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Redescription of *Amphioctopus ovulum* (Sasaki, 1917) (Cephalopoda : Octopodidae) and comparative morphological analyses among three species of violet-ringed octopods

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Abstract. *Amphioctopus ovulum* (Sasaki, 1917) is a small to moderate-sized octopus, which can be identified by the iridescent violet ring present in the dark ocellus on the web between the bases of arms II and III. Comprehensive taxonomic review is required to fully characterise this species because the syntypes are missing and the description is insufficiently complete for modern octopod taxonomy. In this study, the species *A. ovulum* is redescribed with morphological and morphometric characters of 18 specimens collected from the coastal waters of China. The distribution of *A. ovulum* extends from the Gulf of Thailand, through Cambodia, Vietnam, Philippines, through the South China Sea and the East China Sea to Japan. The swollen terminal organ diverticulum and long spermatophores make it possible to distinguish *A. ovulum* clearly from *A. rex* and *A. neglectus*, species with similar morphological characters of violet rings. Moreover, three species of violet-ringed octopods were clearly differentiated by sequences of the partial mitochondrial genes *COI* and *COIII*. Three monophyletic clades resolved in phylogenetic trees. *Amphioctopus rex* and *A. neglectus* clustered into a sister taxon, and clustered with the remaining *Amphioctopus* species.

Keywords: *Amphioctopus ovulum*, comparative morphological analyses, distribution, phylogenetic analyses, redescription, violet-ringed octopods.

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Introduction

Amphioctopus ovulum (Sasaki, 1917) was first reported as *Polypus ovulum* with a brief description that lacks the details necessary for modern systematics. The first review of the taxonomy of this species was that of Sasaki (1929), which was based on morphological characters of 16 specimens collected from the coast of Japan. He pointed out that this small species has shagreen-like skin, slender arms, and a moderate-depth web. It is also characterised by some distinguished features, i.e. two supraorbital cirri, the pair of iridescent cobaltic or violet rings, small eggs, long spermatophores and a well-marked diverticulum. Robson (1929) examined two specimens without giving a specific description, and his two specimens agreed well with *A. ovulum*. However, *A. ovulum* was not considered to be a distinct species, and Sasaki's *A. ovulum was* regarded as a colour variety of *O. areolatus* (which today is called *A. fangsiao*).

Between the 1840s and the 1950s, there were many attempts to clarify different ocellate species, which caused some confusion in this group (Gleadall 1991). Sasaki (1929), Pickford and McConnaughey (1949) proposed the 'two species hypothesis' that there are two types of ocellate octopuses, with egg size as the most consistent difference. However, many researchers overlooked the hypothesis or failed to give it the serious consideration it deserves (Gleadall 1991). Moreover, the syntypes of *A. ovulum* were reported to be lodged in the Tokyo Imperial University but they have not been found (Norman and Hochberg 1994). Toll and Voss (1998) also mentioned that these syntypes are presumed to be no longer extant, contributing another reason why *A. ovulum* needs to be further revised (Toll and Voss 1998; Norman *et al*. 2013).

Since the beginning of the 21st century, there have been few records of *A. ovulum*. Okutani (2000) described *A. ovulum* briefly in his atlas. Kubodera and Lu (2002) revised the cephalopods occurring in the Chinese–Japanese subtropical region, of which *A. ovulum* was confirmed from the South China Sea to the waters around Okinawa Islands and western Japan. The earliest record of *A. ovulum* in China was from Dong (1978). Li (1983) added that this species was common in China and Japan. Subsequently, *A. ovulum* was described by Dong (1987) based on only one male specimen with some key morphological features (e.g. silver ring and gill number of 8–10) inconsistent with the description of Sasaki (1929). So, it is unclear whether the specimen described by Dong is actually *A. ovulum*.

Recently, Kaneko *et al*. (2008) collected three different ocellate octopuses characterised by violet rings on the dark ocellus at the bases of the arms II and III from the coastal waters of Vietnam, which were recognised morphologically as *A*. cf. *ovulum*, *A*. cf. *rex* and *A*. cf. *neglectus*. Subsequently, Kaneko *et al*. (2011) examined the phylogeny of shallow-water benthic octopuses distributed in Japan and adjacent waters, including these three species of violet-ringed octopods, which resolved as separate clades in their phylogenetic analysis. However, the morphological characters for discriminating each species were considered indecisive (Kaneko *et al*. 2011).

In the present study, our molecular data from specimens collected from Chinese coastal waters matched three different sequences from the National Center for Biotechnology Information (NCBI) database (Kaneko *et al*. 2008, 2011), identifying the species as *A. ovulum*, *A. rex* and *A. neglectus*. These three species of violet-ringed octopods have been included in very few molecular phylogenetic studies. Therefore, more molecular data of these violet-ringed octopuses is presented in our work for further evolutionary studies. Moreover, considering the poor description of *A. ovulum*, the limited knowledge of its biology and systematics, and the indistinguishable morphological characters among these three species of violet-ringed octopods, we made morphological measurements to redescribe *A. ovulum* and compared the morphological characters of the three species.

Materials and methods

Material examined

A total of 18 *A. ovulum* specimens were used for morphological analyses. Ten specimens for measurements were collected from a local market in Beihai (Guangxi Province of China). Eight specimens for other morphological analyses were collected from Wenzhou, Zhoushan (Zhejiang Province of China) and Beihai, Nanning (Guangxi Province of China). Ten *A. rex* specimens were collected from coastal waters of India (eight) and Wenzhou (two, Zhejiang Province of China). Ten *A. neglectus* specimens were collected from the fish market in Beihai (Guangxi Province of China). All specimens were fixed in 10% formalin for one week before being transferred to 95% alcohol. These specimens were deposited in the Genetics and Breeding Science of Shellfish (GBSS), Fisheries College, Ocean University of China (OUC), Qingdao, China.

The beaks and radulae were extracted from the buccal mass of some specimens, cleaned and illustrated. Radulae were cleaned with 7% NaOH, critical-point dried, coated with gold before being scanned by a VEGA3 scanning electron microscope (TESCAN, Brno, Czech Republic). Beaks were cleaned and stored in 75% ethanol.

Measurements and description

Morphometric and meristic characters were taken using either a ruler (to the nearest millimetre) or dial callipers (precision of 0.1 mm) following Roper and Voss (1983) and Norman and Sweeney (1997). Indices of morphological measurements and counts are shown in Table S1. In addition, the raw data of measurements and counts were used in our statistical analysis. One-way ANOVA (SPSS, ver. 21.0) was utilised to analyse the data with a Duncan's test to locate differences in 17 morphometric and meristic characters among three species of violet-ringed octopods.

Abbreviations for the institutions consulted are as follows: MSUT, University Museum, University of Tokyo; FMHU, Fisheries Museum, Faculty of Fisheries, Hokkaido University.

Molecular and phylogenetic analyses

All specimens for morphological analyses and another 12 violet-ringed octopuses collected along the coastal waters of China were used for molecular analyses (Table 1). Muscle

Table 1. Species information for phylogenetic analyses
CN, China; VN, Vietnam; JP, Japan

Species	Collecting Site Catalogue number (OUC-)	Gen. Bank Accession number (*COI*/*COIII*)	Collecting sites	Reference
Amphioctopus rex CN	201605160309	MF447874 / MF447874	Wenzhou, Zhejiang, China	Tang *et al*. (2019)
Amphioctopus cf. *rex* VN	–	AB385871 / AB573191	Nha Trang, Vietnam	Kaneko *et al*. (2011)
Amphioctopus rex	201704190301	MN987261 / MN987305	Beihai, Guangxi, China	This study
Amphioctopus rex	201706200302	MN987262 / MN987307	Coastal water of India	This study
Amphioctopus rex	201706200315	MN987271 / MN987306	Coastal water of India	This study
Amphioctopus rex	201706200303	MN987263 / MN987328	Coastal water of India	This study
Amphioctopus rex	201706200304	MN987264 / MN987329	Coastal water of India	This study
Amphioctopus rex	201706200307	MN987265 / MN987330	Coastal water of India	This study
Amphioctopus rex	201706200308	MN987266 / MN987331	Coastal water of India	This study
Amphioctopus rex	201706200310	MN987267 / MN987342	Coastal water of India	This study
Amphioctopus rex	201706200312	MN987268 / MN987332	Coastal water of India	This study
Amphioctopus rex	201706200313	MN987269 / MN987343	Coastal water of India	This study
Amphioctopus rex	201706200314	MN987270 / MN987344	Coastal water of India	This study
Amphioctopus rex	201605160310	MN987260 / MN987340	Wenzhou, Zhejiang, China	This study
Amphioctopus neglectus CN	201104220303	MH899749 / MH899749	Nanning, Guangxi, China	Tang *et al*. (2019)

(*continued next page*)

Table 1. (*continued*)

Species	Collecting Site Catalogue number (OUC-)	Gen. Bank Accession number (*COI*/*COIII*)	Collecting sites	Reference
Amphioctopus cf. *neglectus* VN	–	AB385872 / AB573190	Nha Trang, Vietnam	Kaneko *et al.* (2011)
Amphioctopus neglectus	201811200301	MN987272 / MN987304	Sanya, Hainan, China	This study
Amphioctopus neglectus	201709110301	MN987303 / MN987314	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110302	MN987273 / MN987308	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110303	MN987274 / MN987309	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110304	MN987275 / MN987316	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110305	MN987276 / MN987310	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110306	MN987277 / MN987317	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110307	MN987278 / MN987315	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110308	MN987279 / MN987311	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110309	MN987280 / MN987312	Beihai, Guangxi, China	This study
Amphioctopus neglectus	201709110310	MN987281 / MN987313	Beihai, Guangxi, China	This study
Amphioctopus cf. *ovulum* CN	201605160307	MF447873 / MF447873	Wenzhou, Zhejiang, China	Tang *et al.* (2019)
Amphioctopus ovulum CN	–	AB430523 / AB573198	East China Sea	Kaneko *et al.* (2011)
Amphioctopus ovulum JP	–	AB430524 / AB573197	Tosa Bay, Japan	Kaneko *et al.* (2011)
Amphioctopus cf. *ovulum* VN	–	AB385873 / AB573199	Nha Trang, Vietnam	Kaneko *et al.* (2011)
Amphioctopus ovulum	201104220301	MN987256 / MN987341	Nanning, Guangxi, China	This study
Amphioctopus ovulum	201104220304	MN987257 / MN987346	Nanning, Guangxi, China	This study
Amphioctopus ovulum	201104220305	MN987258 / MN987345	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201104220308	MN987259 / MN987347	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110311	MN987282 / MN987326	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110312	MN987283 / MN987318	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110313	MN987284 / MN987319	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110314	MN987285 / MN987320	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110315	MN987286 / MN987321	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110316	MN987287 / MN987322	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110317	MN987288 / MN987323	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110318	MN987289 / MN987327	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110319	MN987290 / MN987324	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110320	MN987291 / MN987325	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201709110322	MN987292 / MN987350	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201805060311	MN987300 / MN987338	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201805060312	MN987301 / MN987339	Beihai, Guangxi, China	This study
Amphioctopus ovulum	201710200301	MN987293 / MN987348	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200302	MN987294 / MN987349	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200310	MN987295 / MN987333	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200311	MN987296 / MN987334	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200313	MN987297 / MN987335	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200314	MN987298 / MN987336	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201710200318	MN987299 / MN987337	Zhoushan, Zhejiang, China	This study
Amphioctopus ovulum	201811200314	MN987302 / MN987351	Sanya, Hainan, China	This study
Amphioctopus aegina	–	AB385872 / AB385872	Haikou, Hainan, China	Zhang *et al.* (2017)
Amphioctopus marginatus	–	AB385872 / AB385872	Haikou, Hainan, China	Tang *et al.* (2018)
Amphioctopus fangsiao	–	AB240156 / AB240156	Tokyo, Japan	Akasaki *et al.* (2006)
Cistopus chinensis	–	KF017606 / KF017606	Xiamen, Fujian, China	Cheng *et al.* (2013)
Cistopus taiwanicus	–	NC023257 / NC023257	Coastal water of Taiwan	Cheng *et al.* (2013)
Octopus vulgaris	–	NC006353 / NC006353	Tokyo, Japan	Yokobori *et al.* (2004)
Octopus bimaculatus	–	KT581981 / KT581981	Gulf of California	Domínguez-Contreras *et al.* (2016)
Octopus conispadiceus	–	KJ789854 / KJ789854	Haishenwai Amur Bay, Russia	Ma *et al.* (2016)
Octopus minor	–	HQ638215 / HQ638215	Weihai, Shandong, China	Cheng *et al.* (2012)
Eledone cirrhosa	–	AY557520 / HM104251	Great Britain	Strugnell *et al.* (2014)

tissues were taken from the mantles, then specimens were fixed in 10% formalin and stored in 95% ethanol. Total genomic DNA was extracted using the E.Z.N.A.TM Mollusk DNA Kit (OMEGA Bio-Tek Co., Norcross, GA, USA) following the manufacturer's protocol. Approximately 650-base pair (bp) regions of the cytochrome oxidase 1 (*COI*) genes were amplified by Polymerase Chain Reaction (PCR) using primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG

G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer *et al*. 1994). Partial regions of the cytochrome oxidase 3 (*COIII*, ~450 bp long) genes were amplified using primers Oco3F (5′-CAA TGA TGA CGA GAT ATT ATY CG-3′) and Oco3R (5′-CTT CAA ATC CAA AAT GAT GTG A-3′) (Guzik *et al*. 2005). The 10-µL reaction volume contains 1 µL of dNTP (2.5 mM, TransGen Biotech, Beijing, PR China), 1 µL of 10×

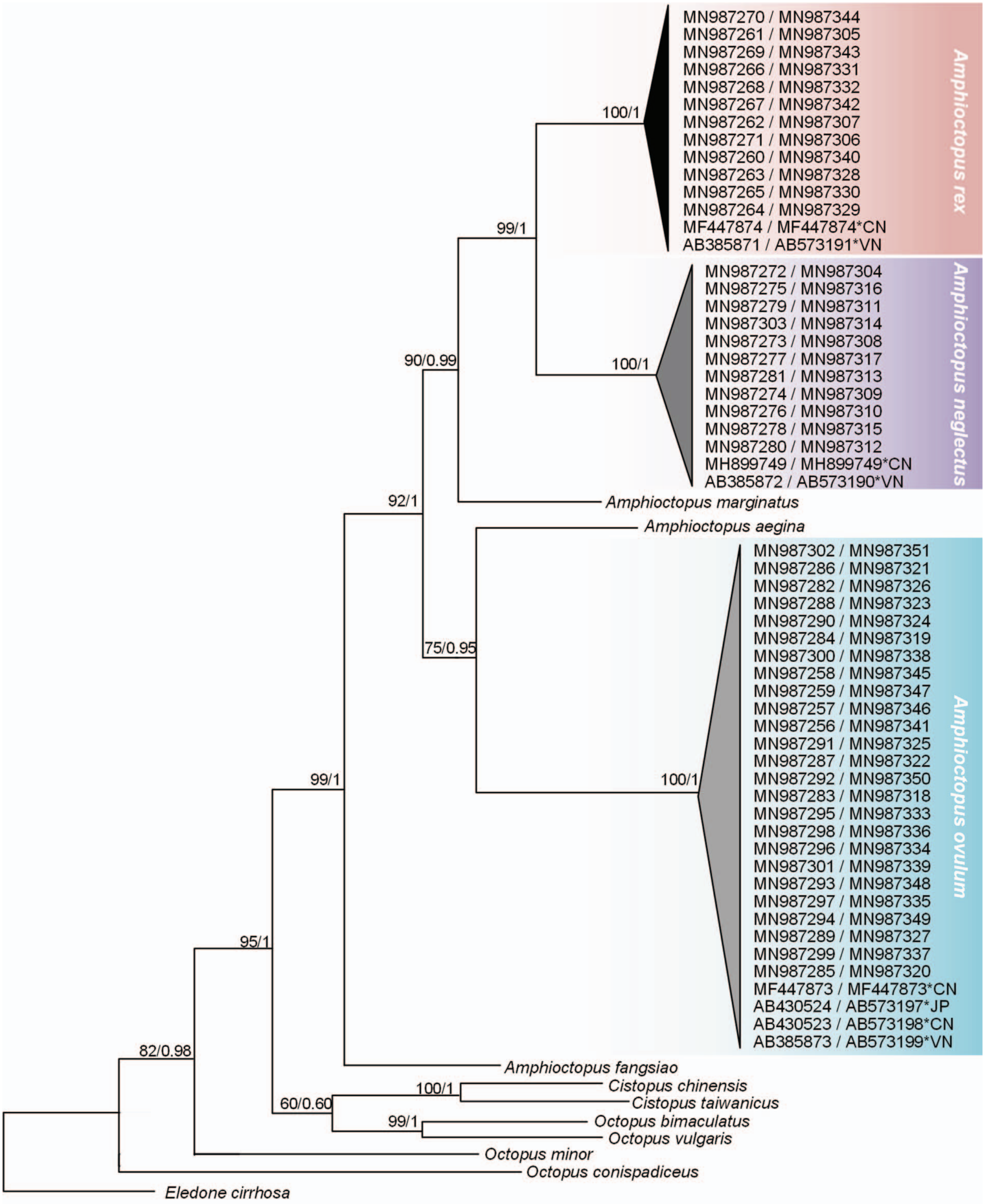

Fig. 1. Phylogenetic trees derived from ML and BI analyses based on partial *COI* and *COIII* sequences. The first number at each node is bootstrap of ML analyses and the second number is Bayesian posterior probability. GenBank accession numbers are presented in order of *COI*/*COIII*. An asterisk indicates the sequence is from GenBank. Country abbreviations are shown in Table 1.

reaction buffer (Mg^{2+} plus, Takara, Shiga, Japan), 0.5 μL of template DNA (~100 ng), 0.2 μL of each primers (20 μM) and 0.1 μL of rTaq DNA polymerase (1 U, Takara). The following PCR conditions were used: 94°C for 3 min, 32 cycles of 94°C for 45 s, 72°C for 1 min, 50°C for 80 s, and a final extension step at 72°C for 5 min. The PCR products were purified using an EZ-10 spin column DNA gel extraction kit (Sangon Biotech, Shanghai, PR China), sent to the Sangon Co. (Shanghai, China) and sequenced using an ABI 3730 XL automatic sequencer (Applied Biosystems, New York, NY, USA) by the primer walking method.

A total of 66 octopods were selected for phylogenetic analyses. *Eledone cirrhosa* was used as outgroup. Details of catalogue numbers, GenBank accession numbers, collecting sites and reference of all individuals is listed in Table 1. ModelFinder (Kalyaanamoorthy *et al*. 2017) plug-in integrated into PhyloSuite (Zhang *et al*. 2020) was used to select the best-fit model using BIC criteria, and the GTR+F+I+G4 model was selected for the *COI* and *COIII* genes. Phylogenetic analyses were performed using two different algorithms: Maximum Likelihood (ML) and Bayesian Inference (BI). ML analysis was carried out on the RAxML web server on the CIPRES Science Gateway (ver. 3.3, see http://www.phylo.org/index.php/; Miller *et al*. 2010) with 1000 replicates. BI analysis was conducted in MrBayes (ver. 3.2.6, see http://nbisweden.github.io/MrBayes/; Ronquist *et al*. 2012) plug-in in PhyloSuite for 100 million generations (sampling every 1000 generations), in which the initial 25% of sampled data were discarded as burn-in. Convergence of the parameters was checked using Tracer (ver. 1.7.1, A. Rambaut and A. J. Drummond, see http://beast.bio.ed.ac.uk/Tracer; Rambaut *et al*. 2018).

Results

Phylogenetic analysis

The ML and BI analyses of *COI* and *COIII* resulted in identical tree topologies with high support values for most internodes (Fig. 1). Violet-ringed octopuses clearly grouped into three clades with strong support values (bootstrap (BS) = 100, posterior probability (PP) = 1), which reflects the distinct genetic differentiation among three morphologically indistinct species (Fig. 1). *Amphioctopus rex* and *A. neglectus* clustered into a sister taxon with very high support value (BS = 100, PP = 1), and clustered with the remaining *Amphioctopus* species, which supported the monophyly of this genus.

Statistical analysis

In this study, 17 morphometric and meristic characters were selected for statistical analysis among three species of violet-ringed octopods (Fig. 2; Tables S2, S3). There are some similarities in mantle width (MWI), head width (HWI), funnel length (FLI) and enlarged sucker diameter (LSDI) among them, reflecting both common overall shapes and close relationship. Fig. 2 shows that *A. ovulum* has the shortest arms I to III (AMI1–3). Hectocotylus suckers in *A. rex* (HASC 69-*77*-85) are more abundant than those in *A. neglectus* (HASC 60-*63*-67) and in *A. ovulum* (HASC 54-*61*-65). The length of ligula in *A. neglectus* (LLI 11.6-*12.1*-12.7) is approximately 2–3 times than that of *A. rex* (LLI 2.6-*3.3*-4.1) or *A. ovulum* (LLI 3.7-*4.3*-5.1). Fig. 2 also shows that the ring of *A. neglectus* is significantly narrower than those of the other two species.

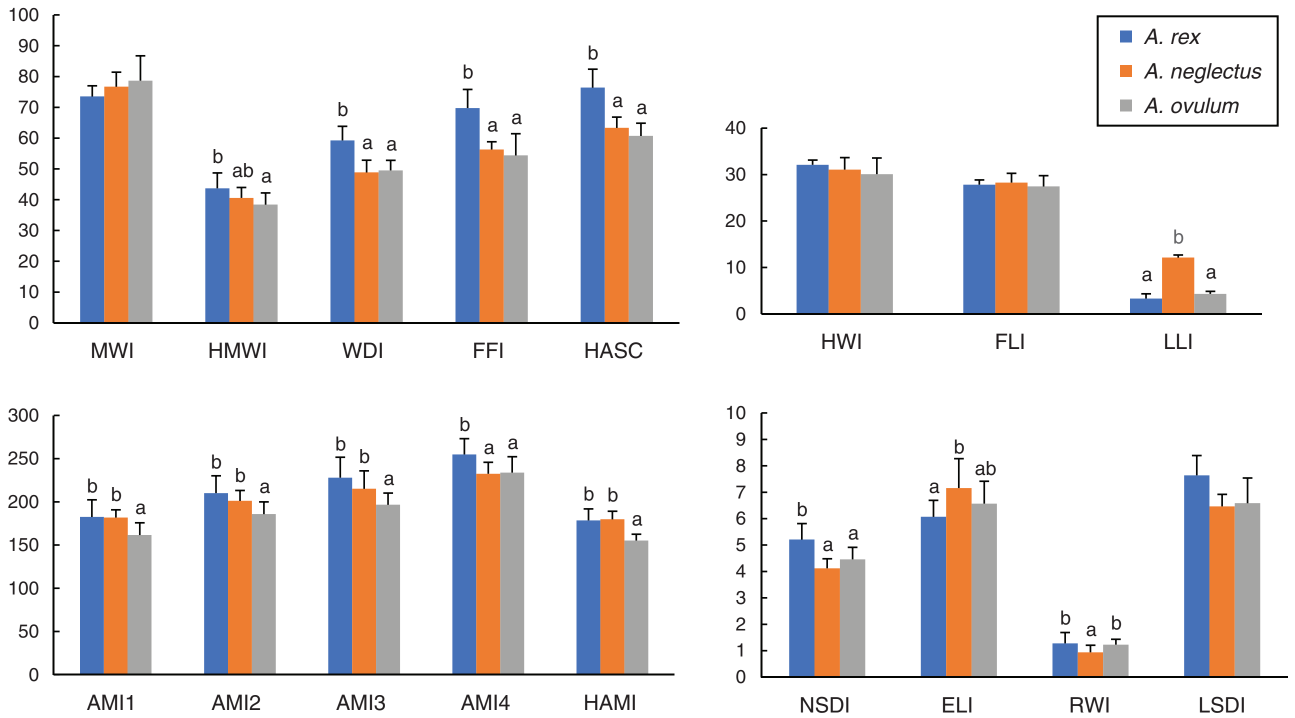

Fig. 2. Statistical analysis of 17 quantitative characters of three species of violet-ringed octopods. Indices refer to Table S1.

Systematics

Order **OCTOPODA** Leach, 1818
Family **OCTOPODIDAE** d'Orbigny, 1840
Genus ***Amphioctopus*** Fischer, 1882

Type species: Octopus membranaceus Quoy & Gaimard, 1832.

Diagnosis

Octopods with biserial arm suckers; skin sculpture of dorsal mantle, head and webs onto oral surface of shallow dorsal web; transverse pair of white spots on dorsal mantle, slightly anterior to midpoint of mantle; colour patterns often incorporate dark leading edges along dorso-lateral face of arms I to III; four short longitudinal ridges of skin in diamond arrangement on dorsal mantle; false eye-spots (ocelli) present in some species, often with gold, blue, violet iridescent ring (Norman *et al*. 2013).

Included species

Amphioctopus aegina (Gray, 1849), *Amphioctopus burryi* (Voss, 1950), *Amphioctopus exannulatus* (Norman, 1993), *Amphioctopus fangsiao* (d'Orbigny, 1839–1841 [in Ferrusac and d'Orbigny, 1834–1848]), *Amphioctopus kagoshimensis* (Ortmann, 1888), *Amphioctopus marginatus* (Taki, 1964), *Amphioctopus mototi* (Norman, 1993), *Amphioctopus neglectus* (Nateewathana and Norman, 1999), *Amphioctopus rex* (Nateewathana and Norman, 1999), *Amphioctopus siamensis* (Nateewathana and Norman, 1999), *Amphioctopus ovulum* (Sasaki, 1917) and some rare species.

Amphioctopus ovulum (Sasaki, 1917)

(Fig. 3, 4, 5, 6, 7, 8*A, B*, 9*A, B*, 10; Tables 2, 3)

Polypus ovulum Sasaki in Notes on the Cephalopoda, 1917: 364–365 [original description, Tokyo, Japan].

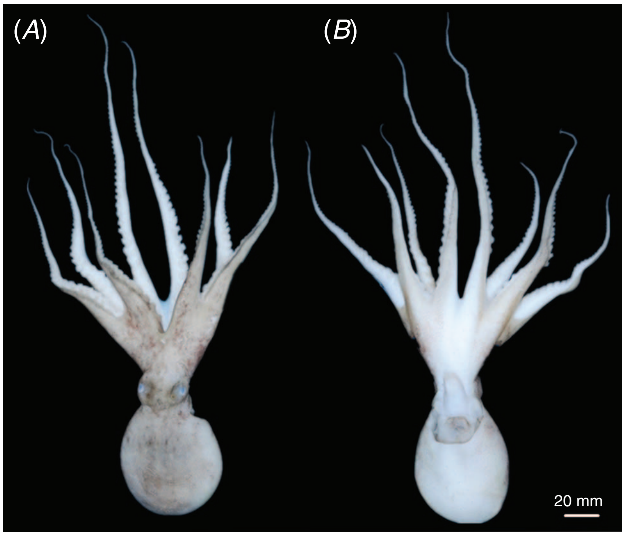

Fig. 3. Whole view of *Amphioctopus ovulum* (OUC-201709110312). (*A*) Dorsal view. (*B*) Ventral view.

Material examined

OUC-201709110311, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 54.2 mm DML, coll. GBSS. OUC-201709110312, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 60.1 mm DML, coll. GBSS (Fig. 3). OUC-201709110313, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 58.8 mm DML, coll. GBSS. OUC-201709110314, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 54.1 mm DML, coll. GBSS. OUC-201709110315, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 53.2 mm DML, coll. GBSS. OUC-201709110316, Beihai, 21°47′N, 109°12′E, 11 September 2017, submature female, 54.9 mm DML, coll. GBSS. OUC-201709110317, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 53.0 mm DML, coll. GBSS. OUC-201709110318, Beihai, 21°47′N, 109°12′E, 11 September 2017, submature female, 58.1 mm DML, coll. GBSS. OUC-201709110319, Beihai, 21°47′N, 109°12′E, 11 September 2017, mature male, 48.3 mm DML, coll. GBSS. OUC-201709110320, Beihai, 21°47′N, 109°12′E, 11 September 2017, submature female, 60.9 mm DML, coll. GBSS. OUC-201710200311, Zhoushan, 30°15′N, 122°12′E, 20 October 2017, mature male, 56.9 mm DML, coll. GBSS. OUC-201710200313, Zhoushan, 30°15′N, 122°12′E, 20 October 2017, mature male, 52.8 mm DML, coll. GBSS. OUC-201710200314, Zhoushan, 30°15′N, 122°12′E, 20 October 2017, mature male, 57.2 mm DML, coll. GBSS. OUC-201710200318, Zhoushan, 30°15′N, 122°12′E, 20 October 2017, mature male, 49.1 mm DML, coll. GBSS. OUC-201104220301, Nanning, 22°86′N, 108°29′E, 22 April 2011, mature female, 74.9 mm DML, coll. GBSS. OUC-201605160307, Wenzhou, 27°60′N, 120°56′E, 16 May 2016, mature male, 40.3 mm DML, coll. GBSS. OUC-201805060311, Beihai, 21°47′N, 109°12′E, 6 May 2018, mature male, 58.0 mm DML, coll. GBSS. OUC-201805060312, Beihai, 21°47′N, 109°12′E, 6 May 2018, mature female, 64.1 mm DML, coll. GBSS.

Diagnosis

Small to medium-sized species; head narrower than mantle width; a pair of small papillae over each eye; transverse pair of white spots on dorsal mantle, slightly anterior to midpoint of mantle; gills with 7–8 lamellae per demibranch; arms short, lengths subequal; narrow dark stripes along dorso-lateral surface of arms I to III; web depths shallow; false-eye spots (ocelli) with a small simple cobaltic/violet iridescent ring; terminal organ diverticulum swollen; spawned eggs small.

Description

Morphometric data

Measurements, counts and morphometric indices are summarised in Tables 2 and 3.

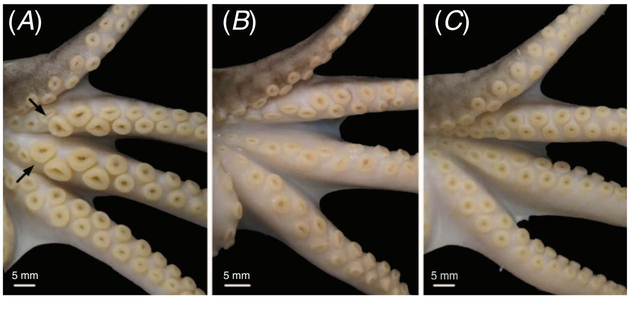

Fig. 4. Arm suckers of *A. ovulum*. (*A*) Mature male (OUC-201710200314); arrows indicate enlarged suckers at arms II and III. (*B*) Mature male (OUC-201709110314). (*C*) Mature female (OUC-201710200310).

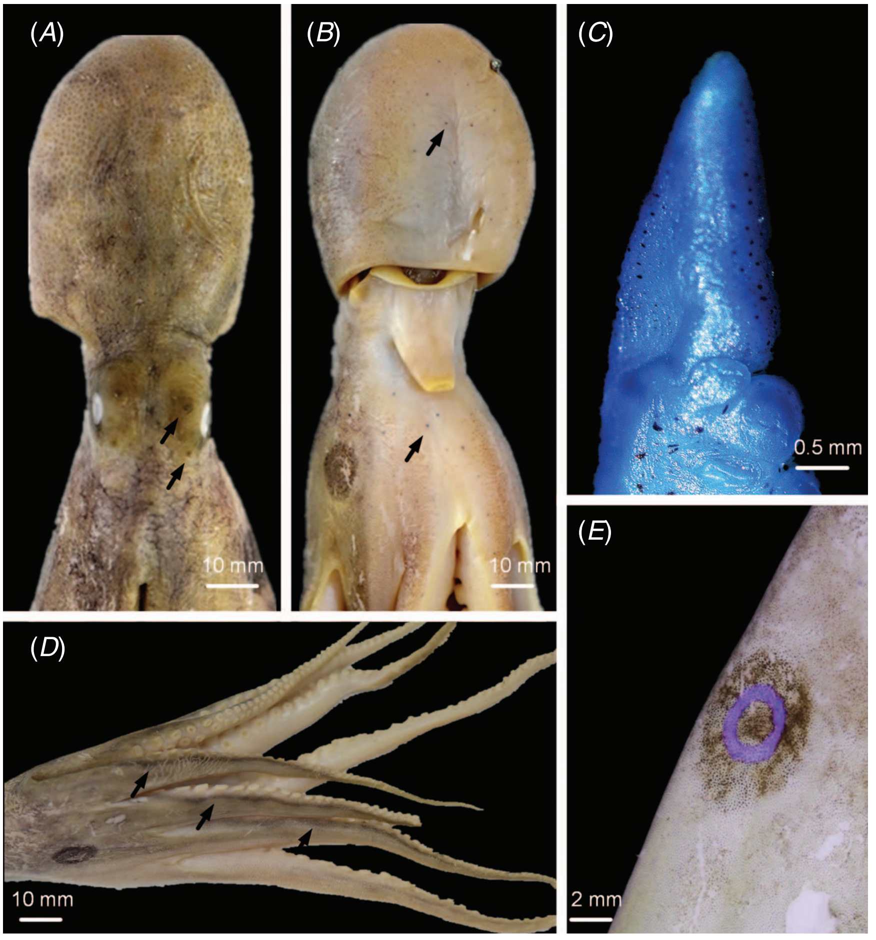

Fig. 5. External characters of *A. ovulum*. (*A*) Partial body in dorsal view (OUC-201710200314); arrows indicate two cirri above each eye. (*B*) Partial body in ventral view (OUC-201710200314); arrows indicate chromatophores on the ventral surface of mantle and base of the arm IV. (*C*) Hectocotylus of mature males (OUC-201710200313); calamus and ligula are dyed by methylene solution. (*D*) Arms in lateral view (OUC-201710200318); arrows indicate stripes along dorsal lateral surface of arms I–III. (*E*) Ocellus (OUC-201710200311).

External features

Small to moderate-sized species (TL 180.0-*195.2*-216.0; ML 48.0-*55.5*-61.0; TWg 19.1-*26.1*-32.3). Mantle oval, dorsally longer than wide (MWI 66.1-*78.7*-89.7). Head obviously narrower than mantle (MWI 26.2-*30.1*-37.5; HMWI 30.8-*38.4*-43.6), a little concave above, clearly demarcated with body. Eyes prominent and small (ELI 4.9-*6.6*-7.8). Funnel of moderate length (Fig. 3*B*) (FLI 24.1-*27.5*-31.3; FFI 11.1-*15.0*-18.2). Arms short (2–3 times mantle length), slender (AWI 12.1-*13.1*-14.0) and tapering to tips. Ventral arms longest, arm formula 4 > 3 > 2 > 1. Webs shallow (WDI 44.3-*49.5*-53.7), dorsal web shallowest, web formula typically D > C > B > E > A or D > C > E > B > A. Arm suckers biserial. Slightly enlarged suckers present in mature males, but not mature females, typically 5th to 8th on arms II and III (Fig. 4*A*, *C*) (LSDI 7.5-*7.8*-8.1; NSDI 3.9-*4.5*-5.4). Diameter of enlarged suckers 1.5–2 times that of adjacent normal suckers. Notably, several mature males showed barely distinguishable enlarged suckers (Fig. 4*B*). Right third arm of mature males hectocotylised, slightly shorter than opposite arm (Fig. 5*C*) (OAI 73.8-*78.8*-86.0). Sucker counts 54–65 on hectocotylus of mature males. Ligula small (LLI 3.7-*4.3*-5.1), with a shallow, conical calamus (CaLI 18.8-*21.5*-24.2) (Fig. 5*C*). Gills with 7–8 lamellae per demibranch.

Fresh and fixed specimens dark or light brown in colour dorsally, shading off to paler on ventral surface (Fig. 3, 4*A*, *B*). Dorsal surface of mantle and head covered with small warts (Fig. 5*A*). Broad dark stripe along dorsal-lateral surface of arms I –III (Fig. 5*D*). A dark ocellar patch with a cobalt or violet ring located antero-ventrally to each eye, between the bases of arms II and III (Fig. 5*E*). A pair of cirri above each eye, the posterior clearly made out, the anterior much smaller (Fig. 5*A*). Chromatophores scattered on the ventral surface of mantle and the base of arm IV (Fig. 5*B*).

Digestive system

Digestive system illustrated in Fig. 6. Buccal mass moderate size. Anterior salivary glands small, approximately one-quarter length of buccal mass. Posterior salivary glands moderate, approximately twice size of anterior salivary glands. Crop diverticulum distinct. Caecum with one whorl. Intestine moderate in length with small anus. Digestive gland well developed. Ink sac faint in fixed specimens (Fig. 6).

Chitinous beaks dark brown. Upper beak (Fig. 7*A*) with a short hooked rostrum, narrow hood, short wings and broad lateral wall. Lower beak with a short rostrum, narrow hood, long and moderately broad wings (Fig. 7*B, C*). Radulae (Fig. 8*A, B*) with seven transverse rows of teeth and two rows of marginal plates. One central rachidian tooth and three lateral teeth on either side, i.e. a 3·1·3 formula. Rachidian tooth with one or two sharp lateral cusps on each side of median cone. Lateral cusps migrating from lateral to medial position over two rows, every two or three forming a repeating unit. First lateral tooth smallest, with one medial cusp, second lateral tooth with wide heel, one dagger-like cusp, base concave, third lateral tooth with a long sabre-like cusp, short base.

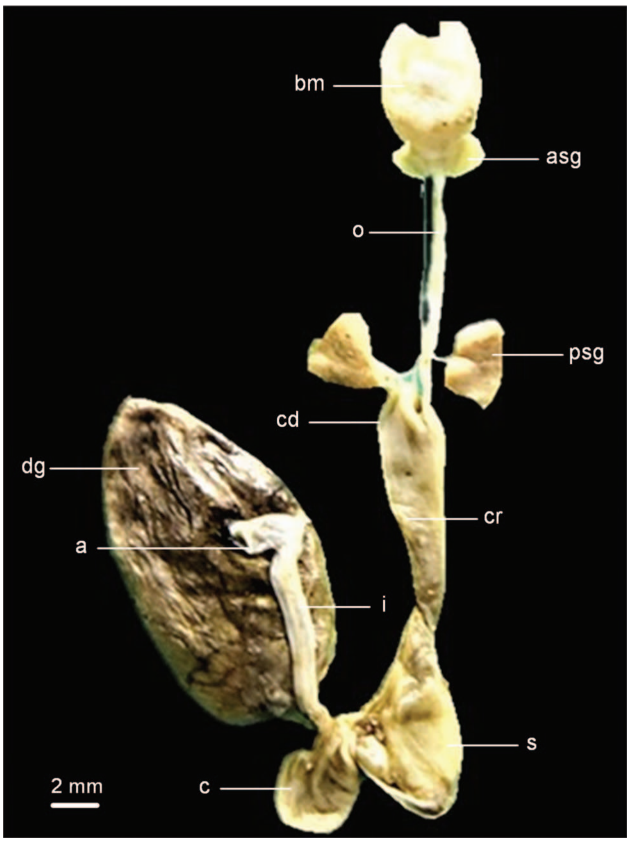

Fig. 6. Digestive system of *A. ovulum* (OUC-201709110318). Abbreviations: a, anus; asg, anterior salivary gland; bm, buccal mass; c, caecum; cd, crop diverticulum; cr, crop; dg, digestive gland; i, intestine; o, oesophagus; psg, posterior salivary gland; s, stomach.

Reproductive system

Male reproductive system illustrated in Fig. 9*A*. Male terminal organ hollow tube, moderate in length (Table 3) (TOLI 9.8-*10.4*-11.0), curved with swollen diverticulum. Vas deferens slender and coiled. Spermatophore storage sac of mature male developed, full of mature spermatophores (Table 3) (SpN 7–8). Spermatophores long (SpLI 96.2–129.6) (Table 3; Fig. 9*B*). Sperm reservoir approximately two-fifths of entire length. Ejaculatory apparatus with ~240 coils.

Female reproductive system illustrated in Fig. 10*A*. Mature ovary as long as broad. Distal oviducts long and thin. Oviducal glands without radiating chambers, wrapped in ligament. Mature eggs numerous and very small (Table 3; Fig. 10*B, C*) (EgLI 1.4–2.1, EgWI 0.4–0.7).

Geographical distribution

Amphioctopus ovulum is a benthic species which is distributed in most coastal waters of eastern continental Asia (Fig. 11): the species has been reported from the Gulf of Thailand (Norman 1992), Cambodia, Vietnam (Kaneko *et al*. 2008, 2011), Philippines (Norman and Hochberg 1994; Norman and Sweeney 1997), through the South China Sea and the East China Sea (Hasegawa *et al*. 2001; Kubodera and Lu 2002), Tokyo Bay to Kyushu in Japan (Sasaki 1917, 1929; Okutani 2000).

Remarks

Sasaki (1917) designated the species name *A. ovulum* on the basis of small eggs. The species is distinct from *A. fangsiao* (large eggs), and shows some similarities with two other Asian violet-ringed *Amphioctopus* species (i.e. *A. rex* and *A. neglectus*). The syntypes (type repository unresolved; MSUT? FMHU?) of the species were purchased from a fish market, Tokyo, Japan, and presumed to be no longer extant (Toll and Voss 1998).

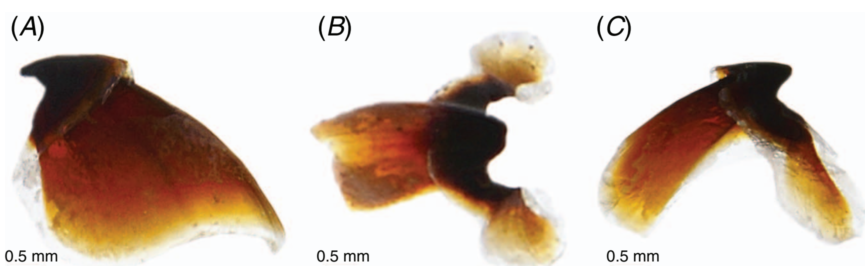

Fig. 7. Beaks of *A. ovulum* (OUC-201605160307). (*A*) Upper beak, lateral view. (*B*) Lower beak, top view. (*C*) Lower beak, lateral view.

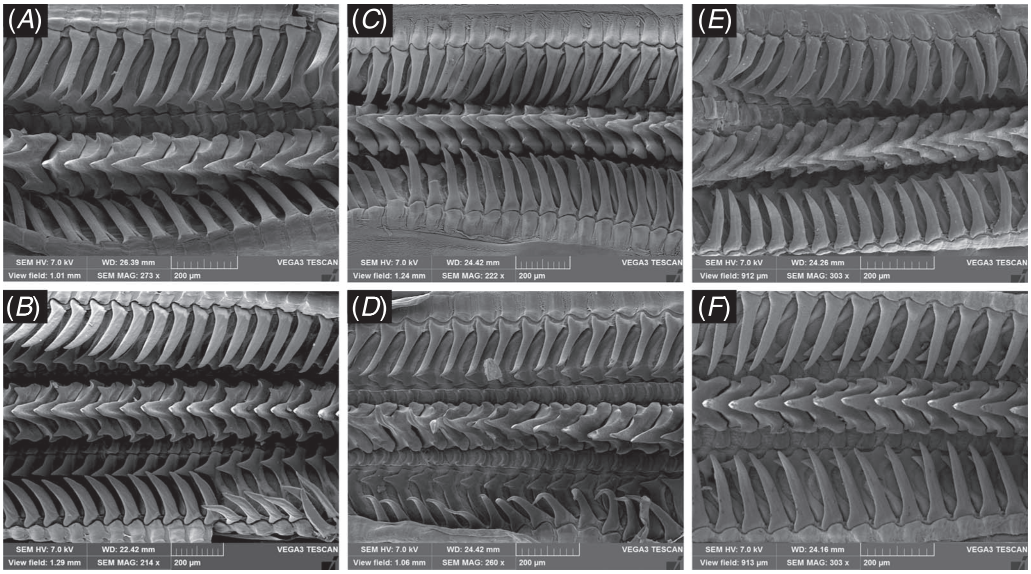

Fig. 8. Radulae. (*A*) *A. ovulum* (OUC-201805060312), female. (*B*) *A. ovulum* (OUC-201805060311), male. (*C*) *A. neglectus* (OUC-201104220303), female. (*D*) *A. neglectus* (OUC-201709110310), male. (*E*) *A. rex* (OUC-201605160310), female. (*F*) *A. rex* (OUC-201605160309), male.

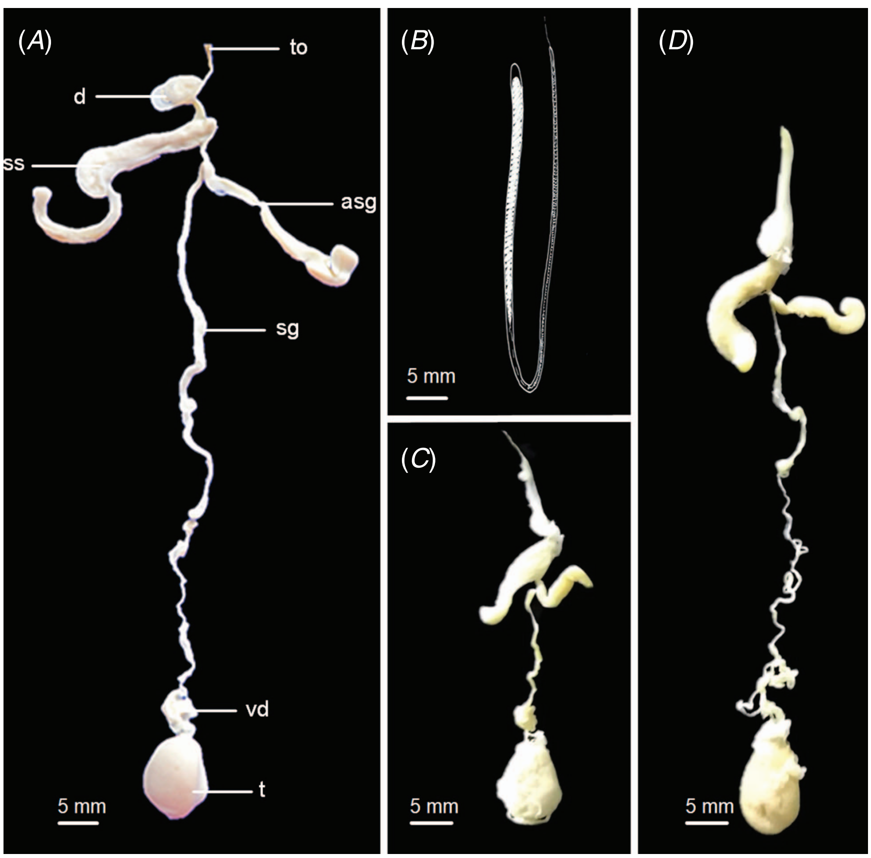

Fig. 9. Male reproductive system. (*A*) *A. ovulum* (OUC-201709110317). (*B*) Spermatophore of *A. ovulum*. (*C*) *A. rex* (OUC-201605160309). (*D*) *A. neglectus* (OUC-201709110307). Abbreviations: asg, accessory spermatophoric gland; d, diverticulum; sg, spermatophoric gland; ss, spermatophore storage sac; t, testis; to, terminal organ; vd, vas deferens.

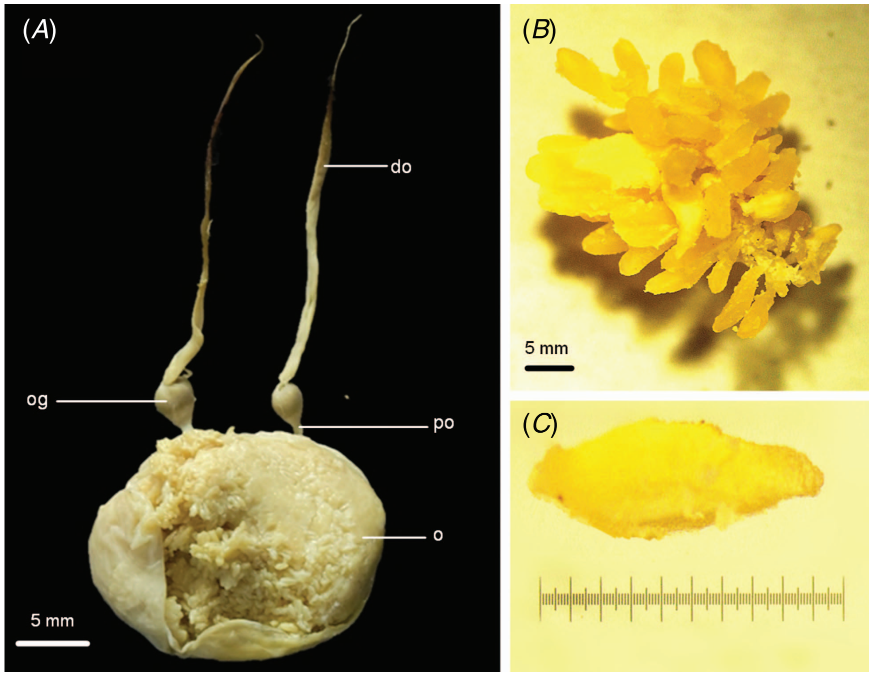

Fig. 10. Female reproductive system of *A. ovulum* (OUC-201104220301). (*A*) Whole view. (*B*) Egg cluster. (*C*) Single laid egg (length = 0.95 mm). Abbreviations: do, distal oviduct; o, ovary; og, oviducal gland; po, proximal oviduct.

Table 2. Measurements, counts and morphometric indices of *Amphioctopus ovulum* for external characteristics analyses

Notes: StM, stage of maturity; M, mature; S, submature; H, hectocotylus; –, not recorded

Number	OUC-20 1709110 311	OUC-20 1709110 312	OUC-20 1709110 313	OUC-20 1709110 314	OUC-20 1709110 315	OUC-20 1709110 316	OUC-20 1709110 317	OUC-20 1709110 318	OUC-20 1709110 319	OUC-20 1709110 320
Sex	♂	♂	♂	♂	♂	♀	♂	♀	♂	♀
StM	M	M	M	M	M	S	M	S	M	S
TL	181.2	209.9	199.3	179.8	184.1	201.2	184.3	216.0	188.1	209.2
TWg	31.6	30.0	27.7	24.2	19.1	25.0	25. 6	32.3	20.0	25.4
ML	54.2	60.1	58.8	54.1	53.2	54.9	53.0	58.1	48.3	60.9
MWI	85.2	68.3	66.1	79.6	75.5	74.5	81.1	89.7	89.6	77.0
HWI	33.3	26.7	28.8	29.6	28.3	30.9	32.1	27.6	37.5	26.2
HMWI	39.1	39.0	43.6	37.2	37.5	41.5	39.5	30.8	41.9	34.0
AMI1	164.8	156.7	147.5	151.9	156.6	174.5	183.0	158.6	181.3	141.0
2	194.4	165.0	179.7	183.3	184.9	185.5	203.8	187.9	208.3	165.6
3	203.7	175.0	193.2	185.2	192.5	192.7	211.3	198.3	222.9	191.8
4	218.5	236.7	215.3	224.1	224.5	245.5	226.4	256.9	270.8	219.7
NSDI	5.4	4.0	4.7	4.6	4.2	4.7	4.0	4.5	4.6	3.9
WDI	53.7	45.0	49.2	50.0	52.8	52.7	50.9	46.6	50.0	44.3
ELI	7.0	5.8	7.8	6.3	7.2	6.9	6.8	5.9	7.1	4.9
FLI	27.8	26.7	27.1	24.1	24.5	29.1	30.2	27.6	31.3	26.2
FFI	46.7	62.5	56.3	46.2	53.8	68.8	50.0	56.3	53.3	50.0
RWI	1.2	1.2	1.1	1.1	1.7	1.4	1.0	1.2	1.3	1.1
LSDI	8.1	5.8	7.5	6.3	6.0	–	5.5	–	6.9	–
LLI	3.7	3.7	4.2	4.3	4.5	–	5.1	–	4.8	–
CaLI	24.2	20.3	18.8	24.1	22.8	–	21.7	–	18.9	–
HAMI	159.3	145.0	145.8	159.3	156.6	–	156.6	–	164.6	–
OAI	78.2	82.9	75.4	86.0	81.4	–	74.1	–	73.8	–
HASC	65H	64H	54H	58H	58H	–	62H	–	64H	–

Table 3. Measurements, counts and morphometric indices of *A. ovulum* for reproductive system characteristics analyses

Notes: StM, stage of maturity; M, mature; –, not recorded

Number	OUC-201709 110311	OUC-201709 110312	OUC-201709 110313	OUC-201709 110314	OUC-201104 220301
Sex	♂	♂	♂	♂	♀
StM	M	M	M	M	M
ML	56.9	54.1	52.8	57.2	74.9
TOLI	9.8	10.2	11.0	10.7	–
SpN	7	7	7	8	–
SpLI	98.6–120.9	100.2–129.6	99.1–120.1	96.2–122.0	–
EgLI	–	–		–	1.4–2.1
EgWI	–	–		–	0.4–0.7

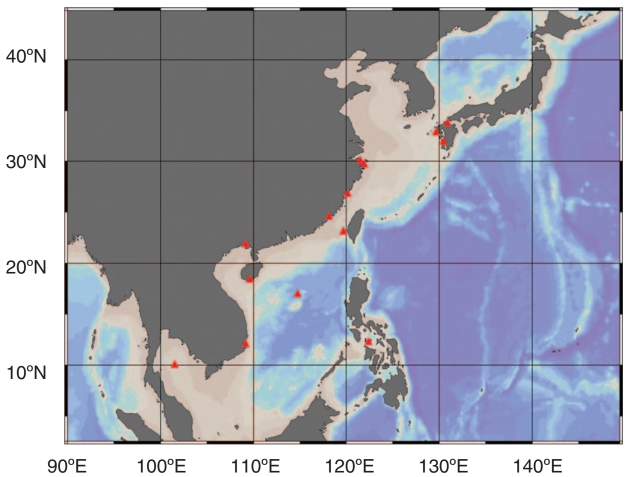

Fig. 11. Geographical distribution of *A. ovulum*. Red triangles indicate the known collecting sites.

Morphological comparison

Previous descriptive characters were summarised for comparison in Table S4. Three species of violet-ringed octopods all have narrow dark stripes along dorsal edges of arms I to III and no significant difference in gill lamellae. The arm formula of 4 > 3 > 2 > 1 is consistent. In contrast, the web formula is variable, with the second deepest sector being any of C or E in most cases for these three species (Table S4). The spermatophores of *A. ovulum* are much longer than either of *A. rex* and *A. neglectus*. The terminal organ diverticulum can be a useful character for distinguishing *A. ovulum* because of its swollen, well-marked diverticulum in comparison to the small diverticula of *A. rex* and *A. neglectus* (Fig. 9*A, C, D*; Table S4). The number of cirri above each eye distinguishes *A. ovulum* easily from *A. rex*, which is two in the former and one in the latter. However, this character cannot be used to distinguish *A. neglectus* from *A. ovulum* and *A. rex*, because it can be one or two in *A. neglectus* (Fig. 5*A*; Table S4).

Radulae of three species of violet-ringed octopods are illustrated in Fig. 8. They exhibit identical teeth patterns. Difference is mainly reflected in the lateral cusps on either side of the rachidian tooth. The lateral cusps are arranged regularly, varying in height. Two or three rachidian teeth form a repeating unit in *A. ovulum* (Fig. 8*A, B*). By contrast, the rachidian teeth repeat every three times in *A. neglectus* (Fig. 8*C, D*), and four or five times in *A. rex* (Fig. 8*E, F*).

Key to the three species of violet-ringed octopods

1 Small white spots on dorsal mantle, ring on ocellus narrow, ligula long, U-shaped transverse bar between eyes .... *A. neglectus*
Transverse pair of white spots on dorsal mantle, ring on ocellus moderate, ligula short, no U-shaped transverse bar between eyes 2

2 One cirrus above each eye, longitudinal brownish bar through eye, terminal organ diverticulum normal, spermatophores moderate.. *A. rex*
Two cirri above each eye, no longitudinal brownish bar through eye, terminal organ diverticulum swollen, spermatophores long .... *A. ovulum*

Discussion

Our redescription was needed as there has been a lot of confusion regarding the diagnostic traits of *A. ovulum*, caused by the loss of the syntypes (Toll and Voss 1998). We believe the most reliable way to identify this species is to trace the description of this species collected from the original location. It was originally purchased by Sasaki from a fish market in Tokyo, Japan. However, this location was questioned by Norman (1992): he proposed that this species might have been collected from outside Japanese waters, especially tropical Indo-West Pacific waters, and then transported to Japan (Norman 1992). We speculate that marine transport in the early 20th century was not sufficient for long-distance transport. Subsequently, the geographical location of Japan for *A. ovulum* was successively confirmed by Okutani (2000), Kubodera and Lu (2002), and Kaneko *et al.* (2008). In this study, the key morphological characters are congruent with previous descriptions (Sasaki 1917, 1929; Okutani 2000; Kaneko *et al.* 2008), especially the violet rings on the dark ocellus, numerous small eggs, a well-marked diverticulum, and two cirri above each eye. *Amphioctopus ovulum* has small eggs, whereas *A. fangsiao* has larger ones, which supports the 'two species hypothesis' (Pickford and McConnaughey 1949; Gleadall 1991).

Norman and Sweeney (1997) distinguished one species from *A. rex* specimens in Philippines. The characters of much shorter ligula and much larger spermatophores with a sperm coiled in many more whorls agreed well with *A. ovulum*, which was also mentioned in the Gulf of Thailand by Norman and Hochberg (1994) before that. Additionally, they were recorded in Cambodia, Vietnam and Japan (Sasaki 1917, 1929; Norman 1992; Okutani 2000; Kaneko *et al*. 2008, 2011). Our results showed that *A. ovulum* specimens were common in the South China Sea and the East China Sea. Consequently, *A. ovulum* is widely distributed along most coastal waters of eastern continental Asia. This can be explained by the fact that octopuses with small eggs can increase dispersal capacity during their planktonic phase (Sasaki 1929; Pickford and McConnaughey 1949).

This study provided the opportunity to compare morphological characters among similar species. Our study showed clearly that what has been considered a single species contains, in fact, three similar but distinct taxa. These three species, *A. ovulum*, *A. rex* and *A. neglectus*, were added to the octopod fauna of China. They share some external characters, such as the violet ring on the ocelli, and a transverse pair of white spots on the dorsal mantle. *Amphioctopus neglectus* has numerous small, rounded white spots on the dorsal mantle and a narrow, small, slightly U-shaped transverse bar between the eyes. *Amphioctopus rex* has a short longitudinal black bar through the eyes. These characters are much more pronounced in fresh, living specimens than in preserved ones (Norman *et al*. 2013). Among the species reported herein, the radula varies in the expression of the lateral cusps on either side of the rachidian tooth. Nixon (1998) proposed that the radulae of Cephalopoda are of great systematic value at the generic and specific levels. The teeth patterns in octopods were similar, but subtle differences were apparent (Nixon 1998), which was used to separate congeneric octopods (Adam 1983; Nixon 1998; Ibáñez *et al*. 2008). The ocellus is an important character and proved valuable for differentiating ocellate species (Huffard and Hochberg 2005). *Amphioctopus neglectus* is distinguished from *A. rex* and *A. ovulum* by the width of the ring on the ocellus, which is much narrower than others. Notably, Sasaki (1929) described the ocellus of *A. ovulum* as being nearer to the web than to the eye, which was regarded as a key morphological character in identifying this species. However, given the variable shape of this character, its systematic value was considered to be of little significance in our study, which was supported by Gleadall (1991).

Molecular study revealed the relationships among three species of violet-ringed octopods and their phylogenetic status in the Octopodidae. Violet-ringed octopuses were consistently recovered as three monophyletic clades. All specimens of *A. ovulum* collected in our study clustered with those analysed previously (Kaneko *et al*. 2011; Tang *et al*. 2019). The topology of this clade provided insights into the distribution of *A. ovulum*, with the extension from Japan to the East China Sea and the South China Sea. It is strongly supported that *A. ovulum* is distantly related to *A. rex* and *A. neglectus*, whereas the latter two cluster into a sister taxon, as indicated with strong support value (BS = 100, PP = 1). The phylogenetic results of (((*A. rex* + *A. neglectus*) + *A. marginatus*) + (*A. aegina* + *A. ovulum*)) + *A. fangsiao* showed that ocellate *Amphioctopus* species do not form a monophyletic clade when non-ocellate *Amphioctopus* are included. Huffard and Hochberg (2005) observed that non-ocellate *Amphioctopus* species such as *A. marginatus* has a transient, and diffuse, dark trellis in the ocellus location, which can exhibit an irregular (approximately circular) darkening when they are alarmed (Norman 1992, 2000). *Amphioctopus marginatus* and *A. aegina* may therefore represent 'incipient' ocellate species and are associated with ocellate species in *Amphioctopus* through the trellis, which is supported by the phylogenetic trees in this study.

Conclusions

In summary, we focused on the redescription of *A. ovulum*, and performed comparative morphological analyses among three species of violet-ringed octopods that have been confused with each other. Both morphological and molecular phylogenetic analyses revealed that *A. ovulum* is common and there is a high diversity of violet-ringed octopuses in coastal waters of China. Descriptive characters seem to be inconclusive, especially when the specimens are in bad condition or fixed in alcohol/formalin. In this study, morphometric and meristic characters can overcome this shortcoming.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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References

Adam, V. W. (1983). Cephalopoda from west and South Africa. *Atlantide Report* **13**, 151–180.

Akasaki, T., Nikaido, M., Tsuchiya, K., Segawa, S., Hasegawa, M., and Okada, N. (2006). Extensive mitochondrial gene arrangements in coleoid Cephalopoda and their phylogenetic implications. *Molecular Phylogenetics and Evolution* **38**, 648–658. doi:10.1016/j.ympev.2005.10.018

Cheng, R., Zheng, X., Lin, X., Yang, J., and Li, Q. (2012). Determination of the complete mitochondrial DNA sequence of *Octopus minor*. *Molecular Biology Reports* **39**, 3461–3470. doi:10.1007/s11033-011-1118-2

Cheng, R., Zheng, X., Ma, Y., and Li, Q. (2013). 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* **8**, e84216. doi:10.1371/journal.pone.0084216

Domínguez-Contreras, J. F., Munguia-Vega, A., Ceballos-Vázquez, B. P., García-Rodriguez, F. J., and Arellano-Martinez, M. (2016). The complete mitochondrial genome of *Octopus bimaculatus* Verrill, 1883 from the Gulf of California. *Mitochondrial DNA* **27**, 4584–4585. doi:10.3109/19401736.2015.1101575

Dong, Z. (1978). On the geographical distribution of the cephalopods in the Chinese waters. *Oceanologia et Limnologia Sinica* **9**, 108–116. [In Chinese with English abstract].

Dong, Z. (1987). *Octopus ovulum* (Sasaki, 1917). In 'Fauna Sinica. Phylum Mollusca: Class Cephalopoda'. pp. 177–178. (Science Press: Beijing, PR China.) [In Chinese with English abstract].

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.

Gleadall, I. G. (1991). The Asian ocellate octopus. I. The two species hypothesis of Sasaki and Pickford. *Annals of Applied Information Sciences* **16**, 161–171.

Guzik, M. T., Norman, M. D., and Crozier, R. H. (2005). Molecular phylogeny of the benthic shallow-water octopuses (Cephalopoda: Octopodinae). *Molecular Phylogenetics and Evolution* **37**, 235–248. doi:10.1016/j.ympev.2005.05.009

Hasegawa, K., Saito, H., Kubodera, T., and Xu, F. (2001). Marine molluscs collected from the shallow waters of Hainan Island, South China Sea, by China–Japan joint research in 1997. *National Science Museum Monographs* **21**, 1–43.

Huffard, C. L., and Hochberg, F. G. (2005). Description of a new species of the genus *Amphioctopus* (Mollusca: Octopodidae) from the Hawaiian Islands. *Molluscan Research* **25**, 113–128.

Ibáñez, C. M., Sepúlveda, R. D., Guerrero, J., and Chong, J. (2008). Redescription of *Robsonella fontaniana* (Cephalopoda: Octopodidae). *Journal of the Marine Biological Association of the United Kingdom* **88**, 617–624. doi:10.1017/S002531540800101X

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., and Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**, 587–589. doi:10.1038/nmeth.4285

Kaneko, N., Kubodera, T., Dihn, T., and Chung, B. D. (2008). Shallow-water benthic octopuses (Cephalopoda, Octopodidae) collected from the coastal waters of Vietnam. *Bulletin of the National Museum of Natural Sciences, Series A* **34**, 105–122.

Kaneko, N., Kubodera, T., and Iguchis, A. (2011). Taxonomic study of shallow-water octopuses (Cephalopoda: Octopodidae) in Japan and adjacent waters using mitochondrial genes with perspectives on octopus DNA barcoding. *Malacologia* **54**, 97–108. doi:10.4002/040.054.0102

Kubodera, T., and Lu, C. C. (2002). A review of cephalopod fauna in Chinese–Japanese subtropical region. *National Science Museum Monographs* **22**, 159–171.

Li, F. (1983). Studies on the cephalopod fauna of the Taiwan Strait. *Journal of Oceanography in Taiwan Straits* **2**, 103–109. [In Chinese with English abstract].

Ma, Y., Zheng, X., Cheng, R., and Li, Q. (2016). The complete mitochondrial genome of *Octopus conispadiceus* (Sasaki, 1917) (Cephalopoda: Octopodidae). *Mitochondrial DNA* **27**, 1058–1059. doi:10.3109/19401736.2014.928866

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 'Gateway Computing Environments Workshop (GCE)', 14 November 2010, New Orleans, LA, USA. INSPEC Accession number 11705685. (IEEE.) doi:10.1109/GCE.2010.5676129

Nateewathana, A. (1997). The octopod fauna (Cephalopoda: Octopoda) of the Andaman Sea, Thailand. *Phuket Marine Biological Center Special Publication* **17**, 407–452.

Nateewathana, A., and Norman, M. D. (1999). On three new species of ocellate octopuses (Cephalopoda: Octopoda) from Thai waters. *Phuket Marine Biological Center Special Publication* **19**, 445–462.

Nixon, M. (1998). The radulae of Cephalopoda. *Smithsonian Contributions to Zoology* **586**, 39–53.

Norman, M. D. (1992). Ocellate octopuses (Cephalopoda: Octopodidae) of the Great Barrier Reef, Australia: description of two new species and redescription of *Octopus polyzenia* Gray, 1849. *Memoirs of the Museum of Victoria* **53**, 309–344. doi:10.24199/j.mmv.1992.53.14

Norman, M. (2000). 'Cephalopods. A World Guide.' (Conch Books: Bad Kreuznach, Germany.)

Norman, M. D., and Hochberg, F. G. (1994). Shallow-water octopuses (Cephalopoda: Octopodidae) from Hong Kong's territorial waters. In 'The Malacofauna of Hong Kong and Southern China III. Proceedings of the Third International Workshop on the Malacofauna of Hong Kong and Southern China', 13 April–1 May 1992, Hong Kong. (Ed. B. Morton.) Vol. 13, pp. 141–160. (Hong Kong University Press: Hong Kong.)

Norman, M. D., and Sweeney, M. J. (1997). The shallow-water octopuses (Cephalopoda: Octopodidae) of the Philippines. *Invertebrate Systematics* **11**, 89–140. doi:10.1071/IT95026

Norman, M. D., Finn, J. K., and Hochberg, F. G. (2013). Family Octopodidae. In 'Cephalopods of the World. An Annotated and Illustrated Catalogue of Cephalopod Species Known to Date: Octopods and Vampire Squids'. (Eds P. Jereb, C. F. E. Roper, M. D. Norman, and J. K. Finn.) FAO species catalogue for fishery purposes, pp. 75–76. (Food and Agriculture Organization of the United Nations: Rome, Italy.)

Okutani, T. (2000). 'Marine Mollusks in Japan.' (University of Tokyo Press: Tokyo, Japan.)

Pickford, G. E., and McConnaughey, B. H. (1949). The *Octopus bimaculatus* problem: a study in sibling species. *Bulletin of the Bingham Oceanographic Collection* **12**, 1–66.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904. doi:10.1093/sysbio/syy032

Robson, G. C. (1929). *Octopus* (*Octopus*) *areolatus* var. *ovulum*, Sasaki. In 'A monograph of the recent Cephalopoda. Part I. Octopodinae'. pp. 123–125. (British Museum (Natural History): London, UK.)

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542. doi:10.1093/sysbio/sys029

Roper, C. F. E., and Voss, G. L. (1983). Guidelines for taxonomic descriptions of cephalopod species. *Early Medieval Europe* **20**, 39–69.

Sasaki, M. (1917). Notes on the Cephalopoda. *Annotationes Zoologicae Japonenses* **9**, 364–365.

Sasaki, M. (1929). A monograph of the dibranchiate cephalopods of the Japanese and adjacent waters. *Journal of the Faculty of Agriculture, Hokkaido Imperial University* **20**, 55–58.

Strugnell, J. M., Norman, M. D., Vecchione, M., Guzik, M., and Allcock, A. L. (2014). The ink sac clouds octopod evolutionary history. *Hydrobiologia* **725**, 215–235. doi:10.1007/s10750-013-1517-6

Tang, Y., Zheng, X., Ma, Y., Cheng, R., and Li, Q. (2018). The complete mitochondrial genome of *Amphioctopus marginatus* (Cephalopoda: Octopodidae) and the exploration for the optimal DNA barcoding in Octopodidae. *Conservation Genetics Resources* **10**, 115–118. doi:10.1007/s12686-017-0777-2

Tang, Y., Zheng, X., Zhong, H., and Li, Q. (2019). Phylogenetics and comparative analysis of the mitochondrial genomes of three violet-ringed octopuses. *Zoologica Scripta* **48**, 482–493. doi:10.1111/zsc.12359

Toll, R. B., and Voss, G. L. (1998). The systematics and nomenclatural status of the Octopodinae described from the West Pacific region. *Smithsonian Contributions to Zoology* **586**, 489–520.

Yokobori, S., Fukuda, N., Nakamura, M., Aoyama, T., and Oshima, T. (2004). Long-term conservation of six duplicated structural genes in cephalopod mitochondrial genomes. *Molecular Biology and Evolution* **21**, 2034–2046. doi:10.1093/molbev/msh227

Zhang, X., Zheng, X., Ma, Y., and Li, Q. (2017). Complete mitochondrial genome and phylogenetic relationship analyses of *Amphioctopus aegina* (Gray, 1849) (Cephalopoda: Octopodidae). *Mitochondrial DNA* **28**, 17–18. doi:10.3109/19401736.2015.1106522

Zhang, D., Gao, F., Li, W. X., Jakovlić, I., Zou, H., Zhang, J., and Wang, G. T. (2020). PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* **20**, 348–355. doi:10.1111/1755-0998.13096

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