pokrovsky 2012), which represents long-term feeding habits. It has been performed on the diets of some marine mammals, e.g. harbor seal (*Phoca vitulina*) (Cullon et al. 2012), spinner dolphins (*Stenella longirostris*) (Dirtu et al. 2016), Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (Dirtu et al. 2016), long-finned pilot whales (*Globicephala melas*) (Pinzone et al. 2015), sperm whales (*Physeter macrocephalus*) (Pinzone et al. 2015), and fin whales (*Balaenoptera physalus*) (Pinzone et al. 2015), but finless porpoises were not included.

In present paper, the diet/feeding habits of narrow-ridged finless porpoises in the East China Sea were studied, with the aim to investigate the short-term feeding habits by morphological and molecular identification on the stomach contents of these porpoises, as well as the long-term feeding habits through the use of stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) analysis.

Materials and Methods

Sample collection

Between 2008 and 2010, a total of 122 dead finless porpoises with stomach contents were collected from the East China Sea including the Nantong, Ningbo, and Pingtan sites of the East China Sea (Fig. 1). Near all porpoises died from fisheries bycatch.

The sample includes individuals of both sexes (68 males, 54 females), with calves (n=19), juveniles (n=56) and adults (n=47) (table 1). Of these porpoises, 119 were narrow-ridged finless porpoises from the areas of Nantong (n=106), Ningbo (n=6), and Pingtan (n=7), and three were Indo-Pacific finless porpoises collected in Pingtan. The Nantong population was chosen for exploring prey variation between maturity categories and sexes, etc. because of the larger sample size (n=106).

Examination of stomach contents and morphologic identification

When porpoise carcasses were collected, the external measurements were taken (Gao and Zhou 1995a). During the necropsy, entire stomachs were excised, and the contents weighed. All the biological prey items were stored at 4°C or frozen at -20°C. Most food in the stomach remained intact or slightly digested, possibly because these porpoises died from fisheries bycatch. We defined “prey item” as the prey sample in the stomach which could be morphologically distinguished as a single individual. Only these prey items were included in the present study, while the relatively small quantity of chyme was excluded.

The intact/undigested and slightly digested prey items were morphologically identified with the aid of local reference collections and published pictorial guides (Song et al. 2006; Ye et al. 2007; Song et al. 2009), and measured (length, width and weight) where possible.

Molecular taxonomic identification

Most previous DNA diet studies have targeted mitochondrial DNA (mtDNA) to increase the likelihood of successful PCR amplification (Dunshea et al. 2009). Mitochondrial 16S has been considered as the most taxonomically informative amplicons (Deagle et al. 2009), and have applied 16S DNA as a marker to identify the prey components in some studies (Deagle et al. 2007; Dunshea et al. 2009). We therefore chose 16S DNA as a marker.

For fish, a fragment of the 3' region of the mitochondrial 16S ribosomal RNA gene was chosen as a PCR target and the primers were (5'-AGACCCTATGGAGCTTTAGAC-3' and (5'-CGCTGTTATCCCTATGGTAACT-3') (Deagle et al. 2005). The squid primers (5'-CGCCGAATCCCGTCGCMAGTAAAMGGCTTC-3' and 5'-CCAAGCAACCCGACTCTCGGATCGAA-3') were used to amplify partial sequence of nuclear 28S ribosomal DNA (c.180 bp product) from each sample (Deagle et al. 2005). The PCR primers for shrimp (5'-CGCCTGTTTAACAAAAACAT-3' and 5'-CCGGTCTGAACTCAGATCATGT-3') were optimized from the initial primers reported in a previous study (Jarman and Wilson 2004) targeting a region of nuclear 16S

ribosomal RNA.

The morphologically unidentifiable prey samples (distinguished as a single individual) were identified using a DNA-based method. All of the tools (scissors, tweezers, scalpel handle) were used after high temperature sterilization. In order to prevent cross contamination between samples, one tool was only used for one sample. When the tools needed to be reused, they were cleaned using pure water and 75% alcohol, then burned with a spirit lamp. After separation, the prey items were washed with double distilled water. Then, a part of prey muscle of the fish and shrimp or the body of squid was cut from the inside for DNA extraction.

Total genomic DNA of the isolated prey sample was extracted with a standard phenol/chloroform procedure followed by an ethanol precipitation (Sambrook and Russell 2001). The 15µL PCRs contained 0.1 µl TaKaRa Taq DNA polymerase(5 U/µl), 1.5 µl 10×PCR Buffer(Mg²⁺ Free), 1.0 µl MgCl₂ (25 mM), 1.0 µl DNTP Mixture (2.5 mM), 0.5 µl DNA template, 0.2 µl each primer and 10.5 µl sterilized distilled water. Thermal cycling conditions were: 94-95 degree centigrade for 3-10min (fish 5 min, squid 10 min, and shrimp 3 min); followed by 35 cycles of 95 degree centigrade for 30-45s; 50-55 degree centigrade for 30-45s; and 72 degree centigrade for 30-60s; with a final extension time of 2-7min (fish 7 min, squid 2 min, and shrimp 7 min) at 72 degree centigrade. The PCR products were electrophorised in a 2% agarose gel and visualized under UV irradiation.

In the present study, the combination of sequence similarity and the NJ tree method were used for species identification of morphologically unidentifiable prey samples,

because they have been successfully conducted for prey identification in many studies (e.g. Deagle et al. 2007; Dunshea et al. 2009; Valentini et al. 2009; Marshall et al. 2010; Ghazali et al. 2016). A sequence was assigned at the species level from the combination of high identify (Dunshea 2009), large score, the lowest E-value (Valentini et al. 2009; Marshall et al. 2010) and the high bootstrap support value on the NJ tree (Ghazali et al. 2016). If two or more taxa could be assigned with the similar identify and score for a given sequence, this sequence was assigned to the higher taxonomic level (genus or family) that included both/these taxa (Valentini et al. 2009). $\geq 98\%$ high identify and ≥ 90 bootstrap support value were used (see examples in Appendix 1).

The taxon was assigned to each sequence by similarity assessment using BLAST (Valentini et al. 2009) and the NJ tree structure (Bhadury et al. 2008). DNA sequences were compared with sequences in GenBank using a BLAST search (Konerding 2004) on the National Center for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov/blast). Closely matched sequences were downloaded from GenBank and sequence data were aligned using MUSCLE (Edgar 2004). A neighbor-joining (NJ) tree was constructed in MEGA 6.0 (Tamura et al. 2013) based on distances calculated using Kimura's two-parameter model (Kimura 1980) to assess the degree of sequence similarity between the sequences. The NJ tree was subsequently validated with bootstrap analysis using 1000 replicates.

Calculation of prey importance

The occurrence and relative importance of the prey items were assessed by using the four different indices below: (1) percentage frequency of occurrence (%FO) (Park et al. 2005; Spitz et al. 2006; Shirakihara et al. 2008), (2) percentage by number (%N) (Park et al. 2005; Spitz et al. 2010), (3) percentage by weight/biomass (%M) (Park et al. 2005), and the index of relative importance (IRI).

Percentage frequency of occurrence (%FO) (Park et al. 2005; Spitz et al. 2006; Shirakihara et al. 2008): $\%FO_i = F_i / N_t \times 100$, where F_i is the number of stomachs where species i was found and N_t is the total number of stomachs. Percentage by number (%N) (Park et al. 2005; Spitz et al. 2010): $\%N_i = n_i / N \times 100$, where n_i is the number of prey i found and N is the total number of prey. The percentage by weight/biomass (%M) (Park et al. 2005) was showed as: $\%M_i = m_i / M \times 100$, where m_i is the mass of prey species i and M the total mass of all prey. Index of relative importance (IRI) of each prey is a combination of the previous three parameters calculated according to Pinkas et al. (1971) and Park et al. (2005) as: $IRI_i = (\%N_i + \%W_i) \times \%FO$.

Influencing factors and statistical analyses

We analyzed dietary variation related to geographical populations, age categories, and body length. The dietary variation refers to these four parameters: change of the prey taxa, number of prey items, prey mass, and prey composition.

The porpoises were classified into three maturity categories, i.e. calves, juveniles and sexually mature. The calves were distinguished as animals with visible foetal folds, or

individuals ≤ 1 year old with a body total length of about 117 cm or less (Gao and Zhou 1993). The juveniles and mature porpoises were also distinguished according to their body length. In this case, 134.5 cm (in our necropsy, a male porpoise with 134.5 cm body length has the 537g of the left testis and epididymis, indicating sexual maturity), and 136 cm (Gao and Zhou 1993) were used as the minimum body length of mature males and females respectively. The correlation between body length and the dietary variation parameters (prey taxa, number of prey items, prey mass, prey length, and prey width) was also explored.

All the tests were performed in SPSS 16.0. The data of prey taxa, number of prey items, and prey mass were all of non-normal distribution (Kolmogorov-Smirnov one-sample test, all $p < 0.05$), and the Mann-Whitney U test was operated for sexual and seasonal variation.

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes analysis

Carbon ratio ($\text{C}^{13}/\text{C}^{12}$) and nitrogen ratio ($\text{N}^{15}/\text{N}^{14}$) were used because they are the most common isotopic for analyzing the relative importance of food resources and trophic position to animals, which is indicative of long-term feeding habits (Kelly 2000; Parnell et al. 2010; Pokrovsky 2012).

2-5 grams of muscle from nine finless porpoises (three adult males, adult females, and young males respectively) and 22 prey items (eleven osteichthyes, $n=29$; four cephalopods, $n=9$; six Crustaceans, $n=12$; one gastropods, $n=2$) in Nantong were prepared for detection of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$).

All samples were rinsed in distilled water and dried in an oven for 24 h at 65 degree

centigrade, then pulverized into a fine powder. The homogenized samples of 2.0 mg were loaded into capsules, after which $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were determined using isotope ratio mass spectrometry (INTEGRA-CN, Sercon Ltd., Cheshire, UK). Results are reported as the isotope ratios in delta notation relative to Pee Dee Belemnite (^{13}C) or atmospheric nitrogen (^{15}N) as follows (Cui et al. 2011; Pokrovsky 2012; Cheng et al. 2013):

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3$$

where, X is ^{13}C or ^{15}N , and R is the corresponding ratio, $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Replicate measurements of the internal laboratory standard (albumen) indicated measurement errors of $\pm 0.1\text{‰}$ and $\pm 0.4\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determinations respectively.

Here, the stable isotopes of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were used to analyze resource use and trophic position, indicating long-term feeding habits. Stable Isotope Analysis in R (SIAR) to estimate the distribution of isotopes amongst all species and proportional contributions of potential prey species in the diet of porpoises was used (Parnell et al. 2010; Pokrovsky 2012). The program was run with the number of interactions, 200,000, using 95% Bayesian confidence intervals for each prey type for these data (Gillespie et al. 2013).

Results

A total of 1671 individual prey items in stomachs were found, with an average of 16.1 ± 92.7 (range 1-218). In general, the number of prey items of nearly 4/5 (80.1%) porpoises were less than 20. All individuals had no more than six identified species in their stomachs. The prey mass of 63.9% of the porpoises weighed no more than 80 g.

A total of 538 tissue samples from prey items were identified into family, genus, or

species level by using morphological methods and molecular methods. Twenty-two families of teleosts, crustaceans, cephalopods, and gastropods (Table 2, Table 3) were identified. A minimum of 33 different species were identified with 19 determined to be teleosts, 7 crustaceans, 5 cephalopods, and 2 gastropods, respectively.

Prey composition and importance

Teleosts occurred in 81 (76.4%) of 106 stomachs examined in Nantong and represented the primary prey items, having the highest number out of all prey items (54.2%), highest prey mass (68.1%), and the highest IRI value of 9,778.4 (67.8% of total IRI) (Fig. 2, Table 2). Crustaceans (IRI=2,354, 16.3%) and cephalopods (IRI=2,292.2, 15.9%) were secondary prey. Somewhat surprisingly, gastropods were found in the stomachs of finless porpoises for the first time. Gastropods accounted for a very low fraction of the diet by number, mass and frequency of occurrence, only having an IRI of 2 (0.01%).

The three most important families were Sciaenidae (IRI=473.3), Engraulidae (IRI=444.3), and Palaemonidae (IRI=478.8), which were represented in greater amounts than all the other families (all IRI= $\leq$ 150). At the species level, the five top prey species in order of importance were oriental shrimp (*Palaemon gravieri*) (IRI=478.8), hairfin anchovy (*Setipinna taty*) (IRI=270.4), dotted gizzard shad (*Konosirus punctatus*) (IRI=90.7), spotted maigre (*Larimichthys polyactis*) (IRI=59.3), and whiparm octopi (*Octopus variabilis*) (IRI=57.9).

Variation in diet composition of calves, juveniles and adults

It was suggested that crustaceans, teleosts, and cephalopods played similar roles in the diet of calves (Fig. 3). In comparison, fish was the dominant prey of juveniles and sexually mature individuals (Fig. 3).

For calves, whitespotted conger (*Conger myriaster*) (18.5% by weight) and Chinese ditch prawn (*Palaemon gravieri*) (15.6% by weight) represented the predominant prey items; for juveniles, hairfin anchovy (15.2% by weight), small yellow croaker (4.2% by weight), and whiparm octopi (3.6%) represented the predominant prey items; for mature porpoises, dotted gizzard shad (13.3% by weight), hairfin anchovy (9.2 % by weight), cresthead cutlassfish (*Eupleurogrammus muticus*) (9.9%) and small yellow croaker (7.2%) showed the highest importance.

Among the three age categories, no significant difference was found in mean prey taxa, number of prey items, or prey mass of porpoise individuals (Kruskal Wallis test, all $p > 0.05$) (Fig. 3), nor for fish length and width, shrimp length and width (t-test, all $p > 0.05$), fish mass, and shrimp mass (Kruskal Wallis test, both $p > 0.05$).

The correlation analyses showed the body length was not significantly correlated with number of prey items (correlation index=-0.06), prey mass (correlation index=0.111), fish weight (correlation index=0.05), fish length (correlation index=0.223), and fish width (correlation index=0.013).

Sexual variation in diet of porpoises

For both male and female porpoises, teleosts were the dominant prey based on all indices (Fig. 4). While crustaceans and cephalopods played secondary roles in the diet,

gastropods had the lowest indices.

For male, hairfin anchovy (15.8% by weight), dotted gizzard shad (8.9% by weight), spotted maigre (7.7% by weight) represented the predominant prey items. While, for female, spotted maigre (3.8% by weight) and whiparm octopi (3.2% by weight) were the predominant prey items.

Mann-Whitney U test results indicated significant differences in the mean prey weight of diet between the sexes (males 92g, females 75.8g) ($p < 0.05$), but a lack of difference in mean number of prey items or prey taxa (Mann-Whitney U test, all $p > 0.05$). Females preyed more on fish by weight (73.6g) than males (70.9g) (Mann-Whitney U, $p < 0.05$), and males preyed more on cephalopods (29.8g) than females (16g) (Mann-Whitney U, $p < 0.05$). Comparisons between the sexes were not statistically significant for fish length and width, or shrimp length and width (T-test, all $p > 0.05$).

Long term feeding habits indicated from stable isotopes analysis

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were highly variable. The gastropods had lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, the adult female finless porpoises and young male finless porpoises had top value of $\delta^{15}\text{N}$ (-17.663 and -17.657‰). The plot in Fig. 5 indicates the significant trophic position of different level of consumers. Particularly, there are separated trophic ecological positions between adult females and adult males, or between adult females and young males.

Overall, the SIAR analysis showed that teleosts were the primary prey items (proportional contribution 52.64%), followed by crustaceans (25.04%), cephalopods

(19.83%), and gastropods (0.03%). At the species level, these prey contributed from 2.9% to 6.54% of food resources of finless porpoises. The cresthead cutlassfish (6.5%), whiparm octopi (6.1%), Chinese ditch prawn (5.8%), and yellow drum (*Nibea albiflora*) (5.0%) were the most important prey species.

With respect to sexual and age-related variation, there were no significant differences among the mean prey contributions of adult males, young males and adult females (T-test, all $p > 0.05$, Fig. 6). However, females had a slightly wider species contribution range (3.4%-5.7%) than adult males (3.8%-5.2%) or young males (3.8%-5.2%) (Fig. 6).

Prey of porpoises in Ningbo and Pingtan

In Ningbo, teleosts were the dominant prey, and only Chinese ditch prawn was identified (Table 3). In Pingtan, teleosts made up 45.5 %N and 57.1 %FO of the diet of narrow-ridged finless porpoises. Cephalopods and crustaceans were present at low levels. For Indo-Pacific finless porpoises in Pingtan, teleosts were the primary prey, and only mitre squid *Loligo chinensis* was identified.

Discussion

General findings

For short term feeding habit analysis, fish, crustaceans, and cephalopods were identified as important and secondary prey items. These findings are generally consistent with the previous reports from Hong Kong (Barros et al. 2002), Japan (Shirakihara et al. 2008),

Korea (Park et al. 2005), Xiamen (Huang et al. 2000), and the Beibu Gulf (Lu and Fang 2006). In particular, crabs were also found in the diet of porpoises, which only represents the second report, following Park *et al.* (2005). Surprisingly, gastropods were also found in the stomach of finless porpoises, which included two species, the bladder moon snail (*Neverita didyma*) and an unidentified species of Turritellidae. Because of the small number of bladder moon snail (n=1), porpoises probably rarely feed on it. The Turritellidae in the stomachs were very small (< 6 mm), suggesting that Turritellidae were probably swallowed incidentally with other prey items.

The present research is the first study to explore the long term feeding habits of finless porpoises, its result is similar with short term feeding habits from stomach content analysis, i.e. teleosts represented the primary prey, cephalopods and crustaceans were secondary prey, and gastropods were only occasional prey. However, the importance index/contribution of species is different between short- and long-term feeding habits (table 2). It was well understood that the prey species varied in different short-term scale (e.g. different week, or month), but in long-term scale (e.g. half and whole year), the prey composition is stable.

General profile of feeding habits and forging ecology

Porpoises that inhabit Hong Kong and the Beibu Gulf represent the Indo-Pacific finless porpoise (wide-ridged species), whereas porpoises that inhabit Nantong, Korea and Japan were the narrow-ridged species (see Jefferson and Wang, 2011). For Indo-Pacific finless

porpoises, the teleosts and cephalopods showed similar higher frequency of occurrence than crustaceans (Table 4). Fish represented dominant prey species of narrow-ridged finless porpoises, higher than cephalopods, crustaceans and gastropods. From the nutritional perspective, the fat content and energy content of fishes in the East China Sea, such as bighead croaker (*Collichthys niveatus*) and small yellow croaker, were higher than those for shrimp or cuttlefish, such as Chinese ditch prawn or juvenile squid (*Loligo japonica*) (Zhang et al. 2008). Therefore, it appears that finless porpoises primarily preyed on fish to get more nutrition.

Adult porpoises preyed on fish predominantly, which is consistent with studies from Hong Kong (Barros et al. 2002), Japan (Shirakihara et al. 2008), and Korea (Park et al. 2005). The diet of juveniles varied largely, e.g. fishes in East China Sea, cephalopods in Japan (Shirakihara et al. 2008), fishes and crustaceans in Korea (Park et al. 2005). Similarly, calves preyed on different items in different sites, i.e. crustaceans, teleosts and cephalopods equally in East China Sea, cephalopods in Japan (Shirakihara et al. 2008), crustaceans in Korea (Park et al. 2005), and fishes and cephalopods in Hong Kong (Barros et al. 2002).

All this variation of diet by age, sex, and geographic region probably was the result of three things. First, stomach contents represent the last food before death (probably several days), which might produce individual variation in short term feeding habits.

Second, the diet of finless porpoises depends somewhat on local food availability, which has geographical variation. The common prey species was consistent with those

primarily caught by trawlers, such as hairfin anchovy, small yellow croaker and Japanese anchovy (Liu et al. 2007). That indicated that Nantong finless porpoises might follow fishing boats like porpoises in Hong Kong (Barros et al. 2002) to target the same high-value fish for maximizing foraging benefits. This behavior was consistent with optimal foraging theory, which states that top marine predators would maximize the ratio between foraging benefits and costs (MacArthur and Pianka 1996). At the same time, association with fishing boats increases the entanglement risk. In 122 finless porpoises, most were collected from the bycatch of fishing boats, although with a few of the sampled animals, the cause of death could not be determined exactly.

Finally, feeding preference does exist. For example, dotted gizzard shad was an important prey of finless porpoises (35.6% IRI), but it was not the dominant species caught by fisheries (low availability) (Liu et al. 2007). On the contrary, although the biomass of Thichiuridae was the highest of the total fisheries catch (%W=26.8) (high availability) in the East China Sea (Liu et al. 2007), it was seldom preyed upon by finless porpoises (%FO=1.7). This is similar to what occurs in the Beibu Gulf (Lu and Fang 2006) and Japan (Shirakihara et al. 2008), which suggests the existence of significant prey preferences by finless porpoises in different waters.

Most of the porpoises included in this study died due to fisheries bycatch, therefore, the present results, might at least in part, represent the diets of porpoises correlated with fisheries. Key information about some of the porpoises, such as were they found stranded,

floating or through bycatch, was not recorded in the original records. Therefore, dietary differences among bycatch, floating and stranded porpoises could not be compared.

Population ecological parameters such as abundance, distribution of finless porpoises in the East China Sea are still unclear due to the deficiency of a dedicated survey. But, the finless porpoise is the most common cetacean in fisheries bycatch in China, and its high fisheries bycatch rate should draw attention to this issue. In 1994, the annual incidental catches of finless porpoises in coastal waters of Zhejiang, Fujian, Guangdong, Hainan and Guangxi were estimated at about 2132 ± 1484 , predominantly in trawl nets (56.8%) and gill nets (26.4%) (Yang et al. 1999). Between 2004 and 2008, 170 porpoises were incidentally caught in Bohai and north Yellow sea (Liu 2006). Here, It is at this point that a dedicated survey on population status should be carried out, especially concerning the correlation between finless porpoise and fishing should be evaluated as soon as possible, to address key issues related to fisheries bycatch.

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References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403-410.
- Amir, O.A., Berggren, P., Ndaró, S.G.M., and Jiddawi, N.S. 2005. Feeding ecology of the indo-pacific bottlenose dolphin (*Tursiops aduncus*) incidentally caught in the gillnet fisheries off zanzibar, Tanzania. *Estuar. Coast. Shelf. S.* **63(3)**: 429-437.
- Barros, N.B., Jefferson, T.A., and Parsons, E.C.M. 2002. Food habits of finless porpoises (*Neophocaena phocaenoides*) in Hong Kong waters. *Raffles Bull. Zool.* **10**: 115-123.
- Bhadury, P., Austen, M.C., Bilton, D.T., Lambshead, P.J.D., Rogers, A.D., and Smerdon, G.R. 2008. Evaluation of combined morphological and molecular techniques for marine nematode (*Terschellingia* spp.) identification. *Mar. Biol.* **154**: 509-518.
- Borrell, A., Tornero, V., Bhattacharjee, D., and Aguilar, A. 2016. Trace element accumulation and trophic relationships in aquatic organisms of the Sundarbans mangrove ecosystem (Bangladesh). *Sci. Total. Environ.* **545-546**: 414-423.

- Bowen, W. D., and Llverson, S. J. 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. *Mar. Mam. Sci.* **29(4)**: 719-754.
- Chen, P., Liu, P., Liu, R., Lin, K., and Pilleri, G. 1979. Distribution, ecology, behaviour and conservation of the dolphins of the middle reaches of Changjiang (Yangtze) River (Wuhan-Yueyang). *Investigations on Cetacea* **10**: 87-103.
- Cheng, Z., Man, Y.B., Nie, X.P., and Wong, M.H. 2013. Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. *Chemosphere* **90**: 2142-2148.
- Cui, B., Zhang, Q., Zhang, K., Liu, X., and Zhang, H. 2011. Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta, China. *Environ. Pollut.* **159**: 1297-1306.
- Cullon, D.L., Yunker, M.B., Christensen, J.R., Macdonald, R.W., Whitticar, M.J., Dangerfield, N.J., and Ross, P.S. 2012. Biomagnification of polychlorinated biphenyls in a harbor seal (*Phoca vitulina*) food web from the strait of Georgia, British Columbia, Canada. *Environ. Toxicol. Chem.* **31(11)**: 2445-2455.
- Deagle, B.E. 2006. DNA-based Methods for Studying the Diet of Marine Predators. thesis, Zoology Department, University of Tasmania.
- Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., and Hindell, M.A. 2007. Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *Plos One* **2(9)**: e831.

- Deagle, B.E., Jarman, S.N., Pemberton, D., and Gales, N.J. 2005. Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). *J. Hered.* **96**: 417-423.
- Deagle, B.E., Kirkwood, R., and Jarman, S.N. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Mol. Ecol.* **18(9)**: 2022-38.
- Dirtu, A.C., Malarvannan, G., Das, K., Dulau-Drouot, V., Kiszka, J.J., Lepoint, G., Mongin, P., and Covaci, A. 2016. Contrasted accumulation patterns of persistent organic pollutants and mercury in sympatric tropical dolphins from the south-western Indian Ocean. *Environ. Res.* **146**: 263-273.
- Dunshea, G. 2009. DNA-based diet analysis for any predator. *PLoS One* **4**: e5252.
- Dunshea, G., Barros, N.B., Berens McCabe, E.J., Gales, N.J., Hindell, M.A., Jarman, S.N., and Wells, R.S. 2013. Stranded dolphin stomach contents represent the free-ranging population's diet. *Biol. Lett.* **9(3)**: 20121036-20121036.
- Edgar, R.C. 2004. Muscle: multiple sequence alignment with high accuracy and high throughput. *Nucleic. Acids. Res.* **32(5)**: 1792-1797.
- Gao, A., and Zhou, K. 1993. Growth and reproduction of three populations of finless porpoise, *Neophocaena phocaenoides*, in Chinese waters. *Aquat. Mamm.* **19**: 3-12.
- Gao, A., and Zhou, K. 1995a. Geographical variation of external measurements and three subspecies of *Neophocaena phocaenoides* in Chinese waters. *Acta Theriol. Sinica.* **15**: 81-92. (in Chinese)

- Gao, A., and Zhou, K. 1995b. Geographical variation of skull among the populations of *Neophocaena* in Chinese waters. *Acta Theriol. Sin.* **15**: 161-169. (in Chinese)
- Ghazali, S.N., Ahmad, A., Quraishia, S.F., Panneerchelvam, S., and Rashid, N.H.A. 2016. Molecular characterization of ornamental fish (Poeciliidae) using mitochondrial DNA 12s rRNA and 16s rRNA genes. *Annals of Biological Research* **7(5)**: 5-11.
- Gillespie, J.H. 2013. Application of stable isotope analysis to study temporal changes in foraging ecology in a highly endangered amphibian. *PLoS One* **8**: e53041.
- Hobson, K.A., and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* **94**: 181-188.
- Huang, Z., Liu, W., Zheng, C., Li, C., Wang, J., and Jefferson, T.A. 2000. Finless porpoises in the southern coastal waters of Fujian, China. *Acta Oceanol. Sin.* (Chin. Ed.) **22**: 114-119. (in Chinese)
- Jarman, S.N., and Wilson, S.G. 2004. DNA-based species identification of krill consumed by whale sharks. *J. Fish. Biol.* **65**: 586-591.
- Jarman, S.N., Deagle, B.E., and Gales, N.J. 2004. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. *Mol. Ecol.* **13(5)**: 1313-1322.
- Jarman, S.N., Gales, N.J., Tierney, M., Gill, P.C., and Elliott, N.G. 2002. A DNA-based method for identification of krill species and its applications to analyzing the diet of marine vertebrate predators. *Mol. Ecol.* **11(12)**: 2679-2690.
- Jefferson, T.A., and Wang, J.Y. 2011. Revision of the taxonomy of finless porpoises

- (genus *Neophocaena*): The existence of two species. J. Mar. Anim. Ecol. **4**, 3-16.
- Kasuya, T. 1999. Finless porpoise *Neophocaena phocaenoides* (G. Guvier, 1829). In Handbook of marine mammals: The second book of dolphins and the porpoises. Edited by S.H. Ridgway and R. Harrison. Academic Press, London, pp. 411-442.
- Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. **78**: 1-27.
- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111-120.
- King, R.A., Read, D.S., Traugott, M., and Symondson, W.O.C. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. **17**: 947-963.
- Konerding, D. 2004. An essential guide to the basic local alignment search tool: blast. Brief. Bioinform. **5**(1): 93-94.
- Liu, Y. 2006. The analyses of genetic diversity MHC-DQB exon 2 of finless porpoises in Bohai and north Yellow Sea. Shandong university master dissertation, 1-57p.
- Liu, Y., Li, S.F., Chen, X.G., and Cheng, J.H. 2007. The structure and diversity of demersal fish communities in winter 2000 in the East China Sea and the Yellow Sea. Mar. Sci. **31**:19-24. (in Chinese)
- Lu, H., and Fang, Z.Q. 2006. Finless porpoises in the coastal waters of Beibu Gulf, South China. Journal of South China Normal University (Natural science edition) **3**: 109-115. (in Chinese)

MacArthur, R.H., and Pianka, E.R. 1966. On the optimal use of a patchy environment.

Am. Nat. **100**: 603-609.

Marshall, H.D., Hart, K.A., Yaskowiak, E.S., Stenson, G.B., Mckinnon, D., and Perry,

E.A. 2010. Molecular identification of prey in the stomach contents of Harp Seals

(*Pagophilus groenlandicus*) using species-specific oligonucleotides. Mol. Ecol.

Resour. **10**: 181-189.

Méheust, E., Alfonsi, E., Ménec, P.L., Hassani, S., and Jung, J.L. 2015. DNA barcoding

for the identification of soft remains of prey in the stomach contents of grey seals

(*Halichoerus grypus*) and harbour porpoises (*Phocoena phocoena*). Mar. Biol.

Res. **11**(4): 385-395.

Park, K.J., Zhang, C.I., Sohn, H., and Kim, Z.G. 2005. Feeding habits of finless porpoise

(*Neophocaena phocaenoides*) in the west coast of Korea. IWC, Impington,

Cambridge, United Kingdom. SC/57/SM17 (unpublished).

Parnell, A.C., Inger, R., Bearhop, S., and Jackson, A.L. 2010. Source Partitioning Using

Stable Isotopes: Coping with Too Much Variation. PLoS One **5**: e 9672.

Pinkas, L., Oliphant, M.S., and Iverson, I.L.K. 1971. Food habits of albacore, bluefin

tuna, and bonito in California waters. Fish. Bull. **152**: 1-105.

Pinzone, M., Budzinski, H., Tasciotti, A., Ody, D., Lepoint, G., Schnitzler, J., Scholl, G.,

Thomé, J., Tapie, N., Eppe, G., and Das, K. 2015. POPs in free-ranging pilot

whales, sperm whales and fin whales from the Mediterranean Sea: Influence of

biological and ecological factors. Environ. Res. **142**: 185-196.

- Pokrovsky, I.G. 2012. A method of stable carbon and nitrogen isotope analysis in assessment of the diet of birds of prey. *Biol. Bull.* **39**: 590-592.
- Praca, E., Laran, S., Lepoint, G., Thomé, J., Quetglas, A., Belcari, P., Sartor, P., Dhermain, F., Ody, D., Tapie, N., Budzinski, H., and Das, K. 2011. Toothed whales in the northwestern Mediterranean: Insight into their feeding ecology using chemical tracers. *Mar. Pollut. Bull.* **62**: 1058-1065.
- Rice, D.W. 1998. Marine mammals of the world: systematics and distribution. Society for Marine Mammalogy Special Publication 4, 1-231.
- Sambrook, J., and Russell, W.D. 2001. Molecular Cloning---A Laboratory Manual. CSHL Press, New York.
- Shirakihara, M., Seki, K., Takemura, A., Shirakihara, K., Yoshida, H., and Yamazaki, T. 2008. Food habits of finless porpoises *Neophocaena phocaenoides* in western Kyushu, Japan. *J. Mammal.* **89**: 1248-1256.
- Song, H.T., Ding, T.M., and Xu, K.D. 2009. The resources of economic cephalopods in East China Sea. The Ocean Press, Beijing. (in Chinese)
- Song, H.T., Yu, C.G., Xue, L.J., and Yao, G.Z. 2006. The economic shrimp and crabs in East China Sea. The Ocean Press, Beijing. (in Chinese)
- Spitz, J., Mourocq, E., Leauté, J.P., Quéro, and Ridoux, V. 2010. Prey selection by the common dolphin: Fulfilling high energy requirements with high quality food. *J. Exp. Mar. Biol. Ecol.* **390**: 73-77.
- Spitz, J., Rousseau, Y., and Ridoux, V. 2006. Diet overlap between harbour porpoise and

- bottlenose dolphin: an argument in favour of interference competition for food? Estuar. Coast. Shelf. S. **70**: 259-270.
- Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. **11**: 627–641.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. **30**: 2725-2729.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids. Res. **25**: 4876-4882.
- Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for ^{13}C analysis of diet. Oecologia **57**: 32-37.
- Valentini, A., Miquel, C., Nawaz, M., Bellemain, E., Crissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J., and Taberlet, P. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Mol. Ecol. Resour. **9**: 51-60.
- Wang, J.Y., Frasier, T.R., Yang, S.C., and White, B.N. 2008. Detecting recent speciation events: the case of the finless porpoise (genus *Neophocaena*). Heredity **101**: 145-155.
- Yang, G., Zhou, K., Xu, X., and Leatherwood, S. 1999. A survey on the incidental

- catches of small cetaceans in coastal waters of China. Chinese Journal of Applied Ecology 10(6): 713-716. (in Chinese)
- Ye, Z.J., Zhu, B.J., and Xue, Y. 2007. Photographic atlas of fish otoliths of the China Sea. Ocean University Press, Qingdao (in Chinese)
- Zhang, B., Zhang, M., Dai, F.Q., and Jin, X.S. 2008. The biochemical compositions and energy content of 16 important resource species in the central and southern Yellow Sea. Mar. Fish. Res. **29**: 11-18.
- Zhang, C.I., Park, K.J., Kim, Z.G., and Sohn, H. 2004. Distribution and abundance of finless porpoise (*Neophocaena phocaenoides*) in the west coast of Korea. J. Kor. Fish. Soc. **37**: 129-136. (in Korean)
- Zhou, K. 2004. Fauna Sinica, Mammalia (Vol.9): Cetacea, Carnivora: Phocoidea, Sirenia. Science Press, Beijing.

Table 1 specimen information of finless porpoises collected between 2008 and 2010 from the East China Sea, China.

	NFP (<i>N. asiaeorientalis</i>)			IFP (<i>N. phocaenoides</i>)	Subtotal
	Nantong	Ningbo	Pingtang	Pingtang	
Male	61	3	3	1	68
Female	45	3	4	2	54
Calf	12	4	3	0	19
Juvenile	49	1	3	3	56
Adult	45	1	1	0	47
Total number	106	6	7	3	122

Note: NFP, narrow-ridged finless porpoises; IFP, Indo-Pacific finless porpoises

Table 2 The prey composition, importance indexes (short term) and species contribution (long term) of finless porpoises collected between 2008 and 2010 in Nantong, East China Sea. %N, the number of prey items; %M, prey mass; %FO, frequency of occurrence; IRI/100, index of relative importance.

Prey		Short term				Long term
		Index of prey importance				Contribution
Class/Family	Genus/Species identified	% M*	% N	% FO	IRI/%	of prey (%)
Crustacea						
Alpheidae	<i>Alpheus digitalis</i>	0.0	0.4	0.9	0.4	3.18
	<i>Alpheus japonicus</i>	0.0	0.2	0.9	0.2	-
Palaemonidae	<i>Palaemon gravieri</i>	4.0	22.7	17.9	478.8	5.80
Penaeidae	<i>Penaeus monodon</i>	0.0	0.2	0.9	0.2	3.95
Squillidae	<i>Oratosquilla oratoria</i>	0.2	0.2	0.9	0.3	4.05
Portunidae	<i>Charybdis variegata</i>	0.1	0.2	0.9	0.3	5.27
	<i>Charybdis bimaculata</i>	0.1	0.2	0.9	0.3	-
Order Subtotal**		8.9	31.4	58.5	2354/16.32%	25.03
Osteichthyes						
Congridae	<i>Conger myriaster</i>	4.1	2.1	5.7	34.8	4.64
Syngnathidae	<i>Syngnathus acus</i>	0.7	0.9	3.8	6.0	4.81
Thichiuridae	<i>Eupleurogrammus muticus</i>	8.9	11.5	1.9	38.5	6.54
Engraulidae	<i>Engraulis japonicus</i>	1.7	2.0	0.9	3.6	-
	<i>Setipinna taty</i>	16.0	12.6	9.4	270.4	4.48
	<i>Coilia mystus</i>	2.8	0.4	1.9	5.9	4.55
	<i>Thryssa kammalensis</i>	0.3	0.4	1.9	1.2	
Sciaenidae	<i>Larimichthys polyactis</i>	9.4	11.5	2.8	59.3	4.57
	<i>Nibea albiflora</i>	3.0	0.2	0.9	3.0	5.03

	<i>Collichthys lucidus</i>	0.3	0.7	1.9	2.0	3.90
Taenioididae	<i>Odontamblyopus rubicundus</i>	0.4	1.1	2.8	4.3	5.0
Gobiidae	Unidentified species	4.6	7.2	1.9	22.4	
Clupeidae	<i>Ilisha elongata</i>	3.2	3.2	0.9	6.0	4.56
	<i>Konosirus punctatus</i>	9.1	4.6	6.6	90.7	-
Scombrida	<i>Scomber sp. Or spp.</i>	2.1	0.6	0.9	2.5	4.56
Triglidae	Unidentified species	0.4	0.2	0.9	0.6	-
	Order Subtotal*	68.1	59.8	76.4	9778.4/67.8%	52.64
Cephalopoda						
Octopodidae	<i>Octopus ocellatus</i>	-	-	-		4.27
	<i>Octopus variabilis</i>	4.9	2.8	7.5	57.9	6.10
Loliginidae	<i>Loligo japonica</i>	1.3	2.0	4.7	16.0	-
	<i>Loligo chinensis</i>	5.3	0.4	0.9	5.4	4.49
Ommastrephidae	<i>Todarodes pacificus</i>	0.3	0.2	0.9	0.5	4.53
	Order Subtotal*	22.9	18.3	55.7	2292.2/15.9%	19.39
Gastropoda						
Naticidae	<i>Neverita didyma</i>	0.1	0.4	0.9	0.5	2.94
Turritellidae	Unidentified species	-	2.8	0.9	2.6	
	Order Subtotal*	0.1	1.0	1.9	2.0 /0.01%	2.94

*The M% was calculated by weighing prey items in different states of digestion. This result cannot be used in terms of food weight. Although the weight of the prey is not exact, it is still valuable information.

** In order to minimize the bias, the order subtotal mass and number included the data of both identified species and unidentified prey (determined order). As a result, the cumulative M% or N% is not equal to the value of the order subtotal. IRI/%: percentage of order divided by total IRI

value (14,426.6).

Table 3 The diets of finless porpoises collected in Ningbo and Pingtan between 2008 and 2010. %N: the number of prey items; %FO: frequency of occurrence.

Research site	Class	Family	species	%N	%FO
Ningbo (n=6)	Crustacea	Palaemonidae	<i>Palaemon gravieri</i>	13.3	33.3
	Osteichthyes	Sciaenidae		80	50
		Unid. eel		6.7	16.7
Pingtan (n=7)	Osteichthyes	Taenioididae	<i>Odontamblyopus</i>	4.5	14.3
			<i>rubicundus</i>		
		Synodontidae	<i>Harpadon nehereus</i>	13.6	14.3
		Clupeidae	<i>Sardinella sp.</i>	14.6	14.3
			<i>Ilisha elongata</i>	4.5	14.3
		Engraulidae	<i>Johnius sp.</i>	4.5	14.3
		Ophichthidae		4.5	14.3
		Sub-total		45.5	57.1
	Cephalopoda	Loliginidae	<i>Loligo chinensis</i>	18.2	28.6
		Unid. squid		9.1	14.3
		Sub-total		27.3	42.9
	Crustacea	Unid. shrimp		27.3	28.6
Pingtan(n=3)	Cephalopoda	Loliginidae	<i>Loligo chinensis</i>	40	66.7
(N. phocaenoides)	Osteichthyes	Unid. fish		40	66.7
		Unid. eel		20	33.3

Table 4 The proportion of the total number of prey (%N) and frequency of occurrence (%FO) of prey taxa of finless porpoises in different waters.

Research area	Species	Osteichthyes %N, %FO	Crustacea %N, %FO	Cephalopoda %N, %FO	Gastropoda %N, %FO	Reference
Korea*	<i>N. asiaeorientalis</i>	28.2, >55.3	63, >42.1	8.8, >36.8	0, 0	(Park et al. 2005)
Ariake Sound, Tachibana Bay, Japan	<i>N. asiaeorientalis</i>	31.2, 88.1	2.8, 28.8	66, 91.5	0, 0	(Shirakihara et al. 2008)
Omura Bay, Japan	<i>N. asiaeorientalis</i>	90.6, 88.9	5.1, 33.3	4.4, 33.3	0, 0	(Shirakihara et al. 2008)
Nantong, China	<i>N. asiaeorientalis</i>	55.0, 70.4	31.4, 53.9	18.3, 51.3	1, 1.7	This paper
Ningbo, China	<i>N. asiaeorientalis</i>	86.7, 60	13.3, 33.3	0, 0	0, 0	This paper
Pingtang, China	<i>N. asiaeorientalis</i>	45.5, 57.1	27.3, 28.6	27.3, 42.9		This paper
Pingtang, China	<i>N. phocaenoides</i>	60, 100	0, 0	40, 66.7	0, 0	This paper
Xiamen	<i>N. phocaenoides</i>	-, 88.9	-, 11.1	-, 66.7	0, 0	(Huang et al. 2000)
Hong Kong, China	<i>N. phocaenoides</i>	-, 88.2	-, 17.6	-, 87.1	0, 0	(Barros et al. 2002)
Beibu Gulf, China	<i>N. phocaenoides</i>	dominant	few	moderate	0, 0	(Lu and Fang 2006)

*: The value of N% and FO% was calculated according to the data of Park et al. (2005), fish was the dominant prey.

Figure captions

Figure 1 Distribution of finless porpoise specimens used in the present study and previous references. Nantong, Ningbo and Pingtan had not been examined before as finless porpoise sampling sites. The research sites information was obtained from published references, i.e. Korea (Park et al. 2005), Japan (Shirakihara et al. 2008), Yangtze (Chen et al. 1979), Xiamen (Huang et al. 2000), Hong Kong (Barros et al. 2002), Beibu Gulf (Lu and Fang 2006).

Figure 2 The importance indices of prey in the stomach of narrow-ridged finless porpoises in Nantong, East China Sea. %N, the number of prey items; %M, prey mass; %FO, frequency of occurrence; %IRI: percentage of order divided by total IRI value.

Figure 3 The importance indices of prey in the stomach of different maturity categories of narrow-ridged finless porpoises in the East China Sea. %N, the number of prey items; %M, prey mass; %FO, frequency of occurrence; %IRI: percentage of order divided by total IRI value.

Figure 4 The importance indices of prey in the stomach of male and female narrow-ridged finless porpoises in the East China Sea. %N, the number of prey items; %M, prey mass; %FO, frequency of occurrence; %IRI: percentage of order divided by total IRI value.

Figure 5 Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) bi-plots of prey species of narrow-ridged finless porpoises in the East China Sea. AF-FP, adult female finless porpoises (n=3); AM-FP, adult male finless porpoises (n=3); YM-FP, young male finless porpoises (n=3); 11 osteichthyes, n=29; four cephalopods, n=9; six Crustaceans, n=12; one gastropod, n=2.

Figure 6 The contribution (%) of prey groups to long term diets of adult female, adult male and young male finless porpoises in the East China Sea (based on the stable isotope ratio of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses).

Appendix 1

We reanalyzed molecular data. In the present study, a combination of the sequence similarity and the NJ tree method were used for prey identification, that have been successfully conducted for prey identification in many studies (e.g. Deagle et al. 2007; Dunshea et al. 2009; Valentini et al. 2009; Marshall et al. 2010; Ghazali et al. 2016). Please see details in Method of manuscript.

The taxon was assigned to each sequence by similarity assessment using blast and the tree structure. DNA sequences were compared with sequences in GenBank using a BLAST search on the National Center for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov/blast). Closely matched sequences were downloaded from GenBank. Sequence data was aligned by MUSCLE. A neighbor-joining (NJ) tree was constructed in MEGA 6.0 based on distances calculated using Kimura's two-parameter model. The NJ tree was subsequently validated with bootstrap analysis using 1000 replicates.

A sequence was assigned as species level from the combination of high identify (98% in present paper), large score, low E-value and large bootstrap support values (90% in present paper) . Please see some examples of species, genus and family identification were provided below.

Species Identification Example 1

A sequence was assigned as *Eupleurogrammus muticus* from the combination of high similarity (100%) to *Eupleurogrammus muticus* sequences and high bootstrap support value (100%). This result was supported by morphological identification.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Eupleurogrammus muticus 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	329	329	97%	1.00E-86	100%	AY212325.1
Achiroides leucorhynchus mitochondrial 16S rRNA gene, clone 83	87.9	87.9	84%	7.00E-14	77%	AM182051.1
Achiroides leucorhynchus mitochondrial 16S rRNA gene, clone 82	87.9	87.9	84%	7.00E-14	77%	AM182050.1
Serranus baldwini voucher KU246 16S ribosomal RNA gene, partial sequence; mitochondrial	78.7	78.7	26%	4.00E-11	96%	HQ731419.1
Oplegnathus insignis 16S ribosomal RNA gene, partial sequence; mitochondrial	78.7	78.7	26%	4.00E-11	96%	HM211188.1
Serranus baldwini 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	78.7	78.7	26%	4.00E-11	96%	AY072681.1
Xystreurus rasile voucher DAAPV F13 16S ribosomal RNA gene, partial sequence; mitochondrial	76.8	76.8	29%	1.00E-10	93%	GU324145.1
Xystreurus liolepis voucher KU465 16S ribosomal RNA gene, partial sequence; mitochondrial	63.9	63.9	25%	1.00E-06	91%	JQ939073.1
Xystreurus liolepis 16S ribosomal RNA gene, partial sequence; mitochondrial	63.9	63.9	25%	1.00E-06	91%	AY952500.2
Xystreurus liolepis 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	63.9	63.9	25%	1.00E-06	91%	AF488454.1
Chascanopsetta lugubris voucher FMNH119729 16S ribosomal RNA gene, partial sequence; mitochondrial	52.8	52.8	15%	0.002	100%	JQ939121.1
Kamoharaia megastoma mitochondrial 16S rRNA gene, clone 193	52.8	52.8	15%	0.002	100%	AM181777.1
Kamoharaia megastoma mitochondrial 16S rRNA gene, clone 192	52.8	52.8	15%	0.002	100%	AM181776.1

Figure A1 An example of a sequence assigned to species of *Eupleurogrammus muticus* with 100% similarity and 100% bootstrap support value.

Species Identification Example 2

Several sequences were respectively assigned as *Setipinna taty* from the combination of high similarity ($\geq 98\%$) to *Setipinna taty* sequences and high bootstrap support value (99%). This result was supported by morphological identification.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Setipinna taty isolate C31 16S ribosomal RNA gene, partial sequence; mitochondrial	366	366	96%	9.00E-98	99%	DQ912091.1
Lycothrissa crocodilus mitochondrial DNA, complete genome	272	272	86%	2.00E-69	93%	AP011562.1
Coilia lindmani mitochondrial DNA, complete genome	231	231	85%	3.00E-57	90%	AP011558.1
Thryssa kammalensis mitochondrion, complete genome	224	224	84%	6.00E-55	89%	KT985048.1
Setipinna melanochir mitochondrial DNA, almost complete genome	224	224	90%	6.00E-55	88%	AP011565.1
Pterengraulis atherinoides voucher H47 16S ribosomal RNA gene, partial sequence; mitochondrial	213	213	85%	1.00E-51	88%	EU552736.1
Pterengraulis atherinoides voucher H44 16S ribosomal RNA gene, partial sequence; mitochondrial	213	213	85%	1.00E-51	88%	EU552734.1
Pterengraulis atherinoides voucher H1 16S ribosomal RNA gene, partial sequence; mitochondrial	213	213	85%	1.00E-51	88%	EU552712.1
Lycengraulis grossidens mitochondrial DNA, complete genome	211	211	85%	5.00E-51	88%	AP011563.1
Anchoiella lepidentostole voucher H45 16S ribosomal RNA gene, partial sequence; mitochondrial	211	211	85%	5.00E-51	88%	EU552735.1
Anchoiella sp. LBP 2297 mitochondrial DNA, complete genome	200	200	84%	1.00E-47	87%	AP011557.1
Anchoa choerostoma isolate C22 16S ribosomal RNA gene, partial sequence; mitochondrial	196	196	73%	1.00E-46	89%	DQ912083.1
Anchoiella sp. CBM-ZF-12586 mitochondrial DNA, complete genome	191	191	85%	6.00E-45	86%	AP012524.1
Anchoa ischana isolate AAI1 16S ribosomal RNA gene, partial sequence; mitochondrial	191	191	73%	6.00E-45	89%	JQ398398.1
Anchoa lyolepis isolate C23 16S ribosomal RNA gene, partial sequence; mitochondrial	191	191	73%	6.00E-45	89%	DQ912084.1
Thryssa baelama mitochondrial DNA, complete genome	187	187	84%	8.00E-44	85%	AP009616.1
Thryssa kammalensis mitochondrion, complete genome	185	185	83%	3.00E-43	85%	KU761588.1
Anchoa delicatissima voucher SJO 02-26 16S ribosomal RNA gene, partial sequence; mitochondrial	185	185	73%	3.00E-43	88%	EU099450.1
Engraulis mordax voucher UW-048857 16S ribosomal RNA gene, partial sequence; mitochondrial	185	185	73%	3.00E-43	88%	EF458389.1
Anchoa mitchilli isolate C16 16S ribosomal RNA gene, partial sequence; mitochondrial	185	185	73%	3.00E-43	88%	DQ912077.1

Figure A2 An example of a sequence assigned to species of *Setipinna taty* with 99% similarity and 99% bootstrap support value.

Species Identification Example 3

The sequence was assigned as *Thryssa kammalensis* from the combination of high similarity (99%) to *Thryssa kammalensis* sequences and high bootstrap support value (100%).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Thryssa kammalensis mitochondrion, complete genome	353	353	91%	7.00E-94	99%	KT985048.1
Coilia grayii mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KP317088.1
Coilia brachygnathus mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KP185129.1
Coilia rarus mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KJ710636.2
Coilia rarus mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KM363243.1
Coilia rarus mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KM257636.1
Coilia rarus mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KM276661.1
Coilia grayii mitochondrion, complete genome	252	252	86%	3.00E-63	91%	KP938994.1
Coilia eclenes mitochondrion, complete genome	252	252	86%	3.00E-63	91%	JX625133.1
Coilia rarus mitochondrial partial 16S rRNA gene	252	252	86%	3.00E-63	91%	AM911208.1
Coilia rarus mitochondrial DNA, complete genome	252	252	86%	3.00E-63	91%	AP009135.1
Coilia mystus isolate MJ4 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	EF422327.1
Coilia mystus isolate MJ1 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	EF422324.1
Coilia rarus isolate C26 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ912087.1
Coilia rarus mitochondrial gene for 16S ribosomal RNA, partial sequence	252	252	86%	3.00E-63	91%	AB246184.1
Coilia grayii 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ315697.1
Coilia grayii 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ315696.1
Coilia grayii 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ315695.1
Coilia grayii 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ315694.1
Coilia mystus 16S ribosomal RNA gene, partial sequence; mitochondrial	252	252	86%	3.00E-63	91%	DQ315693.1

Figure A3 An example of a sequence assigned to species of *Thryssa kammalensis* with 99% similarity and 100% bootstrap support value.

Family or Genus determination

If two or more taxa were assigned with the similar identify and score for a given sequence, we assigned this sequence to the higher taxonomic level (genus or family) that included both/these taxa.

Genus Identification Example 1: *Scomber*

Description	Max score	Total score	Query cover	E value	Ident	Accession
Scomber environmental sample voucher A8 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	KC603515.1
Scomber japonicus mitochondrial DNA, complete genome	372	372	96%	2.00E-99	98%	AB102724.1
Scomber australasicus mitochondrial DNA, complete genome	372	372	96%	2.00E-99	98%	AB102725.1
Scomber japonicus voucher KZN1416S 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	HQ592254.1
Scomber australasicus voucher SA2-WE 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	GU018107.1
Scomber australasicus voucher SA1-WE 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	GU018106.1
Scomber australasicus mitochondrial DNA, complete genome	372	372	96%	2.00E-99	98%	AB488407.1
Scomber colias mitochondrial DNA, complete genome	372	372	96%	2.00E-99	98%	AB488406.1
Scomber japonicus mitochondrial DNA, complete genome	372	372	96%	2.00E-99	98%	AB488405.1
Scomber japonicus voucher SIO 04-102 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	EU099472.1
Scomber japonicus voucher UW 048862 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	EF458394.1
Scomber australasicus 16S ribosomal RNA gene, partial sequence, mitochondrial	372	372	96%	2.00E-99	98%	DQ660418.1
Scomber japonicus 16S ribosomal RNA gene, partial sequence, mitochondrial gene for mitochondrial product	372	372	96%	2.00E-99	98%	AY141455.1
Scomber australasicus mitochondrial gene for 16S rRNA, partial sequence	372	372	96%	2.00E-99	98%	AB032522.1
Scomber japonicus mitochondrial gene for 16S rRNA, partial sequence	372	372	96%	2.00E-99	98%	AB032521.1
Scomber australasicus isolate 114-fish 16S ribosomal RNA gene, partial sequence, mitochondrial	337	337	86%	7.00E-89	98%	GQ346530.1
Scomber scombrus voucher NRM-49618 16S ribosomal RNA gene, partial sequence, mitochondrial	333	333	96%	9.00E-88	95%	KJ128898.1
Scomber scombrus voucher NRM-46843 16S ribosomal RNA gene, partial sequence, mitochondrial	333	333	96%	9.00E-88	95%	KJ128897.1
Scomber scombrus voucher KU5183 16S ribosomal RNA gene, partial sequence, mitochondrial	333	333	96%	9.00E-88	95%	JQ938992.1
Scomber scombrus 16S ribosomal RNA gene, partial sequence, mitochondrial	333	333	96%	9.00E-88	95%	DQ027929.1
Scomber scombrus mitochondrial DNA, complete genome	333	333	96%	9.00E-88	95%	AB120717.1

Figure B1 An example of a sequence assigned to genus *Scomber* based on credible similarity and bootstrap support value.

Genus Identification Example 2: *Odontamblyopus*

Description	Max score	Total score	Query cover	E value	Ident	Accession
Odontamblyopus sp. ZL-2016 mitochondrion, complete genome	346	346	96%	1.00E-91	97%	KT633954.1
Odontamblyopus lacepedii mitochondrial gene for 16S rRNA, partial sequence	337	337	91%	7.00E-89	98%	AB645889.1
Odontamblyopus lacepedii mitochondrion, complete genome	331	331	91%	3.00E-87	97%	KR815520.1
Odontamblyopus rebecca mitochondrion, complete genome	315	315	96%	3.00E-82	94%	KT633953.1
Odontamblyopus rubicundus mitochondrial gene for 16S rRNA, partial sequence	230	230	90%	1.00E-56	89%	AB645890.1
Odontamblyopus sp. NMBE 1066447 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial	224	224	90%	6.00E-55	88%	KF415422.1
Odontamblyopus rubicundus voucher CIFE6 16S ribosomal RNA gene, partial sequence; mitochondrial	209	209	79%	2.00E-50	90%	KC879144.1
Trypauchen sp. NMBE 1066403 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial	207	207	89%	6.00E-50	87%	KF415485.1
Periophthalmodon schlosseri mitochondrion, complete genome	204	204	80%	7.00E-49	88%	KX355324.1
Novem Genus sp. isolate NovGen 16S ribosomal RNA gene, partial sequence; mitochondrial	195	195	88%	4.00E-46	86%	HQ639117.1
Paratrypauchen microcephalus mitochondrial gene for 16S rRNA, partial sequence	193	193	86%	2.00E-45	86%	AB645891.1
Taenioides sp. NMBE 1066434 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial	191	191	93%	6.00E-45	84%	KF415479.1
Sicyopus lord isolate Lord2 16S ribosomal RNA gene, partial sequence; mitochondrial	178	178	88%	5.00E-41	85%	KF668893.1
Oxyurichthys tentacularis voucher CIFE1 16S ribosomal RNA gene, partial sequence; mitochondrial	178	178	80%	5.00E-41	86%	KC879139.1
Sicyopus lord isolate Lord1 16S ribosomal RNA gene, partial sequence; mitochondrial	172	172	88%	2.00E-39	84%	KF668892.1
Leucopsarion petersii voucher NMBE 1066488 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial	172	172	81%	2.00E-39	85%	KF415408.1
Vanderhorstia mertensi voucher Vanhor/724 16S ribosomal RNA gene, partial sequence; mitochondrial	171	171	79%	8.00E-39	85%	FJ517535.1
Vanderhorstia mertensi voucher Vanhor/723 16S ribosomal RNA gene, partial sequence; mitochondrial	171	171	79%	8.00E-39	85%	FJ517534.1
Vanderhorstia mertensi voucher Vanhor/722 16S ribosomal RNA gene, partial sequence; mitochondrial	171	171	79%	8.00E-39	85%	FJ517533.1
Vanderhorstia mertensi voucher Vanhor/715 16S ribosomal RNA gene, partial sequence; mitochondrial	171	171	79%	8.00E-39	85%	FJ460188.1

Figure B2 An example of a sequence assigned to genus *Odontamblyopus* based on credible similarity and bootstrap support value.

Family Identification Example 1: Sciaenidae

Description	Max score	Total score	Query cover	E value	Ident	Accession
Collichthys niveatus mitochondrion, complete genome	357	357	94%	5.00E-95	99%	HM219223.1
Larimichthys polyactis mitochondrion, complete genome	357	357	94%	5.00E-95	99%	GU586227.1
Larimichthys polyactis mitochondrion, complete genome	357	357	94%	5.00E-95	99%	FJ618559.1
Pseudosciaena polyactis haplotype II 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	357	357	94%	5.00E-95	99%	AY336720.1
Pseudosciaena polyactis haplotype I 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	357	357	94%	5.00E-95	99%	AY336719.1
Collichthys lucidus mitochondrion, complete genome	337	337	91%	7.00E-89	98%	JN857362.1
Collichthys niveatus mitochondrion, complete genome	337	337	91%	7.00E-89	98%	JN678726.1
Collichthys lucidus mitochondrion, complete genome	337	337	91%	7.00E-89	98%	HM447239.1
Collichthys lucidus 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	337	337	91%	7.00E-89	98%	AY336721.1
Collichthys lucidus 16S ribosomal RNA gene, partial sequence; mitochondrial	331	331	91%	3.00E-87	97%	KJ439049.1
Collichthys lucidus 16S ribosomal RNA gene, partial sequence; mitochondrial	331	331	91%	3.00E-87	97%	GU952803.1
Larimichthys crocea 16S ribosomal RNA gene, partial sequence; mitochondrial	315	315	91%	3.00E-82	96%	KJ439048.1
Larimichthys crocea 16S ribosomal RNA gene, partial sequence; mitochondrial	315	315	91%	3.00E-82	96%	GU120089.1
Larimichthys crocea mitochondrion, complete genome	315	315	91%	3.00E-82	96%	FJ595214.1
Pseudosciaena crocea 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	315	315	91%	3.00E-82	96%	AY336718.1
Larimichthys crocea mitochondrion, complete genome	309	309	91%	2.00E-80	95%	EU339149.1
Bahaba taipingensis mitochondrion, complete genome	302	302	91%	3.00E-78	95%	JX232404.1

Figure C1 An example of a sequence assigned to family Sciaenidae based on credible similarity and bootstrap support value.

Family Identification Example 2: Gobiidae

Description	Max score	Total score	Query cover	E value	Ident	Accession
Amblychaeturichthys hexanema mitochondrion, complete genome	292	292	93%	2.00E-75	93%	KT781104.1
Chaeturichthys stigmatias mitochondrion, complete genome	292	292	93%	2.00E-75	93%	KC495071.1
Gymnogobius isaza mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG231	231	231	94%	3.00E-57	88%	LC098486.1
Gymnogobius urotaenia mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2106	226	226	94%	2.00E-55	87%	LC098505.1
Gymnogobius urotaenia mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG813	226	226	94%	2.00E-55	87%	LC098503.1
Gymnogobius urotaenia mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2318	226	226	94%	2.00E-55	87%	LC098500.1
Gymnogobius urotaenia mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2176	226	226	94%	2.00E-55	87%	LC098499.1
Gymnogobius isaza mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG240	226	226	94%	2.00E-55	87%	LC098489.1
Gymnogobius isaza mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG209	226	226	94%	2.00E-55	87%	LC098488.1
Gymnogobius isaza mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG235	226	226	94%	2.00E-55	87%	LC098487.1
Gymnogobius urotaenia mitochondrion, complete genome	226	226	94%	2.00E-55	87%	KT601093.1
Gymnogobius urotaenia mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2615	220	220	94%	7.00E-54	87%	LC098501.1
Gymnogobius opperiens mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2307	220	220	94%	7.00E-54	87%	LC098494.1
Gymnogobius opperiens mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2306	220	220	94%	7.00E-54	87%	LC098493.1
Gymnogobius opperiens mitochondrial gene for 16S ribosomal RNA, partial sequence, isolate: TG2305	220	220	94%	7.00E-54	87%	LC098492.1
Periophthalmus argentilineatus mitochondrion, complete genome	220	220	86%	7.00E-54	89%	KT821095.1
Lepidogobius lepidus voucher UW.151092 16S ribosomal RNA gene, partial sequence, mitochondrial	220	220	94%	7.00E-54	87%	KJ010761.1
Lepidogobius lepidus voucher NMBE.1066485 12S ribosomal RNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S ribosomal RNA gene, partial sequence, mitochondrial	220	220	94%	7.00E-54	87%	KF415405.1
Periophthalmus modestus mitochondrial gene for 16S rRNA, partial sequence	220	220	86%	7.00E-54	89%	AB645896.1
Periophthalmus argentilineatus haplotype GP0506 16S ribosomal RNA gene, partial sequence, mitochondrial	215	215	86%	3.00E-52	88%	KP677471.1

Figure C2 An example of a sequence assigned to family Gobiidae based on credible similarity and bootstrap support value.

Draft

Family Identification Example 3: Triglidae

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chelidonichthys lucernus voucher NRM-46290 16S ribosomal RNA gene, partial sequence; mitochondrial	342	342	96%	2.00E-90	96%	KJ128732.1
Chelidonichthys capensis voucher GUIJ116S 16S ribosomal RNA gene, partial sequence; mitochondrial	342	342	96%	2.00E-90	96%	HQ592204.1
Chelidonichthys lucernus voucher 513 16S ribosomal RNA gene, partial sequence; mitochondrial	342	342	96%	2.00E-90	96%	GQ485299.1
Chelidonichthys lucernus 16S ribosomal RNA gene, partial sequence; mitochondrial	342	342	96%	2.00E-90	96%	EF120859.1
Eutrigla gurnardus voucher NRM-53143 16S ribosomal RNA gene, partial sequence; mitochondrial	337	337	96%	7.00E-89	96%	KJ128729.1
Chelidonichthys cuculus voucher NRM-48734 16S ribosomal RNA gene, partial sequence; mitochondrial	337	337	96%	7.00E-89	96%	KJ128728.1
Trigloporus lastoviza voucher NRM-44930 16S ribosomal RNA gene, partial sequence; mitochondrial	331	331	96%	3.00E-87	95%	KJ128731.1
Chelodonichthys lucerna 16S ribosomal RNA gene, partial sequence; mitochondrial gene for mitochondrial product	329	329	96%	1.00E-86	95%	AY141432.1
Lepidotrigla cavillone voucher 354 16S ribosomal RNA gene, partial sequence; mitochondrial	298	298	96%	3.00E-77	92%	GQ485297.1
Lepidotrigla papilio 16S ribosomal RNA gene, partial sequence; mitochondrial	298	298	96%	3.00E-77	92%	EU848437.1
Lepidotrigla argus 16S ribosomal RNA gene, partial sequence; mitochondrial	298	298	96%	3.00E-77	92%	EU848436.1
Lepidotrigla grandis 16S ribosomal RNA gene, partial sequence; mitochondrial	296	296	96%	1.00E-76	92%	EU848438.1
Lepidotrigla spinosa 16S large subunit ribosomal RNA gene, partial sequence; mitochondrial	292	292	96%	2.00E-75	92%	AY539001.2
Trigla lucerna 16S ribosomal RNA gene, partial sequence; mitochondrial	276	276	88%	2.00E-70	92%	KC984266.1
Peristedion gracile 16S large subunit ribosomal RNA gene, partial sequence; mitochondrial	259	259	93%	2.00E-65	90%	AY539003.2
Pallasina barbata voucher UW-119992 16S ribosomal RNA gene, partial sequence; mitochondrial	243	243	93%	2.00E-60	89%	KJ010695.1
Pallasina barbata voucher UW-048794 16S ribosomal RNA gene, partial sequence; mitochondrial	243	243	93%	2.00E-60	89%	EF458357.1
Pallasina barbata voucher UW-119991 16S ribosomal RNA gene, partial sequence; mitochondrial	237	237	93%	8.00E-59	88%	KJ010694.1

Figure C3 An example of a sequence assigned to family Triglidae based on credible similarity and bootstrap support value.