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ARTICLE

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 $\delta^{13}C$ and $\delta^{15}N$ were used to analyze prey resource use and trophic position as a manifestation of their long-term feeding habits. In total, 33 prey species consisting of 19 teleosts, seven crustaceans, five cephalopods, and two 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 differs between the short- and long-term diets. The composition of stomach contents showed sexual and age-related variation. This finding is supported by stable isotopic analyses, which indicated the separation of trophic position of adult males, adult females, and young males. In general, finless porpoises prey on species that are primarily caught by fisheries.

Résumé : La description des habitudes d'alimentation des cétacés est d'importance clé pour la compréhension de leurs stratégies d'alimentation et leur statut de conservation. Des techniques morphologiques et moléculaires sont utilisées pour identifier le contenu stomacal de 122 marsouins (*Neophocaena* spp.) dans la mer de Chine orientale afin d'obtenir de l'information sur leurs habitudes d'alimentation à court terme, et des données d'isotopes stables ($\delta^{13}C$ et $\delta^{15}N$) sont employées pour analyser l'utilisation des ressources de proies et la position trophique comme manifestations de leurs habitudes d'alimentation à long terme. Au total, 33 espèces de proies comprenant 19 poissons téléostéens, sept crustacés, cinq céphalopodes et deux gastéropodes ont été identifiées. Dans les analyses tant à court qu'à long terme, les poissons téléostéens constituaient les principales proies, les céphalopodes et les crustacés étaient des proies secondaires, et les gastéropodes, des proies occasionnelles; toutefois, la composition par espèces des proies principales n'était pas la même pour les régimes alimentaires à court et à long terme. La composition des contenus stomacaux variait selon le sexe et l'âge, ce qui est appuyé par des analyses d'isotopes stables qui indiquent une séparation des positions trophiques des mâles adultes, femelles adultes et jeunes mâles. En général, les proies des marsouins sont des espèces qui sont principalement prises dans les pêches. [Traduit par la Rédaction]

Introduction

Detailed knowledge of the diets and feeding habits of apex predators contributes to a better understanding 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 between the species is the width of the tuberculed area on the dorsal surface, with the former species having a wide area of tubercules (10–18 cm), while the latter shows a narrow tuberculed area (0.3–0.7 cm) (Wang et al. 2008). Based on morphological identification analyses, finless porpoises have been found to feed on a wide variety of fish and cephalopods, and the diet varies among different geographic 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), and *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, including in the East China Sea (Fig. 1). The East China Sea has a dense distribution of finless porpoises, and the Taiwan Strait represents an area of overlap for both species (Gao and Zhou 1995*a*, 1995*b*; Huang et al. 2000; Zhou 2004; Wang et al. 2008).

In this previous research, however, only morphological identification analyses on stomach contents were 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;

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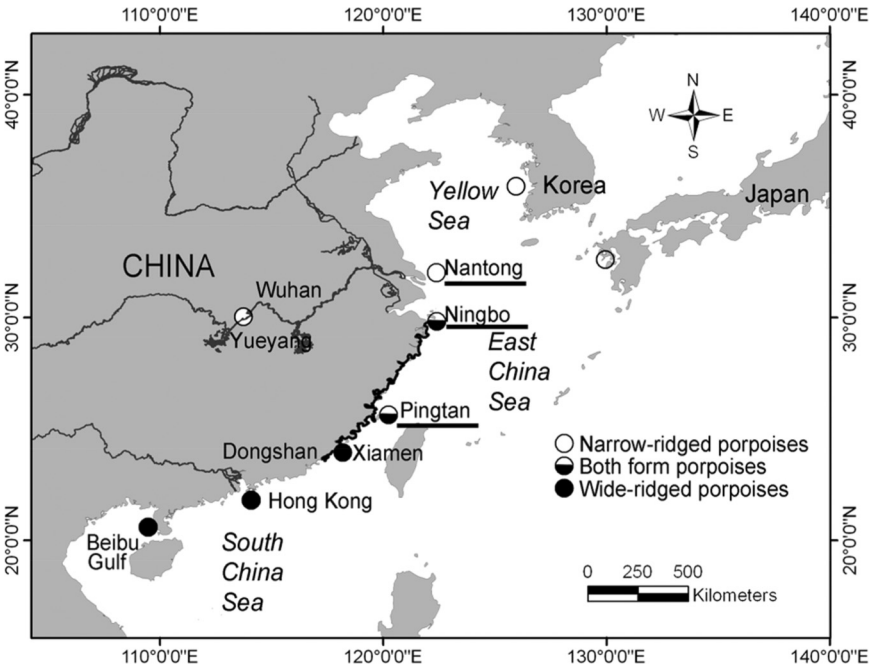
Fig. 1. Distribution of finless porpoise specimens used in the present study and previous references. Nantong, Ningbo, and Pingtan had not been examined before as finless porpoise sampling sites. The research site information was obtained from published references, i.e., Korea (Park et al. 2005), Japan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



Deagle et al. 2005; King et al. 2008; Dunshea 2009). Therefore, DNA-based method is a good supplement to morphological identification. This method is still at a relatively early stage of development, but in terms of providing quantitative estimates of the composition of diets, the results to date are promising (Bowen and Iverson 2013). Although DNA-based methods have rarely been used for analyses of cetacean diets, there are some successful examples. Jarman et al. (2002, 2004) identified the krill components of the pygmy blue whale diet via DNA purified from its feces and then studied prey diversity and identity in the fin whale using this method. Dunshea (2009) and Dunshea et al. (2013) detected diverse prey DNA in fecal and gastric samples to analyze the diet of different species of cetaceans. Méheust et al. (2015) studied the stomach contents of seven harbour porpoises with DNA-based identification of soft tissues using the BOLD system database. Up to this point, DNA-based methods have not been utilized in analyses of the diets of finless porpoises.

Although the identification of stomach contents is the most common method used in diet analyses, the stomach contents only represent the last prey ingested by a predator. The stomach contents are indicative of a predator's diet of no more than several days before death, and therefore they solely represent short-term feeding habits. In fact, dietary inputs can concentrate over time as tissue regenerates, and this turnover can be measured by stable isotopes (Tieszen et al. 1983; Praca et al. 2011). The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can provide information about resource use as indicators of long-term feeding habits, 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). Isotope analyses have been performed on the diets of some marine mammals, e.g., harbour seal (*Phoca vitulina*) (Cullon et al. 2012), spinner dolphin (*Stenella longirostris*) (Dirtu et al. 2016), Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) (Dirtu et al. 2016), long-finned pilot whale (*Globicephala melas*) (Pinzone et al. 2015), sperm whale (*Physeter macrocephalus*) (Pinzone et al. 2015), and fin whale (*Balaenoptera physalus*) (Pinzone et al. 2015), but finless porpoises were not included.

In the present paper, we studied the diet and feeding habits of narrow-ridged finless porpoises in the East China Sea. Specifically, we investigated the short-term feeding habits of these porpoises through morphological and molecular identification of their stomach contents, as well as their long-term feeding habits through the analysis of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes.

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 included 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 to explore prey variation between maturity categories and sexes, etc., because of the larger sample size (n = 106).

Examination of stomach contents and morphological identification

When porpoise carcasses were collected, external measurements were taken (Gao and Zhou 1995*a*). 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 that 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 or undigested and slightly digested prey items were morphologically identified with the aid of local reference collections and published pictorial

Table 1. Specimen information of finless porpoises (*Neophocaena* spp.) collected between 2008 and 2010 from the East China Sea.

	NFP (*N. asiaeorientalis*)			IFP (*N. phocaenoides*)	
	Nantong	Ningbo	Pingtan	Pingtan	Subtotal
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	106	6	7	3	122

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

guides (Song et al. 2006; Ye et al. 2007; Song et al. 2009), then measured (length, width, and mass) where possible.

Molecular taxonomic identification

Most previous DNA diet studies have targeted mitochondrial DNA to increase the likelihood of successful polymerase chain reaction (PCR) amplification (Dunshea 2009). Mitochondrial 16S DNA has been considered the most taxonomically informative amplicon (Deagle et al. 2009) and has been used as a marker to identify the prey components in some studies (Deagle et al. 2007; Dunshea 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; the primer sequences 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 a partial sequence of nuclear 28S ribosomal DNA (~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 targeting a region of nuclear 16S ribosomal RNA reported in a previous study (Jarman and Wilson 2004).

The morphologically unidentifiable prey samples (distinguished as a single individual) were identified using a DNA-based method. All the tools (scissors, tweezers, scalpel handle) were sterilized at high temperature before use. To prevent cross-contamination between samples, one tool per sample was used. 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, for each of the prey species (fish, shrimp, or squid), a sample of muscle 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 precipitation with ethanol (Sambrook and Russell 2001). The 15 μL PCR solution contained 0.1 μL TaKaRa *Taq* DNA polymerase (5 U·μL^{-1}), 1.5 μL 10× PCR Buffer (Mg^{2+}-free), 1.0 μL $MgCl_2$ (25 mmol·L^{-1}), 1.0 μL DNTP mixture (2.5 mmol·L^{-1}), 0.5 μL DNA template, 0.2 μL each primer, and 10.5 μL sterilized distilled water. Thermal cycling conditions were as follows: 94–95 °C for 3–10 min (fish: 5 min; squid: 10 min; shrimp: 3 min); 35 cycles of 95 °C for 30–45 s; 50–55 °C for 30–45 s; and 72 °C for 30–60 s; with a final extension time of 2–7 min (fish: 7 min; squid: 2 min; shrimp: 7 min) at 72 °C. The PCR products were electrophoresed in a 2% agarose gel and visualized under ultraviolet irradiation.

In the present study, a combination of sequence similarity and the neighbor-joining (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 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 identity (Dunshea 2009), large score, lowest Expect value (E value) (Valentini et al. 2009; Marshall et al. 2010), and high bootstrap support value on the NJ tree (Ghazali et al. 2016). If two or more taxa could be assigned a similar identity and score for a given sequence, this sequence was assigned to the higher taxonomic level (genus or family) that included both taxa taxa or the given taxa (Valentini et al. 2009). Criteria included ≥98% identity and ≥90 bootstrap support value (see examples in Appendix A).

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). An 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 was assessed by using the following four indices: (*i*) percentage frequency of occurrence (FO) (Park et al. 2005; Spitz et al. 2006; Shirakihara et al. 2008), (*ii*) percentage by number (*N*) (Park et al. 2005; Spitz et al. 2010), (*iii*) percentage by 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) was calculated as $FO_i = F_i/N_t \times 100\%$, 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) was calculated as $N_i = n_i/N \times 100\%$, where n_i is the number of prey i found and N is the total number of prey. The percentage by biomass (*M*) (Park et al. 2005) was calculated as $M_i = m_i/M \times 100\%$, where m_i is the mass of prey species i and M is 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 $IRI_i = (N_i + M_i) \times FO$.

Influencing factors and statistical analyses

We analyzed dietary variation according to geographic population, age category, and body length. The dietary variation refers to these four parameters: change in prey taxa, number of prey items, prey mass, and prey composition.

The porpoises were classified into three maturity categories: calves, juveniles, and sexually mature adults. The calves were distinguished as animals with visible fetal folds or individuals ≤1 year old with a total body 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 had left testis and epididymis weighing 537 g, 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 statistical tests were performed in SPSS 16.0. The prey taxa, number of prey items, and prey mass variables all had non-normal distributions (Kolmogorov–Smirnov one-sample test, all $p < 0.05$); the Mann–Whitney *U* test was applied for sexual and seasonal variation.

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

Carbon ratio (C^{13}/C^{12}) and nitrogen ratio (N^{15}/N^{14}) were used because they are the most common isotopes for analyzing the relative importance of food resources and trophic position to an-

Table 2. Prey composition, importance indexes (short-term), and species contribution (long-term) of finless porpoises collected between 2008 and 2010 in Nantong, East China Sea.

		Short-term				
		Index of prey importance				
Class or family	Genus or species identified	*M** (%)	*N* (%)	FO (%)	IRI (%)	Long-term contribution of prey (%)
Crustacea						
Alpheidae	*Alpheus digitalis*	0.0	0.4	0.9	0.4	3.18
	Alpheus japonicus	0.0	0.2	0.9	0.2	—
Palaemonidae	*Palaemon gravieri*	4.0	22.7	17.9	478.8	5.80
Penaeidae	*Penaeus monodon*	0.0	0.2	0.9	0.2	3.95
Squillidae	*Oratosquilla oratoria*	0.2	0.2	0.9	0.3	4.05
Portunidae	*Charybdis variegata*	0.1	0.2	0.9	0.3	5.27
	Charybdis bimaculata	0.1	0.2	0.9	0.3	—
	Order subtotal†	8.9	31.4	58.5	2354, 16.32%	25.03
Osteichthyes						
Congridae	*Conger myriaster*	4.1	2.1	5.7	34.8	4.64
Syngnathidae	*Syngnathus acus*	0.7	0.9	3.8	6.0	4.81
Thichiuridae	*Eupleurogrammus muticus*	8.9	11.5	1.9	38.5	6.54
Engraulidae	*Engraulis japonicus*	1.7	2.0	0.9	3.6	—
	Setipinna taty	16.0	12.6	9.4	270.4	4.48
	Coilia mystus	2.8	0.4	1.9	5.9	4.55
	Thryssa kammalensis	0.3	0.4	1.9	1.2	
Sciaenidae	*Larimichthys polyactis*	9.4	11.5	2.8	59.3	4.57
	Nibea albiflora	3.0	0.2	0.9	3.0	5.03
	Collichthys lucidus	0.3	0.7	1.9	2.0	3.90
Taenioididae	*Odontamblyopus rubicundus*	0.4	1.1	2.8	4.3	5.0
Gobiidae	Unidentified species	4.6	7.2	1.9	22.4	
Clupeidae	*Ilisha elongata*	3.2	3.2	0.9	6.0	4.56
	Konosirus punctatus	9.1	4.6	6.6	90.7	—
Scombrida	*Scomber* sp. or spp.	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	*Octopus ocellatus*	—	—	—		4.27
	Octopus variabilis	4.9	2.8	7.5	57.9	6.10
Loliginidae	*Loligo japonica*	1.3	2.0	4.7	16.0	—
	Loligo chinensis	5.3	0.4	0.9	5.4	4.49
Ommastrephidae	*Todarodes pacificus*	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	*Neverita didyma*	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

Note: *N*, the percentage number of prey items; *M*, percentage prey mass; FO, percentage frequency of occurrence; IRI, index of relative importance.

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

†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).

imals, which is indicative of long-term feeding habits (Kelly 2000; Parnell et al. 2010; Pokrovsky 2012).

To detect the levels of $\delta^{13}C$ and $\delta^{15}N$, 2–5 g of muscle from nine finless porpoises (three adult males, three adult females, and three young males) and 22 prey items (11 osteichthyes, $n = 29$; four cephalopods, $n = 9$; six Crustaceans, $n = 12$; one gastropods, $n = 2$) in Nantong were extracted.

All samples were rinsed in distilled water and dried in an oven for 24 h at 65 °C, then pulverized into a fine powder. The homogenized samples of 2.0 mg were loaded into capsules, after which $\delta^{13}C$ and $\delta^{15}N$ 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 PeeDee Belemnite (^{13}C) or atmospheric nitrogen (^{15}N) as follows (Cui et al. 2011; Pokrovsky 2012; Cheng et al. 2013):

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

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

Here, the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were used to analyze resource use and trophic position to assess long-term feeding habits of porpoises. The Stable Isotope Analysis package 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 set to 200 000,

Table 3. The diets of finless porpoises (*Neophocaena* spp.) collected in Ningbo and Pingtan between 2008 and 2010.

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

Note: *N*, the percentage number of prey items; FO, the percentage frequency of occurrence.

using 95% Bayesian confidence intervals for each prey type for these data (Gillespie 2013).

Results

A total of 1671 individual prey items were found in the porpoise stomachs, with a mean of 16.1 ± 92.7 (range 1–218). In general, the number of prey items in nearly four of five (80.1%) porpoises was 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 to the family, genus, or species level using morphological 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 teleosts, seven crustaceans, five cephalopods, and two gastropods.

Prey composition and importance

Teleosts occurred in 81 (76.4%) of 106 stomachs examined in Nantong; they 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 9778.4 (67.8% of total IRI) (Fig. 2; Table 2). Crustaceans (IRI = 2354, 16.3%) and cephalopods (IRI = 2292.2, 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, having an IRI of only 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 other families (all IRI ≤ 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 octopus (*Octopus variabilis*) (IRI = 57.9).

Variation in diet composition of calves, juveniles, and adults

The results suggest that crustaceans, teleosts, and cephalopods played similar roles in the diet of calves (Fig. 3). By comparison, fish were the dominant prey of juveniles and sexually mature individuals (Fig. 3).

For calves, whitespotted conger (*Conger myriaster*) (18.5% by mass) and Chinese ditch prawn (*Palaemon gravieri*) (15.6% by mass) represented the predominant prey items; for juveniles, hairfin anchovy (15.2% by mass), small yellow croaker (4.2% by mass), and

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

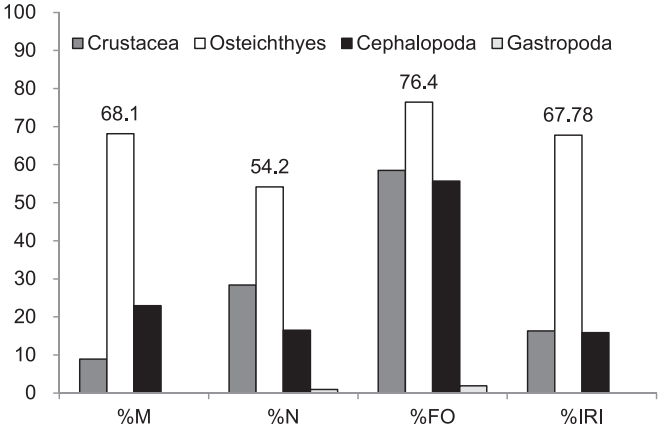


Fig. 3. The importance indices of prey in the stomach of different maturity categories of narrow-ridged finless porpoises in the East China Sea. %*M*, %*N*, %FO, and %IRI are defined in Fig. 2.

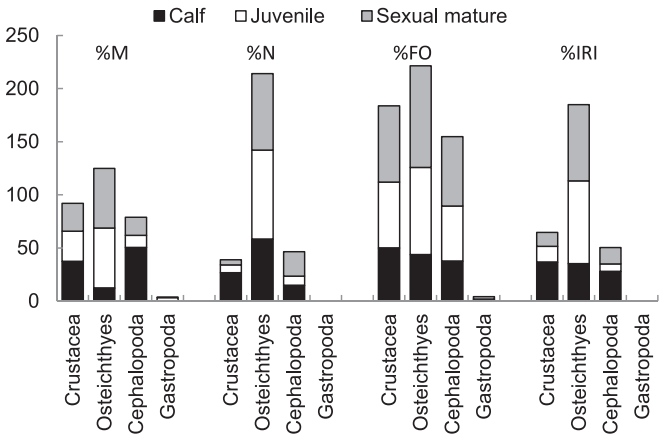
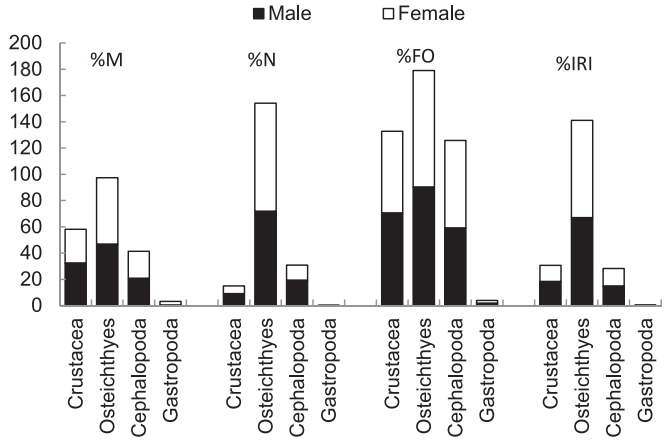


Fig. 4. The importance indices of prey in the stomach of male and female narrow-ridged finless porpoises in the East China Sea. %*M*, %*N*, %FO, and %IRI are defined in Fig. 2.



whiparm octopus (3.6% by mass) represented the predominant prey items; for mature porpoises, dotted gizzard shad (13.3% by mass), hairfin anchovy (9.2% by mass), 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 (Student's *t* test, all $p > 0.05$), fish mass, and shrimp mass (Kruskal–Wallis test, both $p > 0.05$).

The correlation analyses showed that body length was not significantly correlated with number of prey items (correlation index = −0.06), prey mass (correlation index = 0.111), fish mass (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 mass), dotted gizzard shad (8.9% by mass), and spotted maigre (7.7% by mass) represented the predominant prey items. For females, spotted maigre (3.8% by mass) and whiparm octopus (3.2% by mass) were the predominant prey items.

The Mann–Whitney *U* test results indicated significant differences in the mean prey mass of the diet between the sexes (males: 92 g; females: 75.8 g) ($p < 0.05$), 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 mass (73.6 g) than did males (70.9 g) (Mann–Whitney *U*, $p < 0.05$), and males preyed more on cephalopods (29.8 g) than did females (16 g) (Mann–Whitney *U*, $p < 0.05$). Comparisons between the sexes were not statistically significant for fish length and width, or shrimp length and width (Student's *t* test, all $p > 0.05$).

Long-term feeding habits based on stable isotope analysis

The $\delta^{13}C$ and $\delta^{15}N$ data were highly variable. Gastropods had the lowest $\delta^{13}C$ and $\delta^{15}N$, while adult female finless porpoises and young male finless porpoises had the highest value of $\delta^{15}N$ (–17.663‰ and –17.657‰). The plot in Fig. 5 indicates the significant trophic position of different levels of consumers. In particular, trophic ecological positions are distinct for adult females versus adult males or adult females versus young males.

Fig. 5. Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) biplots 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 osteichthyan, $n = 29$; four cephalopods, $n = 9$; six crustaceans, $n = 12$; one gastropod, $n = 2$.

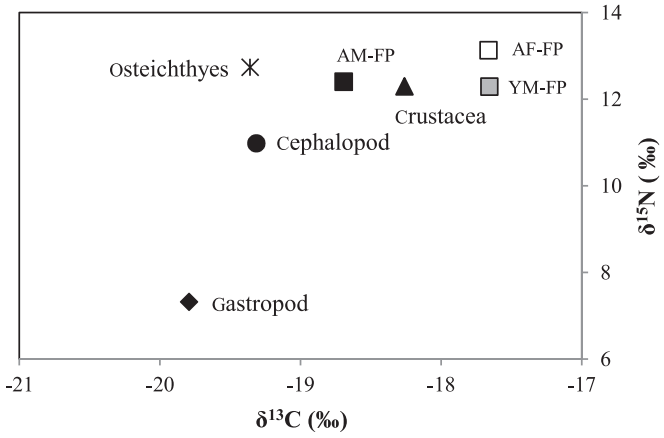
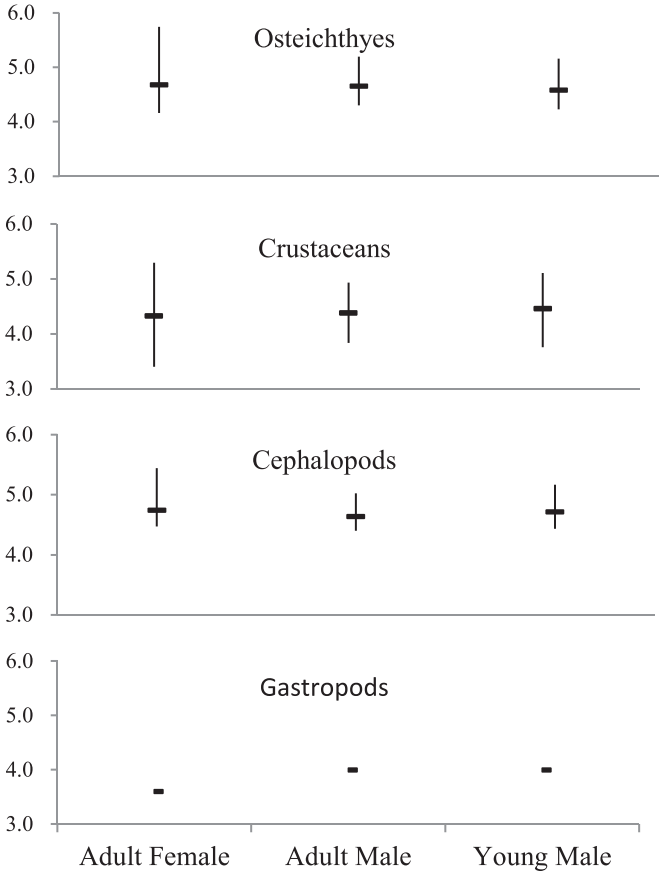


Fig. 6. The contribution (%) of prey groups to long-term diets of adult female, adult male, and young male finless porpoises in the East China Sea (based on analyses of the stable isotope ratio of $\delta^{13}C$ and $\delta^{15}N$).



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 the food resources of finless porpoises. The cresthead cutlassfish

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

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

*The values of *N* and FO were calculated according to the data of Park et al. (2005); fish were the dominant prey.

(6.5%), whiparm octopus (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 (Student's *t* test, all $p > 0.05$; Fig. 6). However, females had a slightly wider species contribution range (3.4%–5.7%) than did 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% of the number of prey and 57.1% of the frequency 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 the most important and secondary prey items. These findings are generally consistent with 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, representing only the second report following Park et al. (2005). Surprisingly, two species of gastropods, the bladder moon snail (*Neverita didyma*) and an unidentified species of Turritellidae, were also found in the stomach of finless porpoises. Because of the small number of bladder moon snail ($n = 1$), porpoises probably rarely feed on it. The Turritellidae found in the stomachs were very small (<6 mm), suggesting that Turritellidae were probably swallowed incidentally with other prey items.

Our study is the first to explore the long-term feeding habits of finless porpoises, with results indicating a similarity between short- and long-term feeding habits based on 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 and contribution of species is different between short- and long-term feeding habits (Table 2). It was well understood that the prey species varied on different short-term scales (e.g., different week or month), but on the long-term scale (e.g., half and whole year) prey composition is stable.

General profile of feeding habits and foraging ecology

Finless porpoises (wide-ridged species) inhabit Hong Kong and the Beibu Gulf represent the Indo-Pacific, 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 a similar higher frequency of occurrence compared with crustaceans (Table 4). Fish were the dominant prey species of narrow-ridged finless porpoises, numbering higher than cephalopods, crustaceans, and gastropods. From a 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 of shrimp or cuttlefish, such as Chinese ditch prawn or juvenile squid (*Loliolus japonica*) (Zhang et al. 2008). Therefore, it appears that finless porpoises primarily preyed on fish for their higher nutritional value.

Adult porpoises predominantly preyed on fish, 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 greatly, e.g., fishes in East China Sea, cephalopods in Japan (Shirakihara et al. 2008), and 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 geographic 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 1966). At the same time, association with fishing boats increases entanglement risk. Of 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 (IRI = 35.6%), 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 (*M* = 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 whether they 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 and 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 individuals, 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 the north Yellow Sea (Liu 2006). A dedicated survey on population status should be carried out as soon as possible, especially an evaluation of the correlation between finless porpoise and fishing, to address key issues related to fisheries bycatch.

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Appendix A

We reanalyzed the molecular data. In the present study, a combination of the sequence similarity and the neighbour-joining (NJ) tree method was used for prey identification, an approach that has been successfully used for prey identification in many studies (e.g., Deagle et al. 2007; Dunshea 2009; Valentini et al. 2009; Marshall et al. 2010; Ghazali et al. 2016). Please see details in the Materials and methods section.

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. An 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 at the species level from the combination of high identity (98% in present paper), large score, low E value, and large bootstrap support values (90% in present paper). Examples of species, genus, and family identifications are provided on the following pages.

Species determination

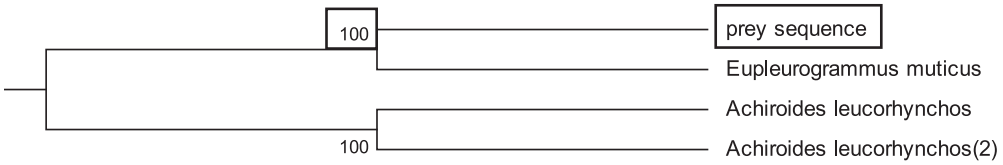
Species identification example 1: **Eupleurogrammus muticus**

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 (Table A1; Fig. A1).

Table A1. Species identification example 1: *Eupleurogrammus muticus*.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
Eupleurogrammus muticus 16S rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	329	329	97	1.00E-86	100	AY212325.1
Achiroides leucorhynchos mitochondrial 16S rRNA gene, clone 83	87.9	87.9	84	7.00E-14	77	AM182051.1
Achiroides leucorhynchos mitochondrial 16S rRNA gene, clone 82	87.9	87.9	84	7.00E-14	77	AM182050.1
Serranus baldwini voucher KU246 16S rRNA gene, partial sequence; mitochondrial	78.7	78.7	26	4.00E-11	96	HQ731419.1
Oplegnathus insignis 16S rRNA gene, partial sequence; mitochondrial	78.7	78.7	26	4.00E-11	96	HM211188.1
Serranus baldwini 16S rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	78.7	78.7	26	4.00E-11	96	AY072681.1
Xystreurys rasile voucher DAAPV F13 16S rRNA gene, partial sequence; mitochondrial	76.8	76.8	29	1.00E-10	93	GU324145.1
Xystreurys liolepis voucher KU465 16S rRNA gene, partial sequence; mitochondrial	63.9	63.9	25	1.00E-06	91	JQ939073.1
Xystreurys liolepis 16S rRNA gene, partial sequence; mitochondrial	63.9	63.9	25	1.00E-06	91	AY952500.2
Xystreurys liolepis 16S rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	63.9	63.9	25	1.00E-06	91	AF488454.1
Chascanopsetta lugubris voucher FMNH119729 16S rRNA 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

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



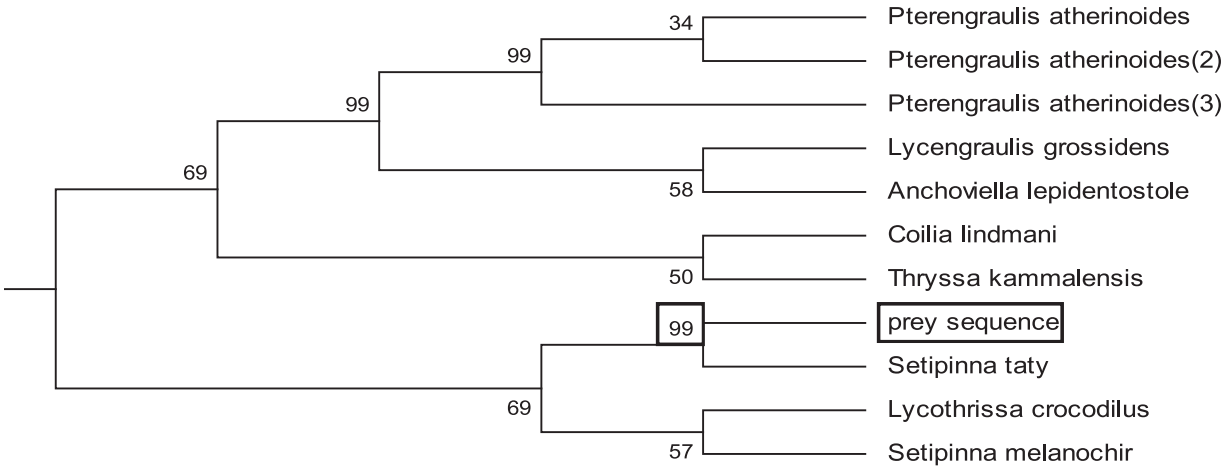
Species identification example 2: **Setipinna taty**

Several sequences were respectively assigned as *Setipinna taty* from the combination of high similarity (≥98%) to *Setipinna taty* sequences and high bootstrap support value (99%). This result was supported by morphological identification (Table A2; Fig. A2).

Table A2. Species identification example 2: *Setipinna taty*.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
Setipinna taty isolate C31 16S rRNA 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 rRNA gene, partial sequence; mitochondrial	213	213	85	1.00E-51	88	EU552736.1
Pterengraulis atherinoides voucher H44 16S rRNA gene, partial sequence; mitochondrial	213	213	85	1.00E-51	88	EU552734.1
Pterengraulis atherinoides voucher H1 16S rRNA 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
Anchoviella lepidentostole voucher H45 16S rRNA gene, partial sequence; mitochondrial	211	211	85	5.00E-51	88	EU552735.1
Anchoviella sp. LBP 2297 mitochondrial DNA, complete genome	200	200	84	1.00E-47	87	AP011557.1
Anchoa choerostoma isolate C22 16S rRNA gene, partial sequence; mitochondrial	196	196	73	1.00E-46	89	DQ912083.1
Anchoviella sp. CBM-ZF-12586 mitochondrial DNA, complete genome	191	191	85	6.00E-45	86	AP012524.1
Anchoa ischana isolate AAi1 16S rRNA gene, partial sequence; mitochondrial	191	191	73	6.00E-45	89	JQ398398.1
Anchoa lyolepis isolate C23 16S rRNA 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 SIO 02-26 16S rRNA gene, partial sequence; mitochondrial	185	185	73	3.00E-43	88	EU099450.1
Engraulis mordax voucher UW:048857 16S rRNA gene, partial sequence; mitochondrial	185	185	73	3.00E-43	88	EF458389.1
Anchoa mitchilli isolate C16 16S rRNA gene, partial sequence; mitochondrial	185	185	73	3.00E-43	88	DQ912077.1

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



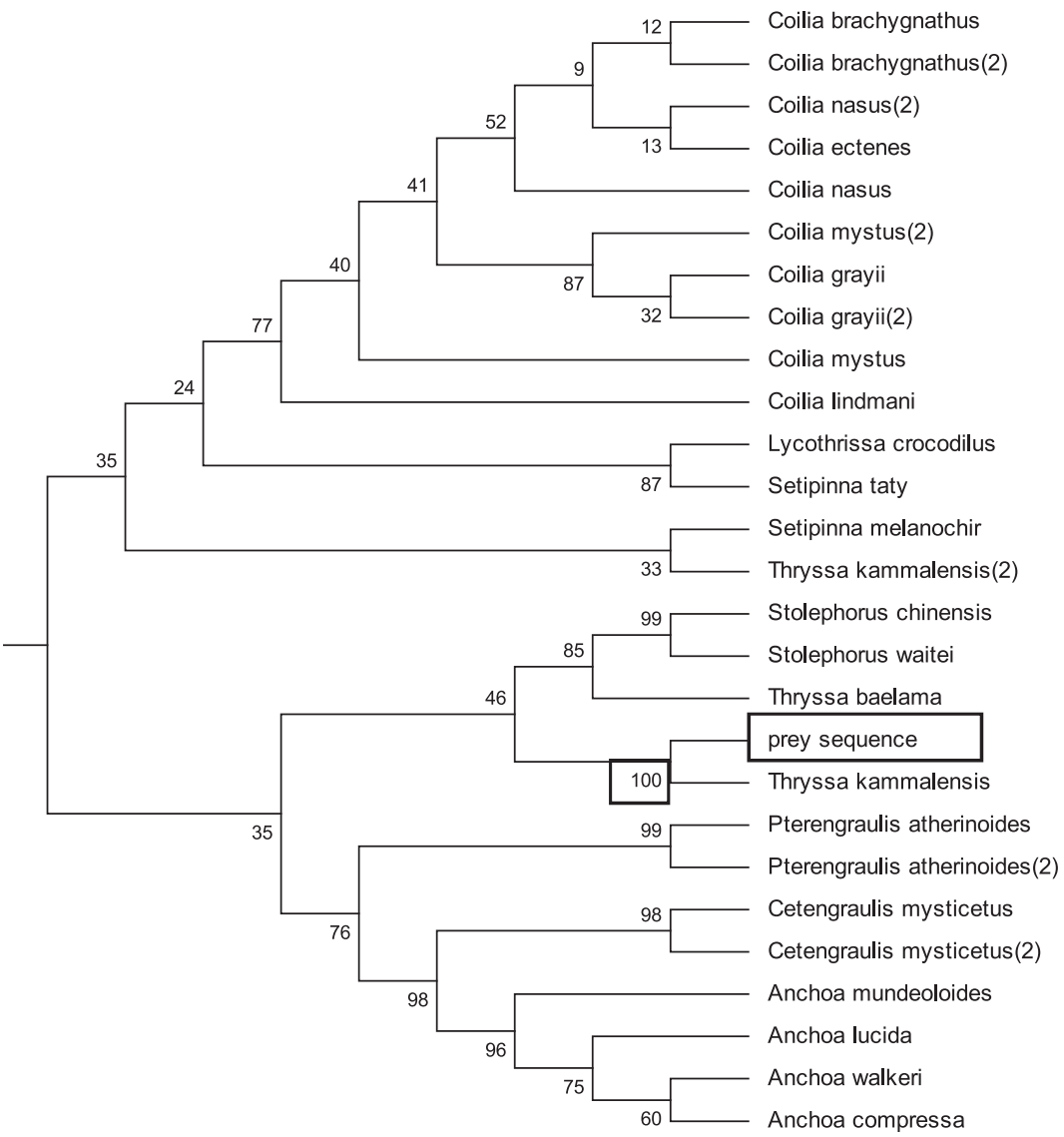
Species identification example 3: **Thryssa kammalensis**

The sequence was assigned as *Thryssa kammalensis* from the combination of high similarity (99%) to *Thryssa kammalensis* sequences and high bootstrap support value (100%) (Table A3; Fig. A3).

Table A3. Species identification example 3: *Thryssa kammalensis*.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
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 nasus mitochondrion, complete genome	252	252	86	3.00E-63	91	KJ710626.2
Coilia nasus mitochondrion, complete genome	252	252	86	3.00E-63	91	KM363243.1
Coilia nasus mitochondrion, complete genome	252	252	86	3.00E-63	91	KM257636.1
Coilia nasus mitochondrion, complete genome	252	252	86	3.00E-63	91	KM276661.1
Coilia grayii mitochondrion, complete genome	252	252	86	3.00E-63	91	KF938994.1
Coilia ectenes mitochondrion, complete genome	252	252	86	3.00E-63	91	JX625133.1
Coilia nasus mitochondrial partial 16S rRNA gene	252	252	86	3.00E-63	91	AM911208.1
Coilia nasus mitochondrial DNA, complete genome	252	252	86	3.00E-63	91	AP009135.1
Coilia mystus isolate MJ4 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	EF422327.1
Coilia mystus isolate MJ1 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	EF422324.1
Coilia nasus isolate C26 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ912087.1
Coilia nasus mitochondrial gene for 16S rRNA, partial sequence	252	252	86	3.00E-63	91	AB246184.1
Coilia grayii 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ315697.1
Coilia grayii 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ315696.1
Coilia grayii 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ315695.1
Coilia grayii 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ315694.1
Coilia mystus 16S rRNA gene, partial sequence; mitochondrial	252	252	86	3.00E-63	91	DQ315693.1

Fig. 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 a similar identity and score for a given sequence, we assigned this sequence to the higher taxonomic level (genus or family) that included both taxa or the given taxa.

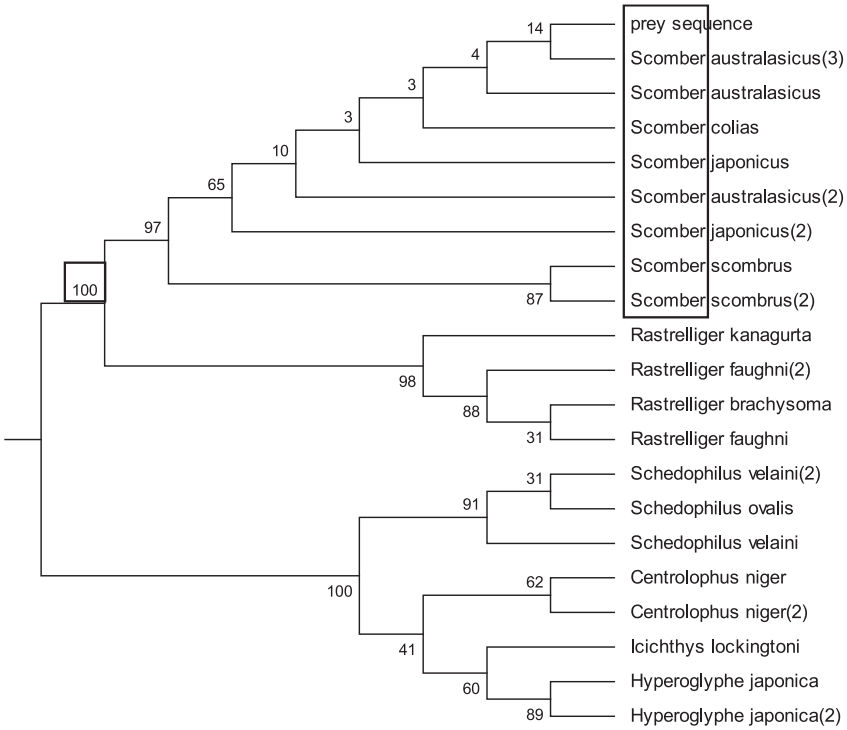
Genus determination

Genus identification example 1: Scomber (Table A4; Fig. A4)

Table A4. Genus identification example 1: *Scomber*.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
Scomber environmental sample voucher A8 16S rRNA 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 rRNA gene, partial sequence; mitochondrial	372	372	96	2.00E-99	98	HQ592254.1
Scomber australasicus voucher SA2-WE 16S rRNA gene, partial sequence; mitochondrial	372	372	96	2.00E-99	98	GU018107.1
Scomber australasicus voucher SA1-WE 16S rRNA 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 rRNA gene, partial sequence; mitochondrial	372	372	96	2.00E-99	98	EU099472.1
Scomber japonicus voucher UW:048862 16S rRNA gene, partial sequence; mitochondrial	372	372	96	2.00E-99	98	EF458394.1
Scomber australasicus 16S rRNA gene, partial sequence; mitochondrial	372	372	96	2.00E-99	98	DQ660418.1
Scomber japonicus 16S rRNA 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 rRNA gene, partial sequence; mitochondrial	337	337	86	7.00E-89	98	GQ365301.1
Scomber scombrus voucher NRM:49618 16S rRNA gene, partial sequence; mitochondrial	333	333	96	9.00E-88	95	KJ128898.1
Scomber scombrus voucher NRM:46843 16S rRNA gene, partial sequence; mitochondrial	333	333	96	9.00E-88	95	KJ128897.1
Scomber scombrus voucher KU5183 16S rRNA gene, partial sequence; mitochondrial	333	333	96	9.00E-88	95	JQ938992.1
Scomber scombrus 16S rRNA 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

Fig. A4. An example of a sequence assigned to genus *Scomber* based on credible similarity and bootstrap support value.

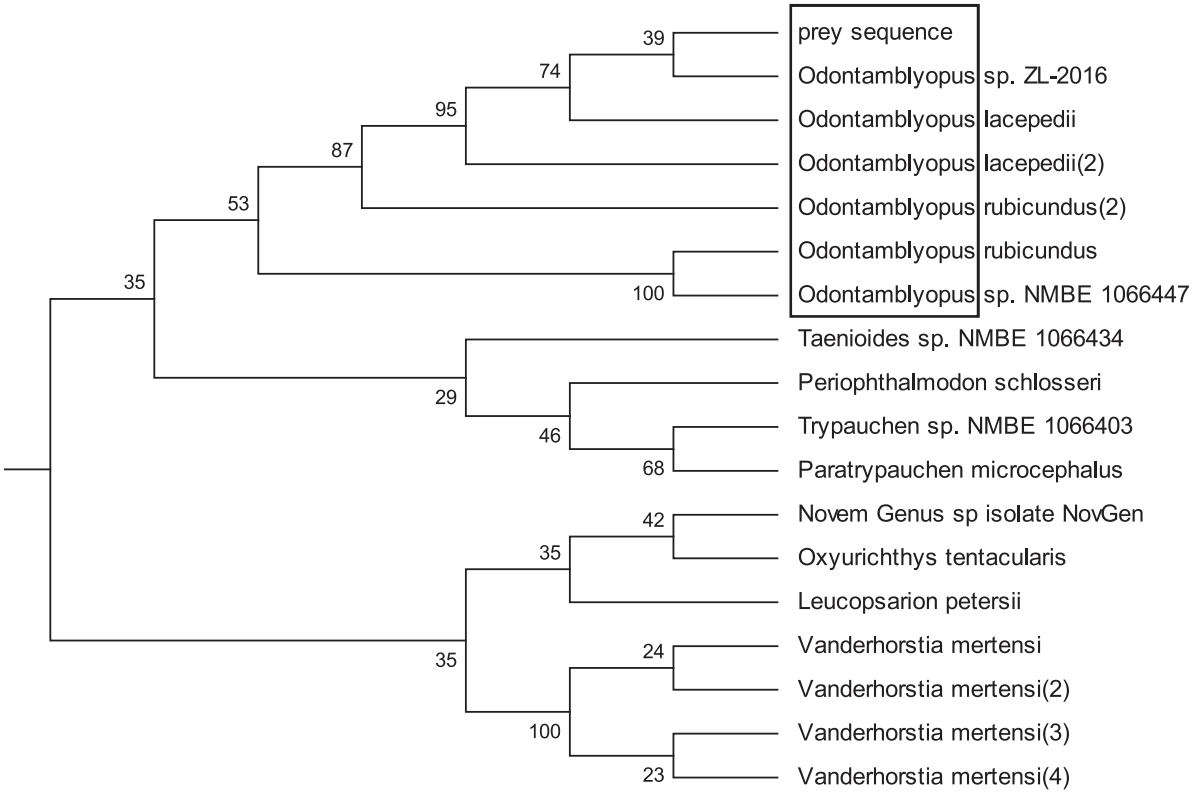


Genus identification example 2: Odontamblyopus (Table A5; Fig. A5)

Table A5. Genus identification example 2: *Odontamblyopus*.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
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 rRNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S rRNA gene, partial sequence; mitochondrial	224	224	90	6.00E-55	88	KF415422.1
Odontamblyopus rubicundus voucher CIFE6 16S rRNA gene, partial sequence; mitochondrial	209	209	79	2.00E-50	90	KC879144.1
Trypauchen sp. NMBE 1066403 12S rRNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S rRNA 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
Genus novum sp. isolate NovGen 16S rRNA 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 rRNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S rRNA gene, partial sequence; mitochondrial	191	191	93	6.00E-45	84	KF415479.1
Sicyopus lord isolate Lord2 16S rRNA gene, partial sequence; mitochondrial	178	178	88	5.00E-41	85	KF668893.1
Oxyurichthys tentacularis voucher CIFE1 16S rRNA gene, partial sequence; mitochondrial	178	178	80	5.00E-41	86	KC879139.1
Sicyopus lord isolate Lord1 16S rRNA gene, partial sequence; mitochondrial	172	172	88	2.00E-39	84	KF668892.1
Leucopsarion petersii voucher NMBE 1066488 12S rRNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S rRNA gene, partial sequence; mitochondrial	172	172	81	2.00E-39	85	KF415408.1
Vanderhorstia mertensi voucher Vanhor/724 16S rRNA gene, partial sequence; mitochondrial	171	171	79	8.00E-39	85	FJ517535.1
Vanderhorstia mertensi voucher Vanhor/723 16S rRNA gene, partial sequence; mitochondrial	171	171	79	8.00E-39	85	FJ517534.1
Vanderhorstia mertensi voucher Vanhor/722 16S rRNA gene, partial sequence; mitochondrial	171	171	79	8.00E-39	85	FJ517533.1
Vanderhorstia mertensi voucher Vanhor/715 16S rRNA gene, partial sequence; mitochondrial	171	171	79	8.00E-39	85	FJ460188.1

Fig. A5. An example of a sequence assigned to genus *Odontamblyopus* based on credible similarity and bootstrap support value.



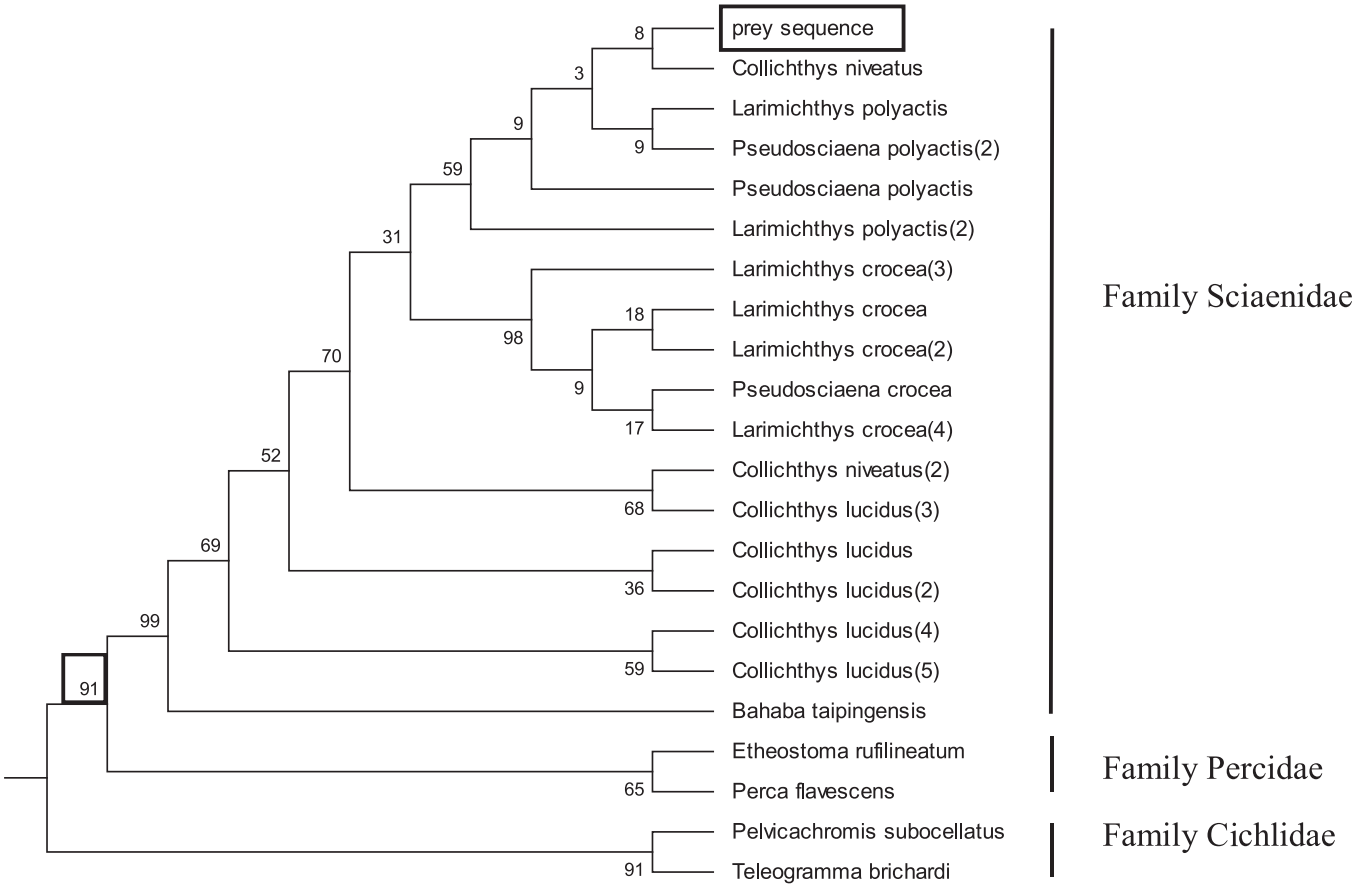
Family determination

Family identification example 1: Sciaenidae (Table A6; Fig. A6)

Table A6. Family identification example 1: Sciaenidae.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
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 rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	357	357	94	5.00E-95	99	AY336720.1
Pseudosciaena polyactis haplotype I 16S rRNA 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 rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	337	337	91	7.00E-89	98	AY336721.1
Collichthys lucidus 16S rRNA gene, partial sequence; mitochondrial	331	331	91	3.00E-87	97	KJ439049.1
Collichthys lucidus 16S rRNA gene, partial sequence; mitochondrial	331	331	91	3.00E-87	97	GU952803.1
Larimichthys crocea 16S rRNA gene, partial sequence; mitochondrial	315	315	91	3.00E-82	96	KJ439048.1
Larimichthys crocea 16S rRNA 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 rRNA 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

Fig. A6. An example of a sequence assigned to family Sciaenidae based on credible similarity and bootstrap support value.

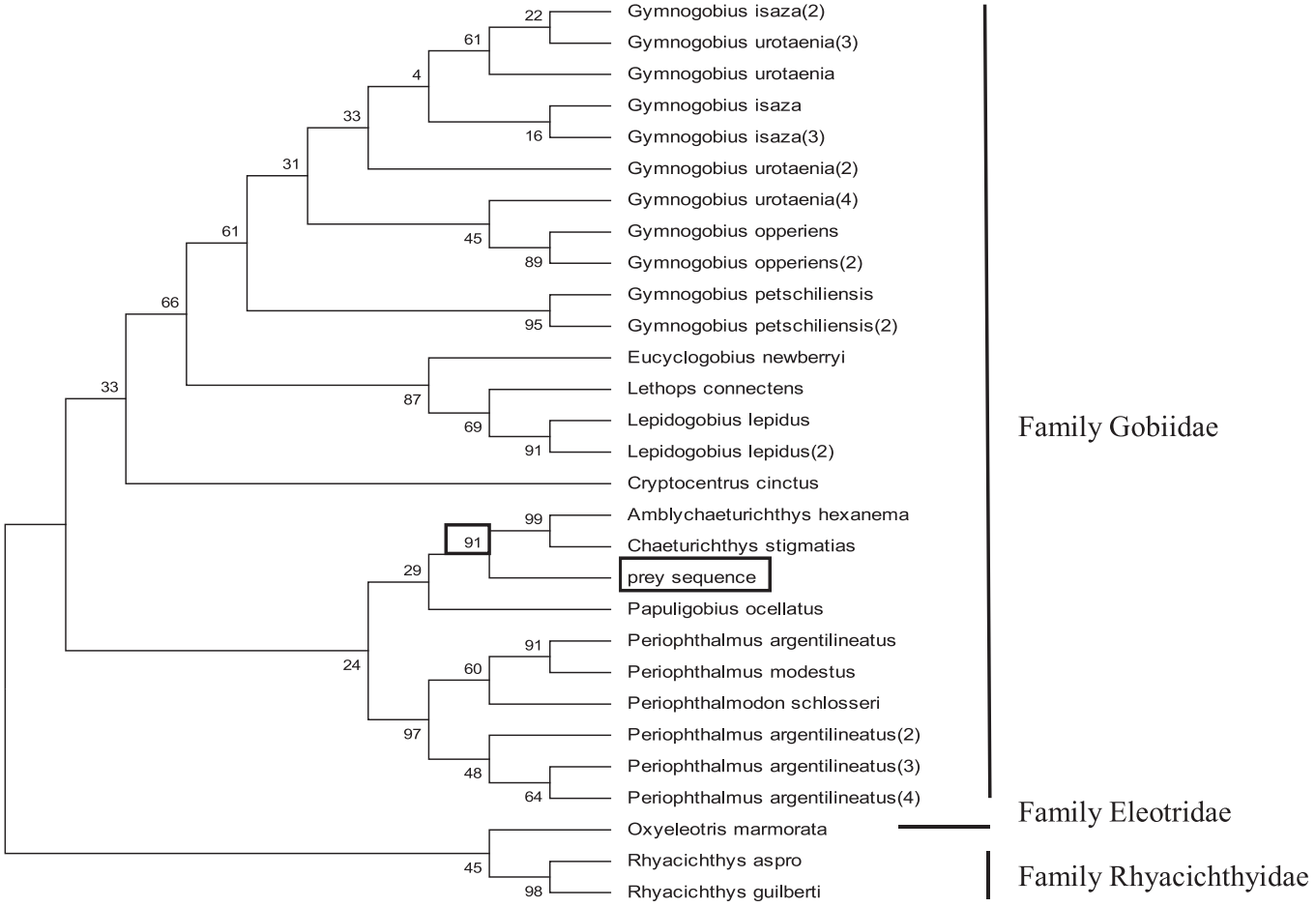


Family identification example 2: Gobiidae (Table A7; Fig. A7)

Table A7. Family identification example 2: Gobiidae.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
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 rRNA, partial sequence, isolate: TG231	231	231	94	3.00E-57	88	LC098486.1
Gymnogobius urotaenia mitochondrial gene for 16S rRNA, partial sequence, isolate: TG2106	226	226	94	2.00E-55	87	LC098505.1
Gymnogobius urotaenia mitochondrial gene for 16S rRNA, partial sequence, isolate: TG813	226	226	94	2.00E-55	87	LC098503.1
Gymnogobius urotaenia mitochondrial gene for 16S rRNA, partial sequence, isolate: TG2318	226	226	94	2.00E-55	87	LC098500.1
Gymnogobius urotaenia mitochondrial gene for 16S rRNA, partial sequence, isolate: TG2176	226	226	94	2.00E-55	87	LC098499.1
Gymnogobius isaza mitochondrial gene for 16S rRNA, partial sequence, isolate: TG240	226	226	94	2.00E-55	87	LC098489.1
Gymnogobius isaza mitochondrial gene for 16S rRNA, partial sequence, isolate: TG209	226	226	94	2.00E-55	87	LC098488.1
Gymnogobius isaza mitochondrial gene for 16S rRNA, 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 rRNA, partial sequence, isolate: TG2615	220	220	94	7.00E-54	87	LC098501.1
Gymnogobius opperiens mitochondrial gene for 16S rRNA, partial sequence, isolate: TG2307	220	220	94	7.00E-54	87	LC098494.1
Gymnogobius opperiens mitochondrial gene for 16S rRNA, partial sequence, isolate: TG2306	220	220	94	7.00E-54	87	LC098493.1
Gymnogobius opperiens mitochondrial gene for 16S rRNA, 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 rRNA gene, partial sequence; mitochondrial	220	220	94	7.00E-54	87	KJ010761.1
Lepidogobius lepidus voucher NMBE 1066485 12S rRNA gene, partial sequence; tRNA-Val gene, complete sequence; and 16S rRNA 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 rRNA gene, partial sequence; mitochondrial	215	215	86	3.00E-52	88	KP677471.1

Fig. A7. An example of a sequence assigned to family Gobiidae based on credible similarity and bootstrap support value.



Family identification example 3: Triglidae (Table A8; Fig. A8)

Table A8. Family identification example 3: Triglidae.

Description	Max. score	Total score	Query cover (%)	E value	Identity (%)	Accession No.
Chelidonichthys lucernus voucher NRM:46290 16S rRNA gene, partial sequence; mitochondrial	342	342	96	2.00E-90	96	KJ128732.1
Chelidonichthys capensis voucher GUIJ116S 16S rRNA gene, partial sequence; mitochondrial	342	342	96	2.00E-90	96	HQ592204.1
Chelidonichthys lucernus voucher 513 16S rRNA gene, partial sequence; mitochondrial	342	342	96	2.00E-90	96	GQ485299.1
Chelidonichthys lucernus 16S rRNA gene, partial sequence; mitochondrial	342	342	96	2.00E-90	96	EF120859.1
Eutrigla gurnardus voucher NRM:53143 16S rRNA gene, partial sequence; mitochondrial	337	337	96	7.00E-89	96	KJ128729.1
Chelidonichthys cuculus voucher NRM:48734 16S rRNA gene, partial sequence; mitochondrial	337	337	96	7.00E-89	96	KJ128728.1
Trigloporus lastoviza voucher NRM:44930 16S rRNA gene, partial sequence; mitochondrial	331	331	96	3.00E-87	95	KJ128731.1
Chelodonichthys lucerna 16S rRNA gene, partial sequence; mitochondrial gene for mitochondrial product	329	329	96	1.00E-86	95	AY141432.1
Lepidotrigla cavillone voucher 354 16S rRNA gene, partial sequence; mitochondrial	298	298	96	3.00E-77	92	GQ485297.1
Lepidotrigla papilio 16S rRNA gene, partial sequence; mitochondrial	298	298	96	3.00E-77	92	EU848437.1
Lepidotrigla argus 16S rRNA gene, partial sequence; mitochondrial	298	298	96	3.00E-77	92	EU848436.1
Lepidotrigla grandis 16S rRNA gene, partial sequence; mitochondrial	296	296	96	1.00E-76	92	EU848438.1
Lepidotrigla spinosa 16S large subunit rRNA gene, partial sequence; mitochondrial	292	292	96	2.00E-75	92	AY539001.2
Trigla lucerna 16S rRNA gene, partial sequence; mitochondrial	276	276	88	2.00E-70	92	KC984266.1
Peristedion gracile 16S large subunit rRNA gene, partial sequence; mitochondrial	259	259	93	2.00E-65	90	AY539003.2
Pallasina barbata voucher UW:119992 16S rRNA gene, partial sequence; mitochondrial	243	243	93	2.00E-60	89	KJ010695.1
Pallasina barbata voucher UW:048794 16S rRNA gene, partial sequence; mitochondrial	243	243	93	2.00E-60	89	EF458357.1
Pallasina barbata voucher UW:119991 16S rRNA gene, partial sequence; mitochondrial	237	237	93	8.00E-59	88	KJ010694.1

Fig. A8. An example of a sequence assigned to family Triglidae based on credible similarity and bootstrap support value.