


## Article

# Morphological Description and Phylogenetic Analyses of a New Species of *Callistoctopus* (Cephalopoda, Octopodidae) from China <sup>†</sup>

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**Abstract:** A new octopus species, *Callistoctopus tenuipes* sp. nov., was formally described from the southeastern coastal waters of China using morphological description and molecular analysis methods. *C. tenuipes* sp. nov. is a small- to moderate-sized octopus, which is characterized by very narrow and long arms. Although it was previously misidentified as the juvenile of *Octopus minor* (Sasaki, 1920), it can be recognised by spots, gill lamellae count, funnel organ shape, enlarged suckers, and ligula shape. *C. tenuipes* sp. nov. differs from the small-sized octopus *Callistoctopus xiaohongxu*, mainly in the gill lamellae count, funnel organ shape, and arm-length index. In the molecular analysis, sequences obtained from the cytochrome c-oxidase subunit I (COI) gene of eight specimens were 590 bp in length. The pairwise Kimura 2-parameter (K2P) genetic distances between Octopodidae species ranged from 8.58 to 23.79% based on the COI gene. The phylogenetic analyses suggested that *C. tenuipes* sp. nov. belonged to the *Callistoctopus* clade and may have a close affinity with *C. xiaohongxu* and *O. minor*. Moreover, three species delimitation methods all strongly supported *C. tenuipes* as a separate species.



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**Keywords:** *Callistoctopus tenuipes*; COI gene; morphological description; new species; taxonomy

## 1. Introduction

The total length of China's coastline is over 32,000 km, of which the mainland coastline is approximately 18,000 km and the island coastline is about 14,000 km. The East China Sea and the South China Sea have a high biological diversity and productivity as a consequence of the warm current flowing from the tropics [1]. In the past 20 years, five new species [2–5], a newly recorded squid [6], and a newly recorded and redescribed octopus *Amphioctopus ovulum* (Sasaki, 1917) [7] have been reported from Chinese waters. In 2012, Lu, et al. [8] recorded 134 species of cephalopods in Chinese waters. In 2022, Xu, et al. [9], using DNA barcoding analysis, found that there was an underestimation of species diversity in cephalopods in Chinese waters. Therefore, there may be far more than 134 species of cephalopods in Chinese waters, and it is important to continuously review and describe the cephalopods of China.

The genus *Callistoctopus* is characterized by a reddish body with white spots or stripes and long arms, often regarded as “*Octopus macropus* group” in the past [10]. Currently, this genus contains 14 members, including *Callistoctopus alpheus* (Norman, 1993), *Callistoctopus aspilosomatis* (Norman, 1993), *Callistoctopus bunurong* (Stranks, 1990), *Callistoctopus dierythraeus* (Norman, 1993), *Callistoctopus graptus* (Norman, 1993), *Callistoctopus lechenaultii* (d'Orbigny, 1826), *Callistoctopus luteus* (Sasaki, 1929), *Callistoctopus macropus* (Risso, 1826), *Callistoctopus nocturnus* (Norman and Sweeney, 1997), *Callistoctopus ornatus* (Gould, 1852), *Callistoctopus rapanui* (Voss, 1979), *Callistoctopus xiaohongxu* Zheng, Xu and Li, 2022, *Callistoctopus furvus* (Gould, 1852), and *Callistoctopus taprobanensis* (Robson, 1926), with the

status of *C. furvus* and *C. taprobanensis* unresolved [11]. In the last few decades, *C. furvus* has been considered a potential name for the “*macropus*” of the West Atlantic. However, Jesus, et al. [12] recently separated this species from *C. macropus* and used integrative methods of ethnoknowledge, classic taxonomy, and molecular analysis to describe a neotype. Thus, two species of *Callistoctopus* are now known to exist in the Mediterranean Sea and the Atlantic Ocean, whereas the other members of the genus are scattered throughout the Indo-Pacific region. In the Indo-Pacific waters, several informally described species of this genus have been reported in recent years. Three unverified species of *Callistoctopus* from the coast of Vietnam were briefly described by Kaneko, et al. [13] and labeled as *Callistoctopus* sp.1, *Callistoctopus* sp.2, and *Callistoctopus* sp.3. Meanwhile, Sreeja [14] found two cryptic *Callistoctopus* species along the coast of Kerala, India.

The diversity and taxonomy of cephalopods in China have been studied over the last 20 years [9,15–17]. The new cryptic *Callistoctopus* species described in this study was first collected in Xiamen, Fujian Province, and then reported in a DNA-barcoding study of cephalopods from Chinese waters in 2011 [15]. In subsequent sample collections, we were fortunate to have collected this octopus species several times on Dongshan Island in Fujian Province. This new species and another similar octopus, *C. xiaohongxu*, which was reported in this area, are both small in size, with reddish-orange skin and long arms. *Octopus minor* (Sasaki, 1920) is a common economic species with red skin and long arms in East Asia, and these two small octopuses were misidentified as juveniles of *O. minor* on Dongshan Island. This new species and *C. xiaohongxu* were called *Xiaohongxu* by the locals in Chinese, which means small and red-skinned octopus.

In this study, this new octopod species is formally described and named *Callistoctopus tenuipes* sp. nov. Phylogenetic trees were reconstructed using COI gene sequences to analyse the phylogenetic position of *C. tenuipes* sp. nov. in the family Octopodidae.

## 2. Materials and Methods

### 2.1. Specimen Collection

All samples were collected from Dongshan Fish Market, Fujian Province, China. After collecting, samples were covered with ice, then fixed in 10% formalin for 7 days, and then preserved in 70% alcohol. The type specimens are deposited in the Specimen Room, Fisheries College, Ocean University of China (OUC), China.

### 2.2. Morphological Analysis

Measurements, counts, and indices follow Roper and Voss [18], Norman and Sweeney [19], and Huffard and Hochberg [20]. Abbreviations and definitions are as follows: ALI—arm length index (arm length/ML  $\times$  100); AWI—arm width index (arm width/ML  $\times$  100); CaLI—calamus length index (calamus length/ligula length  $\times$  100); DWDI—the deepest web depth index (the deepest web depth/the longest arm  $\times$  100); EgC—egg count; EgL—egg length; EgW—egg width; FFLI—free funnel length index (free funnel length/funnel length  $\times$  100); FLI—funnel length index (funnel length/ML  $\times$  100); GC—gill count (number of gill lamellae per outer demibranch, excluding the terminal lamella); HAMI—hectocotylised arm index (hectocotylised arm length/ML  $\times$  100); HASC—hectocotylised arm sucker count; HWI—head width index (head width/ML  $\times$  100); LLI—ligula length index (ligula length/hectocotylised arm length  $\times$  100); ML—dorsal mantle length; MWI—mantle width index (mantle width/ML  $\times$  100); OAI—opposite arm index (hectocotylised arm length/normal third arm length  $\times$  100); PAI—pallial aperture index (pallial aperture length/ML  $\times$  100); SC—sucker count on normal arms; SDIn—normal sucker diameter index (normal sucker diameter/ML  $\times$  100); SpC—spermatophore count; SpL—spermatophore length; SpW—spermatophore width; SWDI—the shallowest web depth index (the shallowest web depth/the longest arm  $\times$  100); TL—total length; TW—total wet weight.

The beaks and radulae were removed from the buccal mass of some specimens, then cleaned and stored in 75% ethanol. The radulae were cleaned with 10% NaOH, air dried, covered with gold, and then scanned using a VEGA3 scanning electron microscope.

### 2.3. DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from muscle tissue using CTAB (Hexadecyl trimethyl ammonium bromide) method [21]. DNA was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The COI fragments were amplified by primers referenced by Folmer, et al. [22]. The amplification was carried out in 50  $\mu$ L reactions, containing 1  $\mu$ L template DNA of 100 ng, 2  $\mu$ L of each primer (10  $\mu$ M), 25  $\mu$ L of 2  $\times$  Hieff<sup>®</sup> PCR Master Mix (With Dye) (Yeasen), and 20  $\mu$ L sterile distilled water. The PCRs were performed under the following conditions: denaturation at 94  $^{\circ}$ C for 5 min, 34 cycles at 94  $^{\circ}$ C for 10 s, annealing at 55  $^{\circ}$ C for 20 s, and extension at 72  $^{\circ}$ C for 30 s, and a final extension of 72  $^{\circ}$ C for 5 min. PCR products were sequenced with the Sanger sequencing method.

### 2.4. Molecular and Phylogenetic Analyses

SeqMan v.7.2 (DNASTAR package) was used to assemble sequences, which were then deposited in GenBank under accession number OP184666-OP184673. The other sequences were downloaded from GenBank (Table 1). All sequences were aligned using the Clustal W in MEGA X [23]. *Vampyroteuthis infernalis* Chun, 1903 was used as an outgroup. The Kimura 2-parameter (K2P) model was used to generate K2P distances in MEGA X [23] as well.

The nucleotide substitution model was selected based on the Bayesian information criterion (BIC) in ModelFinder [24]. The best-fit model GTR + G4 + I + F was selected to construct the BI tree in MrBayes 3.2.6 [25]. The Bayesian analysis was started with four default heat chains, running 1.2 million generations of the Markov Chain Monte Carlo (MCMC) until the standard deviation of split frequencies value was lower than 0.01. The first 25% of generations were discarded as burn-in while the remaining 75% was used to construct the phylogenetic tree. Maximum likelihood phylogenies were inferred using IQ-TREE [26] under the model automatically selected for 1000 ultrafast [27] bootstraps, as well as the Shimodaira–Hasegawa-like approximate-likelihood ratio test [28].

**Table 1.** Information of specimens for the COI gene used in this study.

Species	GenBank Accession No.	Locality	References
<i>Amphioctopus aegina</i>	NC_029702	Haikou, Hainan, China	[29]
<i>Amphioctopus fangsiao</i>	HQ846126	Xiamen, Fujian, China	[15]
<i>Amphioctopus marginatus</i>	NC_036351	Haikou, Hainan, China	[30]
<i>Amphioctopus neglectus</i>	NC_049899	Nanning, Guangxi, China	[31]
<i>Amphioctopus rex</i>	MN987271	Coastal water of India	[7]
<i>Callistoctopus aspilosomatis</i>	AB430525	Miyagi Island, Okinawa, Japan	[32]
<i>Callistoctopus furvus</i>	MT892962	Morro de São Paulo, Bahia, Brazil	[12]
<i>Callistoctopus luteus</i>	MT214050	Zhangzhou, Fujian, China	[9]
<i>Callistoctopus macropus</i>	MN933634	Mediterranean Sea	[33]
<i>Callistoctopus ornatus</i>	HM104257	-	[34]
<i>Callistoctopus tenuipes</i>	OP184666-OP184673	Zhangzhou, Fujian, China	This study
<i>Callistoctopus xiaohongxu</i>	OP135961	Zhangzhou, Fujian, China	[5]
<i>Callistoctopus</i> sp.1	AB385875	Nha Trang, Vietnam	[32]
<i>Callistoctopus</i> sp.2	AB385876	Nha Trang, Vietnam	[32]
<i>Callistoctopus</i> sp.3	AB385877	Nha Trang, Vietnam	[32]
<i>Callistoctopus</i> sp.4	KF489435	Kerala, India	[14]
<i>Callistoctopus</i> sp.5	MN933632	Coastal water of Brazil	[33]
<i>Cistopus chinensis</i>	KF017606	Xiamen, Fujian, China	[35]
<i>Cistopus taiwanicus</i>	HQ846142	Xiamen, Fujian, China	[15]
<i>Octopus cyanea</i>	NC_039847	Xisha Islands, China	Unpublished
<i>Octopus minor</i> 1	NC_015896	Weihai, Shandong, China	[36]
<i>Octopus minor</i> 2	AB191275	Akashi, Osaka, Japan	[37]
<i>Octopus minor</i> 3	MF631967	Penghu Islands, China	[16]
<i>Octopus sinensis</i>	OK001740	Wenzhou, Zhejiang, China	[38]
<i>Octopus vulgaris</i>	KU525762	Galicia, Spain	[39]
<i>Vampyroteuthis infernalis</i>	NC_009689	Ogasawara Island, Japan	[40]

### 2.5. Species Delimitation

We implemented three widely used molecular methods for species delimitation, including Automatic Barcode Gap Discovery (ABGD), Generalized Mixed Yule Coalescent model (GMYC), and Bayesian Poisson Tree Processes (bPTP). The ABGD analysis was performed on the online server (<https://bioinfo.mnhn.fr/abi/public/abgd/>) using Kimura (K80) distance model with the relative gap width (X) set to 1.0 and the other parameters maintained as default [41]. The GMYC model was conducted in the split package in R using single-threshold and multiple-threshold analyses (<https://www.R-project.org/>) [42], respectively. GTR + G4 + I + F was selected as the substitution model, which was evaluated by ModelFinder [24]. The ultrametric tree was generated in BEAST 2.7.1 with a Yule model and strict clock as an assumption [43], running 30 million generations and sampling every 10,000 generations. Then Tracer v1.7.2 was used to assess the convergence by effective sample size (ESS) of each value [44]. The bPTP analysis was carried out on the bPTP online server (<https://species.h-its.org/ptp/>) with 10,000 generations and 0.1 burn-in [45].

## 3. Results

### 3.1. Molecular and Phylogenetic Analyses

Fragments obtained from eight specimens were 590 bp in length. The K2P genetic distances between *C. tenuipes* sp. nov. and the other species in Octopodidae based on the COI gene are shown in Table 2, ranging from 8.58 to 23.79%.

The COI gene dataset represented four genera of Octopodidae. The Maximum-Likelihood tree (Figure 1) showed that Octopodidae were divided into two clades. One clade comprised the *Callistoctopus* species and *O. minor*, with a bootstrap probability (BP) of 84%. The other clade included *Octopus*, *Amphioctopus*, and *Cistopus*. Eight specimens formed a highly supported monophyletic clade, with a BP of 95%. *C. tenuipes* sp. nov. had a close relationship with *C. xiaohongxu* and *O. minor* group, whereas the BP value was  $\leq 50\%$ . The topology of the Bayesian Inference analysis is presented in Figure 2. Similarly, *C. tenuipes* sp. nov. fell into a monophyletic branch with a posterior probability (PP) of 1. *C. tenuipes* sp. nov., *C. xiaohongxu*, and *O. minor* group converged into a single clade. In both phylogenetic trees, species of the genus *Amphioctopus* and *Cistopus* formed a monophyletic clade, respectively. However, *Octopus* was observed to be polyphyletic.

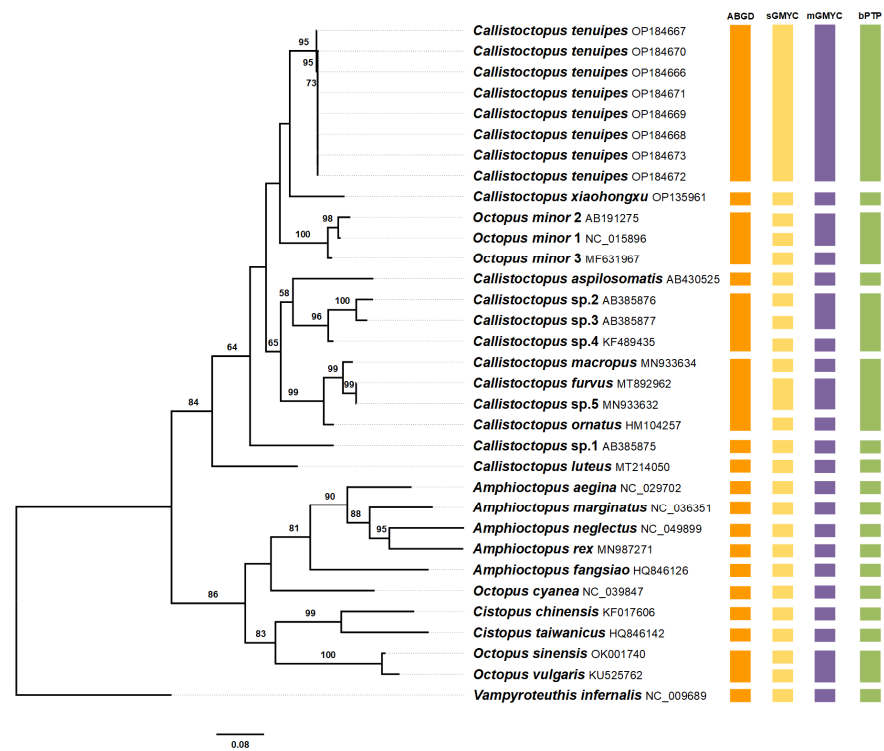
### 3.2. Species Delimitation

The results of the three species delimitation methods of ABGD, GMYC, and bPTP are shown in Figure 1. Eight specimens of *C. tenuipes* sp. nov. were recognised as a separate molecular operational taxonomic unit (MOTU) by all three methods. ABGD and bPTP methods showed similar results, with a total of 18 MOTUs identified. GMYC retrieved 25 and 22 MOTUs for the single-threshold model and multiple-threshold model, respectively.

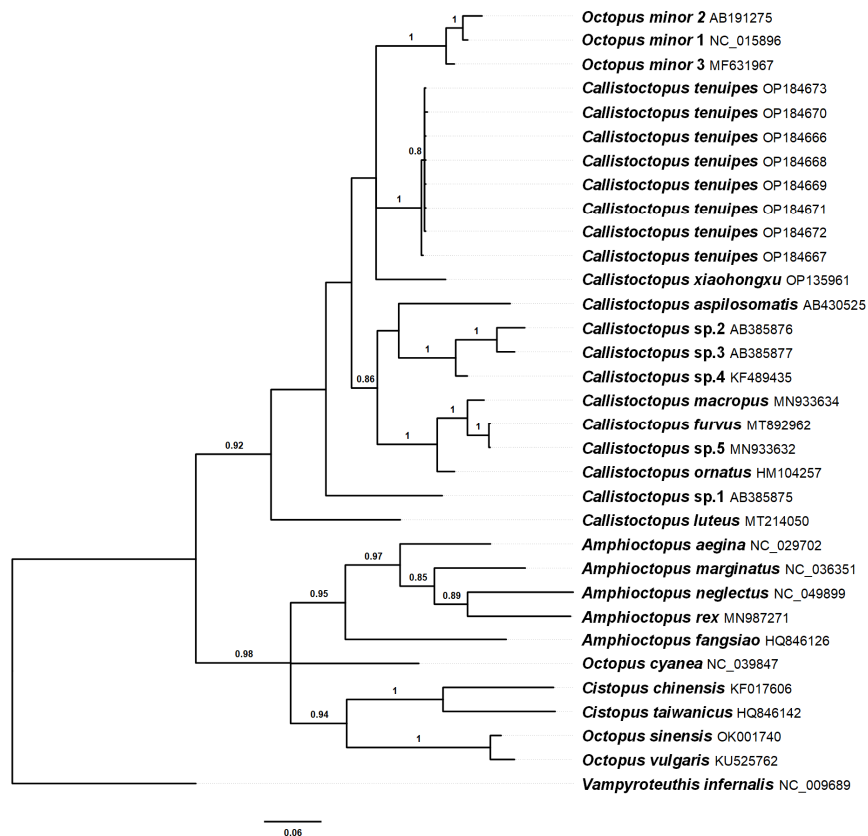
**Table 2.** Pairwise Kimura 2-parameter (K2P) genetic distance (%) between Octopodidae species analysed in this study based on the COI gene.

	<i>A. a</i>	<i>A. f</i>	<i>A. m</i>	<i>A. n</i>	<i>A. r</i>	<i>Ca. a</i>	<i>Ca. f</i>	<i>Ca. l</i>	<i>Ca. m</i>	<i>Ca. o</i>	<i>Ca. t</i>	<i>Ca. x</i>	<i>Ca. sp.1</i>	<i>Ca. sp.2</i>	<i>Ca. sp.3</i>	<i>Ca. sp.4</i>	<i>Ca. sp.5</i>	<i>Ci. c</i>	<i>Ci. t</i>	<i>O. c</i>	<i>O. m 1</i>	<i>O. m 2</i>	<i>O. m 3</i>	<i>O. s</i>	
<i>A. a</i>	-																								
<i>A. f</i>	14.40	-																							
<i>A. m</i>	11.05	14.67	-																						
<i>A. n</i>	14.11	16.24	12.65	-																					
<i>A. r</i>	11.87	16.46	13.95	11.84	-																				
<i>Ca. a</i>	20.25	20.27	20.76	22.96	21.28	-																			
<i>Ca. f</i>	20.20	20.97	21.20	21.95	20.00	12.78	-																		
<i>Ca. l</i>	20.36	19.95	21.54	20.97	19.95	15.32	15.19	-																	
<i>Ca. m</i>	19.54	20.51	20.29	21.49	19.82	12.32	3.39	15.47	-																
<i>Ca. o</i>	20.97	20.31	20.53	20.53	20.58	11.03	4.75	16.27	5.05	-															
<i>Ca. t</i>	20.27	20.54	20.09	23.79	18.86	13.15	12.40	15.00	11.08	11.09	-														
<i>Ca. x</i>	18.43	19.22	19.26	21.10	18.50	14.02	11.69	15.74	12.12	11.92	8.58	-													
<i>Ca. sp.1</i>	18.65	17.28	19.70	20.90	19.69	14.85	13.89	15.76	13.83	12.10	11.26	11.89	-												
<i>Ca. sp.2</i>	21.26	19.95	22.10	23.60	20.84	12.30	13.03	13.95	13.45	13.24	12.53	12.59	12.55	-											
<i>Ca. sp.3</i>	21.00	17.78	21.33	21.58	21.10	12.53	14.15	15.67	13.21	12.78	11.65	11.70	12.12	3.91	-										
<i>Ca. sp.4</i>	19.32	19.38	20.07	22.04	21.55	10.83	12.60	14.70	11.71	10.86	10.42	10.44	13.17	6.02	5.63	-									
<i>Ca. sp.5</i>	20.01	20.77	20.76	21.74	19.82	12.74	0.00	15.19	3.32	4.85	12.37	11.67	13.61	13.20	14.31	12.56	-								
<i>Ci. c</i>	18.19	18.73	18.96	22.57	19.16	21.58	21.77	21.12	19.86	20.39	19.38	18.00	19.87	21.23	18.51	18.50	21.55	-							
<i>Ci. t</i>	16.36	18.94	17.53	20.13	17.51	20.04	18.33	21.46	18.65	17.72	21.43	19.34	19.62	19.39	19.38	18.65	17.96	13.21	-						
<i>O. c</i>	15.07	16.88	15.78	18.06	18.05	17.11	17.19	17.72	17.75	17.08	17.07	15.33	19.13	19.91	18.70	18.73	17.29	17.09	16.15	-					
<i>O. m 1</i>	19.80	19.37	19.82	23.74	21.74	13.61	11.75	14.44	10.86	11.52	10.28	11.07	13.60	14.60	13.90	11.97	11.72	18.43	20.77	17.51	-				
<i>O. m 2</i>	20.98	20.54	20.76	24.46	22.70	14.92	12.60	15.62	11.69	12.35	10.89	11.91	14.69	15.71	15.00	13.03	12.56	19.58	21.72	18.19	2.00	-			
<i>O. m 3</i>	19.10	19.12	20.07	22.73	20.28	13.40	11.96	15.19	10.23	10.45	8.82	10.23	12.73	13.71	13.02	11.32	11.93	18.20	21.00	17.27	2.38	3.70	-		
<i>O. s</i>	14.42	18.05	15.95	18.54	18.52	21.01	21.49	21.00	20.28	20.29	20.30	20.54	17.99	20.79	20.80	20.08	21.28	17.60	17.85	17.13	20.74	21.94	20.02	-	
<i>O. v</i>	14.37	17.51	18.01	17.50	18.29	21.51	21.00	21.04	20.14	20.15	19.90	20.95	16.44	21.84	20.71	20.43	21.00	18.87	18.60	18.30	20.69	22.08	19.31	2.97	

Abbreviations: *A. a*, *Amphioctopus aegina*; *A. f*, *Amphioctopus fangsiao*; *A. m*, *Amphioctopus marginatus*; *A. n*, *Amphioctopus neglectus*; *A. r*, *Amphioctopus rex*; *Ca. a*, *Callistoctopus aspilosomatis*; *Ca. f*, *Callistoctopus furvus*; *Ca. l*, *Callistoctopus luteus*; *Ca. m*, *Callistoctopus macropus*; *Ca. o*, *Callistoctopus ornatus*; *Ca. t*, *Callistoctopus tenuipes*; *Ca. x*, *Callistoctopus xiaohongxu*; *Ca. sp.1*, *Callistoctopus sp.1*; *Ca. sp.2*, *Callistoctopus sp.2*; *Ca. sp.3*, *Callistoctopus sp.3*; *Ca. sp.4*, *Callistoctopus sp.4*; *Ca. sp.5*, *Callistoctopus sp.5*; *Ci.c*, *Cistopus chinensis*; *Ci. t*, *Cistopus taiwanicus*; *O. c*, *Octopus cyanea*; *O. m 1*, *Octopus minor 1*; *O. m 2*, *Octopus minor 2*; *O. m 3*, *Octopus minor 3*; *O. s*, *Octopus sinensis*; and *O. v*, *Octopus vulgaris*.



**Figure 1.** Phylogenetic tree based on Maximum Likelihood of the mitochondrial COI gene. The values (%) at each node show the bootstrap probability (BP) of ML analyses (only values  $\geq 50\%$  are shown). The coloured rectangles on the right present results of species delimitation, including ABGD, sGMYC, mGMYC, and bPTP.



**Figure 2.** Phylogenetic tree based on Bayesian Inference of the mitochondrial COI gene. The values (%) at each node show the posterior probability (PP) of BI analyses (only values  $\geq 0.8$  are shown).

### 3.3. Taxonomy

Order Octopoda Leach, 1818.

Family Octopodidae d'Orbigny, 1840.

Genus *Callistoctopus* Taki, 1964.

Type species: *Callistoctopus ornatus* (Gould, 1852).

*Callistoctopus tenuipes* sp. nov. (Figures 3–6).



**Figure 3.** *Callistoctopus tenuipes* sp. nov. (OUC-201812050307). (A). Dorsal view; (B). ventral view. Scale bar = 10 mm.

**Holotype.** OUC-201812050307, mature male, 37.6 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll.

**Paratypes.** OUC-201812050308, mature male, 41.4 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll. OUC-201812050309, mature male, 42.8 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll. OUC-201812050310, mature male, 41.1 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll. OUC-201808200302, mature male, 30.5 mm ML, 23.42° N, 117.85° E, 20 August 2018, coll. OUC-201812050311, mature female, 54.7 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll. OUC-201812050312, mature female, 57.3 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll. OUC-201812050313, immature female, 34.6 mm ML, 23.42° N, 117.85° E, 5 December 2018, coll.

**Diagnosis:** Small- to medium-sized species. Mantle ovoid to elongate. Stylets absent. Arms long and slender, with dorsal arms longest (arm formula 1 > 2 > 3 > 4). Web shallow,

web formula typically  $A > B > C > D > E$ . Suckers small and biserial, enlarged suckers absent. Hectocotylied arm with 67–73 suckers. Ligula moderate size (around 5% of hectocotylied arm length), triangular with shallow groove. Funnel organ W-shaped. Gills with 8 to 10 lamellae per demibranch. Colour typically reddish-orange to reddish-brown in fresh specimens. Lateral margins of arms start with two lines of chromatophores under the skin, then increase to three rows and end in a single line.

Description: The following description is based on five mature males and three females (two mature, one immature). Counts, measurements, and indices are presented in Table 3. Small- to moderate-sized species (ML 30.5–57.3 mm). Total length 146.5–318.8 mm. Body weight to 27.3 g. Skin smooth. Mantle ovoid to elongate. Mantle opening length moderate (PAI 63.1–101.1). Head width narrower than mantle width (HWI 25.6–40.7). Funnel short (FLI 27.7–38.9), free funnel length around 33–58% funnel length (FFLI 33.3–58.4). Funnel organ W-shaped (Figure 4D), outer limbs approximately equal in length with medial ones. Arms long (ALI 148.9–635.7) and slender (AWI 11.4–20.7), dorsal arms longest (typical arm formula  $1 > 2 > 3 > 4$ ). Webs shallow (WDI 3.5–13.8) (Figure 4B). Dorsal webs deepest, ventral webs shallowest (web formula  $A > B > C > D > E$ ).

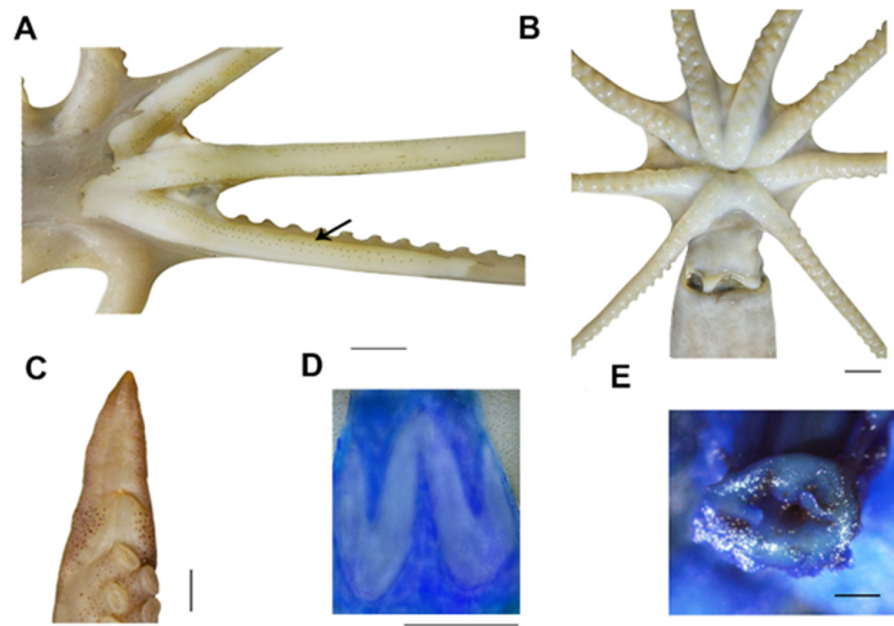
**Table 3.** Measurements (mm), counts, and morphometric indices of *Callistoctopus tenuipes* sp. nov.

Catalogue Number	OUC-201812050307	OUC-201812050308	OUC-201812050309	OUC-201812050310	OUC-201808200302	OUC-201812050311	OUC-201812050312	OUC-201812050313
Status	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype
Sex	♂	♂	♂	♂	♂	♀	♀	♀
Maturity	Mature	Mature	Mature	Mature	Mature	Mature	Mature	Immature
TL	232.6	318.8	216.8	214.4	146.5	284.0	283.4	156.1
TW(g)	7.9	11.3	11.6	9.3	12.3	27.3	20.6	8.2
ML	37.6	41.4	42.8	41.1	30.5	54.7	57.3	34.6
MWI	52.4	59.4	60.7	48.7	95.1	56.7	58.3	60.7
PAI	72.3	101.1	63.1	67.4	87.5	72.5	71.6	69.7
HWI	30.6	26.8	30.8	36.7	40.7	25.6	30.2	31.5
ALII (L/R)	500.3/478.5	635.7/D	388.8/377.6	395.1/373.7	D/401.3	385.4/401.3	D/364.7	D/263.6
AL2I (L/R)	356.6/345.5	473.9/439.4	331.5/304.4	273.5/319.7	379.3/D	331.3/318.4	D/322.7	322.3/332.9
AL3I (L/R)	359.6/186.4	419.1/192.3	293.7/154.4	298.1/148.9	D/193.8	235.7/230.7	264.0/D	278.0/273.1
AL4I (L/R)	310.4/299.7	345.2/D	D/D	D/280.5	335.4/334.8	211.8/225.6	254.4/247.8	D/253.5
AWI	11.4	12.1	13.8	11.4	20.7	13.2	13.4	15.9
DWDI	A:8.7	A:6.8	A:10.9	A:9.7	A:13.8	A:9.2	A:8.6	A:10.2
SWDI	E:3.5	E:3.9	E:6.5	E:5.0	E:9.0	E:7.1	E:6.0	E:6.9
FLI	29.0	38.9	31.1	31.1	31.5	38.7	30.9	27.7
FFLI	53.2	58.4	46.6	38.3	55.2	44.8	47.5	33.3
SDIn	4.8	4.8	4.4	4.4	6.2	3.4	3.3	4.0
HAMI	186.4	192.3	154.4	148.9	193.8	-	-	-
OAI	51.8	45.9	52.6	50.0	D	-	-	-
HASC	68H	73H	68H	67H	72H	-	-	-
LLI	6.3	5.2	7.2	4.4	6.8	-	-	-
CaLI	34.1	24.4	30.4	44.4	35.0	-	-	-
GC	8/9	9/9	8/8	8/8	8/8	9/10	8/9	8/9
SpC	-	4	-	-	-	-	-	-
SpL	-	59.9	-	-	-	-	-	-
SpW	-	1.6	-	-	-	-	-	-
EgC	-	-	-	-	-	114	111	-
EgL	-	-	-	-	-	13.7	15.1	-
EgW	-	-	-	-	-	4.2	4.5	-

D: Damaged.

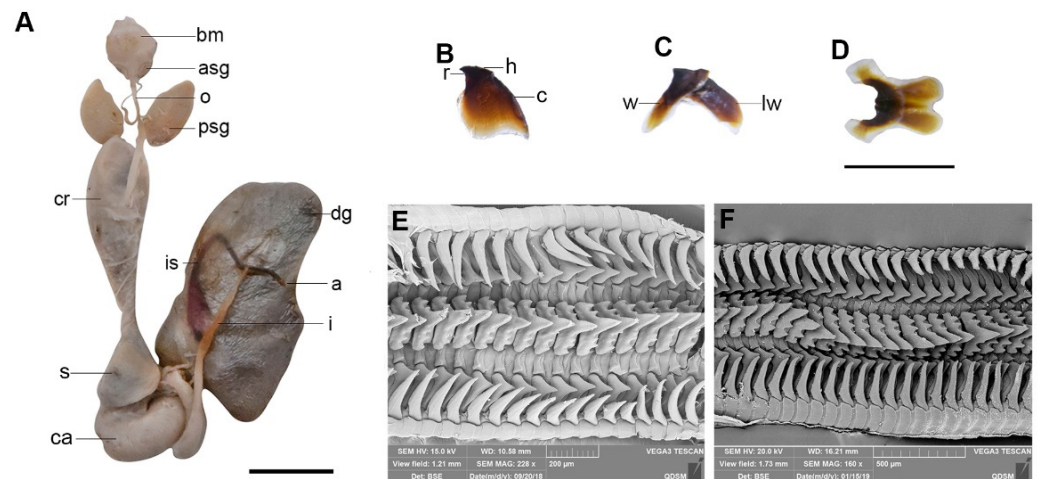
Suckers in two rows, small-sized (SDIn 3.3–6.2) (Figure 4B), enlarged suckers absent. The third right arm of mature males hectocotylied with 67–73 suckers, approximately 50% length of the opposite arm (OAI 45.9–52.6). Ligula small, triangular with shallow groove (Figure 4C), about 5% of arm length (LLI 4.4–7.2). Calamus of moderate size (CaLI 24.4–44.4). Gills with 8–10 lamellae per demibranch.





**Figure 4.** *Callistoctopus tenuipes* sp. nov. (A). Chromatophores under the arm skin (OUC-201812050311); (B). oral view (OUC-201812050311); (C). hectocotylus (OUC-201812050309); (D). funnel organ (OUC-201812050311); (E). anal flaps (OUC-201812050311). Scale bars: (A–D) = 10 mm, (E) = 200  $\mu$ m.

Digestive tract (Figure 5A): Buccal mass moderate size. Anterior salivary glands small. Posterior salivary glands large, triangular, and as long as buccal mass. Crop well developed. Stomach about as large as caecum. Caecum with about one whorl. Intestine long. Digestive gland well developed, dark grey. Ink sac present, embedded in digestive gland, and attached to the intestine posteriorly, then opening into anus. Anal flaps small (Figure 4E).

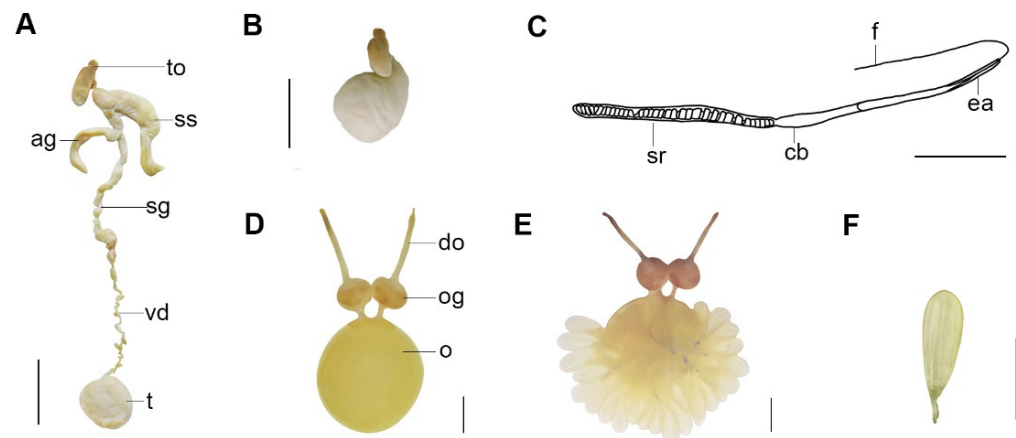


**Figure 5.** *Callistoctopus tenuipes* sp. nov. (A). Digestive system (OUC-201812050311); (B). upper beak (OUC-201808200302), lateral view; (C). lower beak (OUC-201808200302), lateral view; (D). lower beak (OUC-201808200302), ventral view; (E,F). radulae (OUC-201812050308). Scale bars: (A–D) = 10 mm; (E,F) = 200  $\mu$ m. Abbreviations: a, anus; asg, anterior salivary gland; bm, buccal mass; c, crest; ca, caecum; cr, crop; dg, digestive gland; h, hood; I, intestine; is, ink sac; o, oesophagus; psg, posterior salivary gland; r, rostrum; s, stomach; w, wing; lw, lateral wall.

Chitinous beaks dark brown. Upper beak (Figure 5B) with short hood, sharp and small rostrum. Crest curved and longer than total wing length. Lower beak (Figure 5C,D) with a medium-sized hood, moderate broad wings, and short lateral wings. Radula (Figure 5E,F)

with seven teeth and two marginal plates per transverse row. Rachidian teeth with two or three lateral cups on each side. First lateral teeth small and sharp. Second lateral teeth larger than the first, sharp. Third lateral teeth long, curved, sharply pointed, longer than second lateral teeth. Marginal plates flat.

Male reproductive tract (Figure 6A): Terminal organ ‘—’ shaped (Figure 6B) in mature males. Spermatophore gland long. Accessory gland curved, almost same length as the Needham’s sac. Spermatophore storage sac long and slender. Vas deferens short, narrow. Testis roundish and large. Spermatophores narrow (Figure 6C), average length 46.4 mm, few (4–6 in storage sac).



**Figure 6.** *Callistoctopus tenuipes* sp. nov. (A,B). Male reproductive tract (OUC-201812050310); (C). spermatophore (OUC-201812050310), (D). female reproductive tract (OUC-201812050312), (E,F). eggs (OUC-201812050312). Scale bars: (A–F) = 10 mm. Abbreviations: ag, accessory gland; cb, cement body; do, distal oviduct; ea, ejaculatory apparatus; o, ovary; og, oviducal gland; sg, spermatophore gland; sr, sperm reservoir; ss, spermatophore storage sac; t, testis; f, filament; to, terminal organ (penis); vd, vas deferens.

Female reproductive tract (Figure 6D–F): Ovary large and round in mature females. Proximal oviducts short and narrow. Distal oviducts long and thin. About 110 eggs in ovary of a single mature female. Largest mature ovarian eggs approximately 15.1 mm.

Colouration: Fresh specimens reddish orange. Skin smooth with no papillae on dorsal mantle. Two or more lines of chromatophores obviously presented on the lateral margins of arms under the skin (Figure 4A).

Etymology: The species name *tenuipes* is proposed for its very narrow arms.

Distribution: Through the survey of fishermen and our sampling collection, this species is mainly found in the East China Sea to the South China Sea, and potentially south to Hainan Province, including Beibu Gulf.

Remarks: The species reported in this study have differences between males and females. Generally, mature females are larger than males. To determine the maturation times, we collected samples three times from the same sea area and found that males and females mature synchronously.

#### 4. Discussion

As mentioned above, *C. tenuipes* sp. nov. was misidentified as the juvenile of *O. minor*. To prove the validity of the nominal taxon *C. tenuipes* sp. nov., we compared it with the original descriptive features of three unsolved subspecies of *O. minor*. Table 4 shows that *C. tenuipes* sp. nov. differs from the other three subspecies, mainly in spots, gill count, funnel organ shape, enlarged suckers, and ligula shape. Meanwhile, *C. tenuipes* sp. nov. is distinct from *C. xiaohongxu* in body size (mature individuals to 39.2 g versus 27.3 g), funnel organ shape (W-shaped versus  $\wedge$  /-shaped), web depth index (WDI 3.5–13.8 versus 15.7–22.9), arm length index (ALI 247.8–635.7 versus 154.9–336.3), and ligula index (LLI

4.4–7.2 versus 7.0–11.6). On the other hand, *C. tenuipes* sp. nov. resembles *C. xiaohongxu* in orange skin as in the fish market. However, in mature individuals, *C. tenuipes* sp. nov. has a smaller body size and longer arms than *C. xiaohongxu*. Notably, chromatophores on the lateral margins of arms under the skin were also found in *O. minor* and *C. xiaohongxu*. However, the arrangement of chromatophores in the three species was not consistent. In addition, small chromatophores of an unresolved octopus *Callistoctopus* sp.1, collected by Kaneko, et al. [13] from Vietnamese waters, were scattered on the surface of the arms. It remains to be seen whether chromatophores will be found in more species and whether there is a pattern of arrangement of chromatophores in certain taxa.

**Table 4.** Comparison of *Callistoctopus tenuipes* sp. nov., *Callistoctopus xiaohongxu*, and three subspecies of *Octopus minor* group.

Item	<i>Callistoctopus tenuipes</i> sp. nov.	<i>Callistoctopus xiaohongxu</i>	<i>Octopus minor typicus</i>	<i>Octopus minor pardalis</i>	<i>Octopus minor minor</i>
Data source	This study	[5]	[46]	[46]	[47]
Body weight	to 27.3 g	to 39.2 g	-	-	-
Skin	smooth, reddish-orange	smooth, reddish-orange to reddish-brown	smooth, red-brown	slightly firm, dark brown in alcohol	slightly warty, grey brown
Spots	no spot	no spot	light yellow spots on mantle surface	five transverse streaks on mantle surface	irregular marbled stripe
GC	8–10	8–9	10–12	14	7–9
Funnel organ	W-shaped	\ ^ /-shaped	V V-shaped	U U-shaped	V V-shaped
WDI	3.5–13.8	15.7–22.9	deepest around 10	-	deepest around 12
ALI	247.8–635.7	154.9–336.3	400–500	around 400–700	around 300–700
Enlarged suckers	absent	absent	present	-	-
Ligula	triangular with shallow groove, LLI 4.4–7.2	cylindrical with groove, LLI 7.0–11.6	spoon-like with wide hollow groove, LLI around 18–23	-	-

In the molecular analysis, the pairwise K2P genetic distances displayed in Table 3 indicate that *C. tenuipes* sp. nov. can be separated as a new species since the genetic distance between other sequences of Octopodidae analysed in this study ranged from 8.58 to 23.79%. Three species delimitation analyses also strongly supported *C. tenuipes* sp. nov. as a separate new species. Phylogenetic topologies showed that *C. tenuipes* sp. nov. may have a close relationship with *C. xiaohongxu* and *O. minor*. Regarding the taxonomic status of *O. minor*, Kaneko, et al. [32] placed it into *Callistoctopus* based on its morphological characteristics and DNA-barcoding analyses. In this study, three *O. minor* sequences converged to the *Callistocotpus* clade, which may indicate that *O. minor* had a closer affinity to the genus *Callistocotpus* than *Octopus*. However, the taxonomic classification of *O. minor* still needs further study.

In summary, we described a new octopod species, *C. tenuipes* sp. nov., using morphological and molecular methods. The South China Sea coastal current flows from Guangdong Province, and one branch flows southwest along the Hainan coast. Considering the current system, we speculate that this new octopus will be collected in Hainan and Beibu Gulf after a comprehensive resource survey. Our work adds some useful information to the classification of octopuses. Moreover, this study increases the knowledge of species diversity in the Southeast China Sea.

**Author Contributions:** Conceptualization, X.Z., J.L. and C.X.; methodology, J.L. and C.X.; software, J.L. and C.X.; validation, J.L., X.Z. and C.X.; formal analysis, J.L. and X.Z.; investigation, J.L. and X.Z.; resources, X.Z. and C.X.; data curation, J.L. and X.Z.; writing—original draft preparation, J.L.; writing—review and editing, X.Z.; visualization, J.L., X.Z. and C.X.; supervision, X.Z. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The animal study protocol was approved by the Experimental Animal Ethics Committee of the Ocean University of China.

**Data Availability Statement:** The molecular data presented in this study are openly available in the National Center for Biotechnology Information under the GenBank accession numbers OP184666–OP184673.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Liu, J.Y. Status of marine biodiversity of the China seas. *PLoS ONE* **2013**, *8*, e50719. [[CrossRef](#)] [[PubMed](#)]
- Ho, C.-W.; Lu, C.-C. Two new species of *Sepia* (*Doratosepion*) (Cephalopoda: Sepiidae) from Taiwan, based on morphological and molecular data. *Phuket Mar. Biol. Cent. Res. Bull.* **2005**, *66*, 51–69.
- Liao, J.X.; Lu, C.C. A new species of *Cistopus* (Cephalopoda: Octopodidae) from Taiwan and morphology of mucous pouches. *J. Molluscan Stud.* **2009**, *75*, 269–278. [[CrossRef](#)]
- Zheng, X.; Lin, X.; Lu, C.; Ma, R. A new species of *Cistopus* Gray, 1849 (Cephalopoda: Octopodidae) from the East and South China Seas and phylogenetic analysis based on the mitochondrial COI gene. *J. Nat. Hist.* **2012**, *46*, 355–368. [[CrossRef](#)]
- Zheng, X.; Xu, C.; Li, J. Morphological description and mitochondrial DNA-based phylogenetic placement of a new species of *Callistoctopus* Taki, 1964 (Cephalopoda, Octopodidae) from the southeast waters of China. *ZooKeys* **2022**, *1121*, 1–15. [[CrossRef](#)]
- Lee, K.-S.; Lu, C.-C. *Histioteuthis miranda* (Berry, 1918) (Cephalopoda: Histioteuthidae) from the North Pacific Ocean. *Collect. Res.* **2019**, *33*, 9–18.
- Tang, Y.; Zheng, X.; Li, Q. Redescription of *Amphioctopus ovulum* (Sasaki, 1917) (Cephalopoda: Octopodidae) and comparative morphological analyses among three species of violet-ringed octopods. *Invertebr. Syst.* **2020**, *34*, 823–836. [[CrossRef](#)]
- Lu, C.C.; Zheng, X.D.; Lin, X.Z. Diversity of Cephalopoda from the waters of the Chinese mainland and Taiwan. In *Proceedings of the 1st Taiwan Symposium of Marine Biodiversity Studies*; Lin, M., Wang, C.G., Eds.; Ocean: Beijing, China, 2012; pp. 76–87.
- Xu, R.; Lü, Y.; Tang, Y.; Chen, Z.; Xu, C.; Zhang, X.; Zheng, X. DNA Barcoding reveals high hidden species diversity of Chinese waters in the Cephalopoda. *Front. Mar. Sci.* **2022**, *9*, 830381. [[CrossRef](#)]
- Norman, M.D. *Cephalopods: A World Guide*; ConchBooks: Hackenheim, German, 2000; pp. 1–320.
- Norman, M.D.; Hochberg, F.G. The current state of octopus taxonomy. *Phuket Mar. Biol. Cent. Res. Bull.* **2005**, *66*, 127–154.
- Jesus, M.D.; Sales, J.B.; Martins, R.S.; Ready, J.S.; Costa, T.A.S.; Ablett, J.D.; Schiavetti, A. Traditional knowledge aids description when resolving the taxonomic status of unsettled species using classical and molecular taxonomy: The case of the shallow-water octopus *Callistoctopus furvus* (Gould, 1852) from the western Atlantic Ocean. *Front. Mar. Sci.* **2021**, *7*, 595244. [[CrossRef](#)]
- Kaneko, N.; Kubodera, T.; Dinh, T.; Chung, B.D. Shallow-water benthic octopuses (Cephalopoda, Octopodidae) collected from the coastal waters of Vietnam. *Bull. Natl. Mus. Nat. Sci. Ser. A* **2008**, *34*, 105–122.
- Sreeja, V. Taxonomy and Diversity of Cephalopods (Mollusca: Cephalopoda) of Kerala Coast. Ph.D. Thesis, University of Kerala, Thiruvananthapuram, India, 2013; pp. 138–141.
- Dai, L.; Zheng, X.; Kong, L.; Li, Q. DNA barcoding analysis of Coleoidea (Mollusca: Cephalopoda) from Chinese waters. *Mol. Ecol. Resour.* **2012**, *12*, 437–447. [[CrossRef](#)] [[PubMed](#)]
- Xu, R.; Bo, Q.; Zheng, X. A divergent lineage among *Octopus minor* (Sasaki, 1920) populations in the Northwest Pacific supported by DNA barcoding. *Mar. Biol. Res.* **2018**, *14*, 335–344. [[CrossRef](#)]
- Tang, Y.; Zheng, X.; Liu, H.; Sunxie, F. Population genetics and comparative mitogenomic analyses reveal cryptic diversity of *Amphioctopus neglectus* (Cephalopoda: Octopodidae). *Genomics* **2020**, *112*, 3893–3902. [[CrossRef](#)]
- Roper, C.F.E.; Voss, G.L. Guidelines for taxonomic descriptions of cephalopod species. *Mem. Natl. Mus. Vic.* **1983**, *44*, 48–63. [[CrossRef](#)]
- Norman, M.D.; Sweeney, M.J. The shallow-water octopus (Cephalopoda: Octopodidae) of the Philippines. *Invertebr. Taxon.* **1997**, *11*, 89–140. [[CrossRef](#)]
- Huffard, C.L.; Hochberg, F.G. Description of a new species of the genus *Amphioctopus* (Mollusca: Octopodidae) from the Hawai'ian Islands. *Molluscan Res.* **2005**, *25*, 113–128.
- Winnepeninckx, B. Extraction of high molecular weight DNA from molluscs. *Trends Genet.* **1993**, *9*, 407. [[CrossRef](#)]
- Folmer, O.; Black, M.B.; Vrijenhoek, R.C. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299.
- Kumar, S.; Stecher, G.; Li, M.; Niyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)]

24. Kalyaanamoorthy, S.; Bui Quang, M.; Wong, T.K.F.; von Haeseler, A.; Jermini, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [[CrossRef](#)] [[PubMed](#)]
25. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [[CrossRef](#)] [[PubMed](#)]
26. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [[CrossRef](#)] [[PubMed](#)]
27. Minh, B.Q.; Nguyen, M.A.T.; von Haeseler, A. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.* **2013**, *30*, 1188–1195. [[CrossRef](#)] [[PubMed](#)]
28. Guindon, S.; Dufayard, J.-F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* **2010**, *59*, 307–321. [[CrossRef](#)] [[PubMed](#)]
29. Zhang, X.; Zheng, X.; Ma, Y.; Li, Q. Complete mitochondrial genome and phylogenetic relationship analyses of *Amphioctopus aegina* (Gray, 1849) (Cephalopoda: Octopodidae). *Mitochondrial DNA Part A* **2017**, *28*, 17–18. [[CrossRef](#)]
30. Tang, Y.; Zheng, X.; Ma, Y.; Cheng, R.; Li, Q. The complete mitochondrial genome of *Amphioctopus marginatus* (Cephalopoda: Octopodidae) and the exploration for the optimal DNA barcoding in Octopodidae. *Conserv. Genet. Resour.* **2017**, *10*, 115–118. [[CrossRef](#)]
31. Tang, Y.; Zheng, X.; Zhong, H.; Li, Q. Phylogenetics and comparative analysis of the mitochondrial genomes of three violet-ringed octopuses. *Zool. Scr.* **2019**, *48*, 482–493. [[CrossRef](#)]
32. Kaneko, N.; Kubodera, T.; Iguchis, A. Taxonomic study of shallow-water octopuses (Cephalopoda: Octopodidae) in Japan and adjacent waters using mitochondrial genes with perspectives on octopus DNA barcoding. *Malacologia* **2011**, *54*, 97–108. [[CrossRef](#)]
33. Lima, F.D.; Strugnell, J.M.; Leite, T.S.; Lima, S.M.Q. A biogeographic framework of octopod species diversification: The role of the Isthmus of Panama. *PeerJ* **2020**, *8*, e8691. [[CrossRef](#)]
34. Strugnell, J.M.; Norman, M.D.; Vecchione, M.; Guzik, M.; Allcock, A.L. The ink sac clouds octopod evolutionary history. *Hydrobiologia* **2014**, *725*, 215–235. [[CrossRef](#)]
35. Cheng, R.; Zheng, X.; Ma, Y.; Li, Q. The complete mitochondrial genomes of two octopods *Cistopus chinensis* and *Cistopus taiwanicus*: Revealing the phylogenetic position of the genus *Cistopus* within the order Octopoda. *PLoS ONE* **2013**, *8*, e84216. [[CrossRef](#)] [[PubMed](#)]
36. Cheng, R.; Zheng, X.; Lin, X.; Yang, J.; Li, Q. Determination of the complete mitochondrial DNA sequence of *Octopus minor*. *Mol. Biol. Rep.* **2011**, *39*, 3461–3470. [[CrossRef](#)] [[PubMed](#)]
37. Takumiya, M.; Kobayashi, M.; Tsuneki, K.; Furuya, H. Phylogenetic relationships among major species of Japanese coleoid cephalopods (Mollusca: Cephalopoda) using three mitochondrial DNA sequences. *Zool. Sci.* **2005**, *22*, 147–155. [[CrossRef](#)] [[PubMed](#)]
38. Li, J.; Chen, S.; Chen, W.; Xie, S.; Ni, X.; Zheng, X. Morphological and genetic diversity analysis of *Octopus sinensis* in Nanji Islands. *Oceanol. Limnol. Sin.* **2022**, *53*, 486–495. (In Chinese) [[CrossRef](#)]
39. Amor, M.D.; Norman, M.D.; Roura, A.; Leite, T.S.; Gleadall, I.G.; Reid, A.; Perales-Raya, C.; Lu, C.C.; Silvey, C.J.; Vidal, E.A.G.; et al. Morphological assessment of the *Octopus vulgaris* species complex evaluated in light of molecular-based phylogenetic inferences. *Zool. Scr.* **2016**, *46*, 275–288. [[CrossRef](#)]
40. Yokobori, S.; Lindsay, D.J.; Yoshida, M.; Tsuchiya, K.; Yamagishi, A.; Maruyama, T.; Oshima, T. Mitochondrial genome structure and evolution in the living fossil vampire squid, *Vampyroteuthis infernalis*, and extant cephalopods. *Mol. Phylog. Evol.* **2007**, *44*, 898–910. [[CrossRef](#)]
41. Puillandre, N.; Lambert, A.; Brouillet, S.; Achaz, G. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* **2012**, *21*, 1864–1877. [[CrossRef](#)]
42. Ezard, T.; Fujisawa, T.; Barraclough, T. *Splits: Species' Limits by Threshold Statistics*, version 1.0-20. 2021.
43. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchene, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kuhnert, D.; De Maio, N.; et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [[CrossRef](#)]
44. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [[CrossRef](#)]
45. Zhang, J.; Kapli, P.; Pavlidis, P.; Stamatakis, A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **2013**, *29*, 2869–2876. [[CrossRef](#)] [[PubMed](#)]
46. Sasaki, M. A monograph of dibranchiate cephalopods of the Japanese and adjacent waters. *J. Coll. Agric. Hokkaido Imp. Univ.* **1929**, *20*, 90–96.
47. Sasaki, M. Report on cephalopods collected during 1906 by the United States Bureau of Fisheries steamer “Albatross” in the northwestern Pacific. *Proc. United States Natl. Mus.* **1920**, *57*, 181–182. [[CrossRef](#)]