

Shallow-water Benthic Octopuses (Cephalopoda, Octopodidae) Collected from the Coastal Waters of Vietnam

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Abstract Twelve benthic octopus species assigned to four genera were collected from the coastal waters of Vietnam through cooperative research between Vietnamese institutions (Research Institute of Marine Products, Haiphong Institute of Oceanography, Institute for Ecology and Biological Resources Research, National Center for Natural Science and Technology, and Nha Trang Institute of Oceanography) and the National Museum of Nature and Science, Japan. The collection includes five *Amphioctopus* species (*A. aegina*, *A. marginatus*, *A. cf. neglectus*, *A. cf. ovulum*, and *A. cf. rex*), four *Callistoctopus* species (*C. luteus*, *C. sp. 1–3*), one *Cistopus* species (*Cistopus cf. indicus*), and two *Octopus* species (*O. sp. 1–2*). Diagnostic characters, illustrations, and photographs are provided for these taxa. We also performed nucleotide sequence analysis of the mitochondrial cytochrome oxidase I (COI) and evaluated phylogenetic relationships of these species to complement the results of morphological analysis.

Key words: benthic octopus, coastal water, Vietnam.

Introduction

Vietnam is located at the eastern end of the Indochina Peninsula and its long north-south coast faces the South China Sea. There have been a few studies of the shallow-water benthic octopuses of this region despite their importance in subsistence and commercial fisheries. Rochebrune (1882) listed two species from this region, *Cistopus indicus* (Rapp, 1835) and *Octopus harmandi* Rochebrune, 1882. Khromov (1996) investigated the cephalopods in Vietnam, and listed two unidentified octopus (*Octopus sp. 1* and *2*). In the 1999 workshop of the Tropical Marine Mollusc Program (TMMP), three nominal species were

recognized (Hylleberg, 2000): *O. aegina* Gray, 1849, *O. marginatus* Taki, 1964, and *C. indicus* (Rapp, 1835). In these studies, no morphological descriptions or figures were presented based on the specimens examined. Therefore, little information has been available to date on the benthic octopus fauna of Vietnam.

In 2002 and 2003, several Vietnamese research institutions (Research Institute of Marine Products, Haiphong Institute of Oceanography, Institute for Ecology and Biological Resources Research, National Center for Natural Science and Technology, and Nha Trang Institute of Oceanography) and the Department of Zoology of the National Museum of Nature and Science, Japan, conducted a cooperative research project on the marine fauna of Vietnam. The objective of the project was to gain a better understanding of the marine fauna of Vietnam by collecting marine

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organisms from several locations and fishing ports along the Vietnamese coast.

In this component of the project, we examine the shallow-water benthic octopuses of Vietnam and provide a list of recognized species along with diagnostic descriptions and figures. To complement the results of morphological identification, we examined the relationships of species collected in Vietnam based on phylogenetic analyses of DNA sequence data from the cytochrome *c* oxidase subunit I (COI) gene.

Materials and Methods

Sampling

Sampling was conducted at Nha Trang of south Vietnam in January 2002 and December 2003, and at Cat Ba of North Vietnam in September 2002 (Fig. 1). Both locations are centers for local fisheries, predominantly by small fishing boats that undertake day trip bottom trawls in shallow coastal waters. Octopus specimens were sorted from the bottom trawl catches landed at these local fishing ports as well as purchased directly from the local fish markets.

Preliminary species identifications were made where possible in the field and photographs were taken for each taxon in fresh condition. Small pieces of tissue samples were taken from the mantle or damaged arms of some specimens and stored in 99% ethanol for DNA extraction. All samples were subsequently fixed in 10% formalin and later stored in 40% isopropanol. The samples were divided between Vietnamese and Japanese collections and deposited in the Research Institute of Marine Products, Haiphon City, Vietnam and the National Museum of Nature and Science, Tokyo, Japan, respectively. A single specimen of each taxon was brought to the National Museum of Nature and Science for a detailed systematic study.

Morphological observations

Counts and measurements from preserved materials were taken following Roper and Voss (1983) and Norman and Sweeney (1997). All

specimens examined in this study were deposited in the National Museum of Nature and Science (NSMT).

Abbreviations and definitions of indices in the text are as follows: AF—arm formula (comparative length of arms expressed numerically in decreasing order); ASIe—enlarged arm sucker index (diameter of an enlarged sucker as a percentage of mantle length); ASI_n—arm sucker index (diameter of a normal sucker as a percentage of mantle length); AWI—arm width index (stoutest arm width as a percentage of mantle length); CaLI—calamus length index (calamus length as a percentage of ligula length); EgL—egg length; EgLI—egg length index (length of an egg as a percentage of mantle length); FFuI—free funnel length index (free funnel length as a

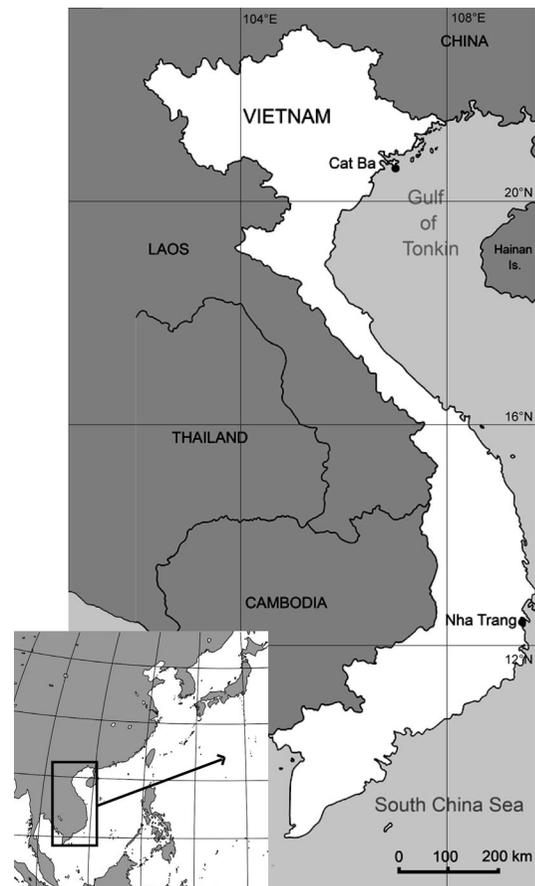


Fig. 1. Map of the collection sites.

percentage of mantle length); FLI—funnel length index (funnel length as a percentage of mantle length); HWI—head width index (head width as a percentage of mantle length); LLI—ligula length index (ligula length as a percentage of hectocotylied arm length); ML—mantle length; MWI—mantle width index (mantle width as a percentage of mantle length); OAI—opposite arm index (length of hectocotylied arm as a percentage of its fellow arm on the opposite side); TOLI—terminal organ length index (length of terminal organ and diverticulum as a percentage of mantle length); and WDI—web depth index (web depth as a percentage of the longest arm).

PCR amplification and sequencing

DNA was extracted by following a phenol/chloroform extraction protocol. A partial segment of the mitochondrial cytochrome oxidase I (COI) was amplified with primers LCO1490 and HCO2198 (Folmer *et al.*, 1994).

PCR was performed in volumes of 50 μ L containing 0.2 mM of each dNTP, 1.5 mM of MgCl₂, 0.4 μ M of each primer, and 1.0 unit of Taq DNA polymerase (Takara Ex-taq). Amplifications were performed using a Takara PCR Thermal Cycler MP (Takara) under the following conditions: 30 sec at 94°C, 30 sec at 45°C, and 60 sec at 72°C (35 cycles). The samples were incubated at 94°C for 3 min before the cyclic reactions and at 72°C for 5 min after completion. We placed 5 μ L of the reaction product on a 1.5% agarose gel in 0.5 \times TBE buffer to check the success of the reaction. The remainder of the PCR product was purified using ExoSAP-IT (Amersham Bioscience, USA).

PCR products were sequenced with the BigDye Terminator Cycle Sequencing v3.1 Cycle sequencing Kit (Applied Biosystems, USA) on an ABI PRISM™ 3100-Avant Genetic Analyzer (Applied Biosystems, USA). Sequencing reactions were performed in volumes of 20 μ L containing 3.0 μ L of Terminator Ready Reaction Mix, 2.5 μ L of 5 \times sequencing buffer, and 3.2 pmol of the sequencing primer. The reactions were carried out under the following conditions

using a Takara PCR Thermal Cycler MP: 30 sec at 96°C, 15 sec at 50°C, and 4 min at 60°C (25 cycles). Samples were incubated at 96°C for 1 min before the cyclic reactions. Products were purified using ethanol/EDTA/sodium acetate precipitation according to the sequencing kit manual. Sequence data were deposited in the DDBJ database. Accession numbers and museum numbers of voucher specimens are provided in Appendix 1. Sequences were aligned by eye with ATGC v4.0.10 and Genetix ver.7.0.8 (Genetix) and then imported into MEGA 4 (Tamura *et al.* 2007) to calculate genetic distance (Kimura's two-parameter distance) and construct a neighbor-joining (NJ) phylogenetic tree.

Results

Species accounts and systematics

A total of twelve species assigned to four genera were recognized in this study including three nominal species and nine unidentified species (Table 1). *Amphioctopus aegina* and *A. marginatus* have previously been reported from Vietnam (Hylleberg, 2000). *Callistoctopus luteus* is a first

Table 1. List of shallow-water benthic octopus collected from Vietnam.

Species name	Collection site
Genus <i>Amphioctopus</i> Fischer, 1882	
<i>Amphioctopus aegina</i> (Gray, 1949)	Nha Trang
<i>Amphioctopus marginatus</i> (Taki, 1964)	Nha Trang
<i>Amphioctopus</i> cf. <i>neglectus</i> (Nateewathana and Norman, 1999)	Nha Trang
<i>Amphioctopus</i> cf. <i>ovulum</i> (Sasaki, 1917)	Nha Trang
<i>Amphioctopus</i> cf. <i>rex</i> (Nateewathana and Norman, 1999)	Nha Trang
Genus <i>Callistoctopus</i> Taki, 1964	
<i>Callistoctopus luteus</i> (Sasaki, 1929)	Nha Trang
<i>Callistoctopus</i> sp. 1	Nha Trang
<i>Callistoctopus</i> sp. 2	Nha Trang
<i>Callistoctopus</i> sp. 3	Nha Trang
Genus <i>Cistopus</i> Gray, 1849	
<i>Cistopus</i> cf. <i>indicus</i> (Rapp, 1835)	Nha Trang, Cat Ba
Genus <i>Octopus</i> Cuvier, 1797	
<i>Octopus</i> sp. 1	Nha Trang
<i>Octopus</i> sp. 2	Cat Ba

record for this region. Four species requires further research to clarify identification and are presented here as *Amphioctopus* cf. *rex*, *A.* cf. *neglectus*, *A.* cf. *ovulum* and *Cistopus* cf. *indicus*. The remaining five species of two genera, *Callis-*

toctopus and *Octopus*, show no similarities with species described to date and are treated here by species number (*Callistoctopus* sp. 1–3, *Octopus* sp. 1–2).

Key to genera of shallow-water benthic octopus collected from Vietnam

1. Dorsal pair of arms longer than the others, and dorsal web sector deeper than the other sectors 2
 Lateral pair longer than dorsal pair, and dorsal web sector shallower than lateral sector 3
2. Water pouches present between base of arms on oral surface. Ligula tiny (LLI: <0.5%) and calamus absent. Genus *Cistopus*
 Water pouches absent. Ligula moderate and calamus distinct Genus *Callistoctopus*
3. Arms short (2–3×ML). Skin sculptured in regular patches defined by distinct grooves.
 Genus *Amphioctopus*
 Arms moderate to long. Skin not highly sculptured in obvious patch and groove system.
 Genus *Octopus*

Family Octopodidae

Genus *Amphioctopus* Fischer, 1882

Diagnosis. Small to medium sized body (140 mm>ML); short arms (2–3×ML), subequal in length, arm formula typically 4>3>2>1 or 4=3=2>1; moderate depth web with sector A shallowest; moderate number of gill lamellae (6–11 in ocellates; 9–11 in non-ocellates); well developed patch and groove system on skin surface.

Remarks. Genus *Amphioctopus* was used to be referred to as *Octopus aegina* species group (Robson, 1929). Huffard and Hochberg (2005) reinstate genus to include member species of this species group. From Vietnam, we found five species possessing these morphological characters.

***Amphioctopus aegina* (Gray, 1849)**

(Figs. 2a, 4a–b)

Octopus aegina Gray, 1849: 7 (type locality: unknown); Robson, 1929: 113–115, pl. 5, fig.1, text-figs. 31–32; Norman and Hochberg, 1994: 149–150, fig. 3A; Nateewathana, 1997: 417–421, figs. 4A–B; Norman and Sweeney, 1997: 104–105, fig. 2i, 4h; Kubodera and Lu,

2002: 163.
Octopus dollfusi Robson, 1928: 43; Voss and Williamson 1971: 82, 84, fig. 33, pl. 25
Octopus hardwickei Gray, 1849: 8; Robson, 1929: 115–116, pl. 3, fig. 2.
Amphioctopus aegina: Norman and Hochberg, 2005: 132.

Material examined. NSMT–Mo.75211: 2 female (sub-mature), 75, 52 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 3 December 2003. NSMT–Mo.75212: 1 female and 2 males (mature), 92, 68, 67 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 4 December 2003.

Diagnosis. Body of moderate size (52–92 mm ML); ovoid (MWI: 42.2–73). Head narrow (HWI: 30.3–39). Arms short (2–3×ML); width narrow (AWI: 9–10.9); AF typically 4>3>2>1. Arm suckers in two rows; normal arms with 110–164 suckers (ASIn: 8–9.3). Enlarged suckers on arms 2 and 3 of male at 7–9th from arm base but not dramatically enlarged (ASIE: to 12.4). Webs of moderate depth (WDI to 23.6), web formula typically D>C>E>B>A. Right third arm of male hectocotylized; shorter than opposite arm (OAI: 75.3–80.2) with 66–72 suckers. Ligula of moderate size (LLI: 4.9–7.5); cala-

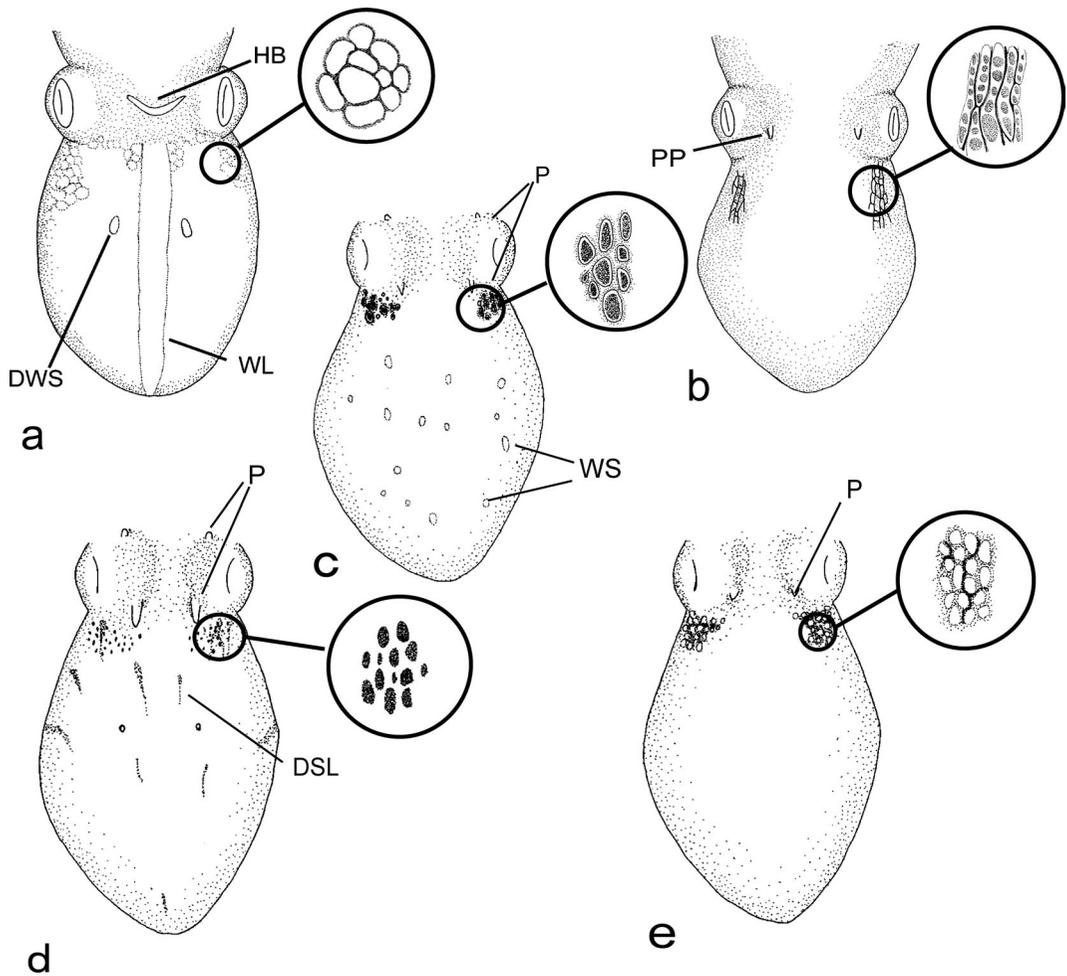


Fig. 2. Skin texture, color pattern, and detailed skin sculpture (shown in circle) of *Amphioctopus* species from Vietnam. a, *A. aegina* showing dorsal white spot (DWS), head bar (HB) and white line (WL); b, *A. marginatus* showing superocular primary papillae (PP); c, *A. cf. neglectus*, showing numerous small white spots (WS) and papillae (P); d, *A. cf. ovulum*, showing dark short line (DSL) and papillae (P); e, *A. cf. rex* with papillae (P).

mus moderate to large (CaLI: 27.1–27.8). Gills with 7–8 lamellae per demibranch. Funnel length short to moderate (FLI: 33.5–49.6); free funnel length short (FFuL: 17.1–28.7). Funnel organ W-shaped. Male terminal organ moderate in length (TOLI: 32.6–34.5), straight; diverticulum small and rounded. Eggs in ovary small (EgLI: 3 in 92 mm ML female), numerous. Body of fresh and fixed specimens brown in color. Dorsal surface sculptured with regular round patches; defined by distinct grooves, edged with black lines

especially on dorsal surface of arms; cream yellow longitudinal line along the midline of dorsal mantle, cream transverse head bar visible (sensu Norman and Sweeney, 1997); dorsal white spot present (Fig. 2a).

Remarks. Norman and Sweeney (1997) characterized *Amphioctopus aegina* by the two distinct color patterns visible in the female holotype: (1) pale circular patches defined by dark reticulations on the dorsal surface of body and (2) a pale longitudinal stripe along the dorsal

midline. The specimens from Vietnam clearly show these diagnostic characters.

***Amphioctopus marginatus* (Taki, 1964)**

(Figs. 2b, 4c–d)

Octopus marginatus Taki, 1964: 304–305, pl. 5, text-figs. 47–48 (type locality: near Komae town, Minami-Amabe County, Oita Prefecture, Japan); Norman and Hochberg, 1994: 152–153; Nateewathana, 1997: 429–432, figs. 8A–B; Hasegawa *et al.*, 2001: 38, pl. 9, figs. 5–6; Kubodera and Lu, 2002: 163.

Octopus aegina (non Gray): Voss and Williamson, 1971: 80, 82, pl. 24, fig. 32.

Octopus striolatus Dong, 1976: 212–213, figs. 3–4.

Amphioctopus marginatus: Norman and Hochberg, 2005: 139.

Material examined. NSMT–Mo.74816: 1 male (mature), 53 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 14 January 2002. NSMT–Mo.74817: 1 male (mature), 70 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp fish market, 17 January 2002. NSMT–Mo.75213: 1 male (mature), 68 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 3 December 2003. NSMT–Mo.75214: 1 female (submature), 83 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 4 December 2003.

Diagnosis. Body of moderate size (53–83 mm ML); ovoid (MWI: 51.3–58.8). Head narrow (HWI: 31.7–39). Arms short (2–3.5×ML); width narrow to moderate (7.5–12.7); subequal in length (AF variable, typically dorsal pair shortest). Arm suckers in two rows; normal arms with 128–211 suckers (ASIn: 7.3–9.9). Distinct enlarged suckers visible on arms 2 and 3 of only one male specimen (NSMT–Mo. 74817) at level 8–9th suckers from arm base (ASIE: 12.7). Webs of moderate depth (WDI: to 17.4); web formula typically D>E>C>B>A. Right third arm of male hectocotylized; shorter than opposite arm (OAI: 87–90.3) with 110–123 suckers. Ligula small (LLI: 1.3–2.1); calamus moderate to large (CaLI: 28.9–51.4). Gills with 9 lamellae per

demibranch. Funnel length short (FLI: 31.3–37.1); free funnel length short (FFuI: 15.5–21.9). Funnel organ W-shaped. Male terminal organ moderate in length (TOLI: 23–35), straight, diverticulum well-developed, coiled. Body of fresh and fixed specimen dark brown in color. Dorsal surface sculptured in relatively regular elongate patches to ridges defined by distinct darkened and branching grooves, most obvious as branching reticulations on lateral arm faces. Single superocular primary papilla present above each eye (Fig. 2b). Dorso-lateral faces of suckers white or pale pink, forming contrasting white strip against dark brown or purple of aboral surfaces of arms.

Remarks. *Amphioctopus marginatus* was described based on a single sub-mature female specimen (Taki, 1964). Though descriptions of males were not provided in the original description, identification is possible based on the diagnostic color patterns in Arakawa's illustrations of the live animal presented by Taki, along with agreement in morphological measurements. On the basis of other published descriptions of this species (Norman and Hochberg, 1994; Nateewathana, 1997; Norman, 1998), we identify these four specimens as *A. marginatus*.

Amphioctopus cf. neglectus

(Nateewathana and Norman, 1999)

(Figs. 2c, 3a–b, 4e)

Material examined. NSMT–Mo.74818: 1 male (mature), 52 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 14 January 2002. NSMT–Mo.74819: 2 females (mature), 52, 49 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 14 January 2002.

Diagnosis. Body of moderate size (49–52 mm ML); ovoid (MWI: 56.8–65.6). Head narrow (HWI: 25.4–32.2). Arms short (2–3×ML); width narrow to moderate (7.1–19.1); AF 4>3>2>1. Arm suckers in two rows; normal arms with 96–151 suckers (ASIn: 6.5–12.8). Distinctly enlarged suckers absent. Webs shallow (WDI: to 19.6); web formula D>E>C>B>A. Right third

arm of male hectocotylized; with 67 suckers. Ligula long and slender (LLI: 14.4); calamus small (CaLI: 12.3) (Fig. 3a). Gills 7–8 lamellae per demibranch. Funnel medium (FLI: 31.9–37.3); free funnel length short (FFuL: 16.1–20). Funnel organ W-shaped. Male terminal organ moderate in length (TOLI: 31.7) straight; single non-coiled diverticulum present (Fig. 3b). Eggs in ovary very small (EgLI: 0.2 in 52 mm ML female). Body of fresh and fixed specimen light brown or pale grey in color. Dorsal surface sculptured with regular patches; numerous small white rounded spots on mantle in fresh specimen (Fig. 2a), unclear in fixed specimen. A pair of ocelli on the arm base between II and III; black rim and iridescent purple ring. Small papillae present above and under each eyes.

Remarks. *Amphioctopus cf. neglectus* was distinguished from other ocellate *Amphioctopus* species found in Vietnam in this study by long and slender ligula, non-coiled diverticulum, and numerous small white spots scattered on dorsal mantle. Although these characters are quite similar to the original description of *A. neglectus* (Nateewathana and Norman, 1999), lack of distinctive enlarged sucker on male arms and absence of transverse white bar between eyes are disagreed. Thus we treat this species as *Amphioctopus cf. neglectus* here.

Amphioctopus cf. ovulum (Sasaki, 1917)

(Figs. 2d, 3c–d; 4f)

Material examined. NSMT–Mo.74820: 1 female and 1 male (mature), 45, 50 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 19 January 2002.

Diagnosis. Body of moderate size (45–50 mm ML); ovoid (48.2–52.9). Head narrow (HWI: 25.8–34.8). Arms short (2–3×ML); width narrow (AWI: 7.6–8); AF 4>3>2>1. Arm suckers in two rows; normal arms with 95–132 suckers (ASIn: 4.9–6.1). 5–6th sucker on male 2 or 3 arms slightly enlarged (ASIE: 8.1). Webs shallow (WDI: to 18.9); web formula D>C>B>E>A. Right third arm of male hectocotylized; shorter than opposite arm (OAI: 63.5) with 55 suckers. Ligula small (LLI: 5.6); calamus small (CaLI: 22.2) (Fig. 3c). Gills 7–8 lamellae per demibranch. Funnel length short (FLI: 30.9–33.2); free funnel length short (FFuL: 20.5–23.5). Funnel organ W-shaped. Male terminal organ moderate in length (TOLI: 36.6) straight; well developed (47.3% of terminal organ length) and coiled diverticulum present (Fig. 3d). Eggs small, numerous in ovary (EgLI: 0.4 in 45 mm ML female). Body of fresh and fixed specimen dark or light brown in color. Dorsal surface covered with regular fine minute warts, with a few dark broad

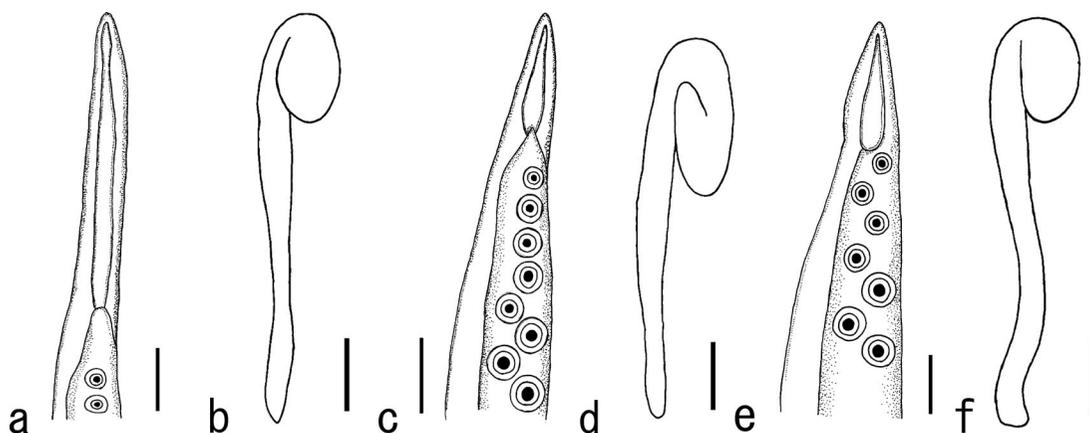


Fig. 3. Male organs of ocellate *Amphioctopus* species from Vietnam. a, Hectocotylus of *A. cf. neglectus*; b, Terminal organ of *A. cf. neglectus*; c, Hectocotylus of *A. cf. ovulum*; d, Terminal organ of *A. cf. ovulum*; e, Hectocotylus of *A. cf. rex*; f, Terminal organ of *A. cf. rex*. Scale bars: 3 mm.

short lines (Fig. 2d). Two cirri present above the each eyes. A pair of ocelli on the arm base between II and III; black rim and iridescent purple ring. Small papillae present above and under each eyes.

Remarks. *Amphioctopus cf. ovulum* was distinguished from other ocellate *Amphioctopus* species found in Vietnam in this study by coiled diverticulum, and texture pattern of dorsal mantle surface. In accordance with Sasaki (1929), ligula size and terminal organ shape of this specimen were well agreed to those of Sasaki's description. Although other morphological characters are also generally agreed, we considered that further investigation is required to identify these complicated ocellate *Amphioctopus* species. Here we treat this species as *Amphioctopus cf. ovulum*.

Amphioctopus cf. rex

(Nateewathana and Norman, 1999)

(Figs. 2e, 3e–f, 4g–h)

Material examined. NSMT–Mo.75215: 2 females (1 mature, 1 sub-mature) and 1 male (mature), 71, 67, 56 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 3 December 2003. NSMT–Mo.75216: 2 females (1 mature, 1 sub-mature), 94, 65 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 4 December 2003. NSMT–Mo.75217: 1 female (sub-mature), 60 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 6 December 2003.

Diagnosis. Body moderate (56–94 mm ML); ovoid (MWI: 49.1–62). Head narrow (HWI: 25.1–33.7). Arms short (2–3×ML); width narrow (AWI: 6.4–8.3); AF 4>3>2>1. Arm suckers in two rows; normal arms with 100–196 suckers; base of 2 and 3 arm of male slightly large, but not distinctively enlarged (ASIn: 7.3–10.3). Webs shallow (WDI: to 23.5), web formula D>E>C>B>A or D>C>E>B>A. Right third arm of male hectocotylized; slightly shorter than opposite arm (OAI: 82.1) with 77 suckers. Ligula small (LLI: 3.4); calamus not

clear (Fig. 3e). Gills 7–8 lamellae per demibranch. Funnel medium (FLI: 30.6–38.2); free funnel length short (FFuI: 16.5–21.6). Funnel organ W-shaped. Male terminal organ moderate (TOLI: 27.5%), thin and straight; diverticulum round and small (Fig. 3f). Eggs in ovary small; numerous (EgLI: 2.5 in 94 mm ML female). Body of fresh and fixed specimen brown in color. Dorsal surface sculpture in regular round patches partially edged with black lines (Fig. 2e). A pair of ocellus on the arm base between II and III; black rim and iridescent purple ring. Small single papillae present above each eyes.

Remarks. This species was distinguished from other Vietnamese ocellate *Amphioctopus* by small ligula, small diverticulum of male terminal organ, lack of numerous small spots on dorsal mantle, and large maturity size. These characters are agreed to those of *A. rex* (Nateewathana and Norman, 1999), but further investigation is required to identify this species especially in female specimens. We treat this species as *Amphioctopus cf. rex* here.

Genus *Callistoctopus* Taki, 1964

Diagnosis. Medium to large sized body; elongate arms with the dorsal pair longest; moderate to high gill counts (10–14); moderate to large ligula and distinct calamus.

Remarks. The generic name *Callistoctopus* was resurrected by Norman and Hochberg (2005). It was used to be referred to as *Octopus macropus* species group (Robson, 1929). Four species including three unidentified species were found from the coastal water of Vietnam in this study.

***Callistoctopus luteus* (Sasaki, 1929)**

(Figs. 5a–b, 6a–b)

Polypus luteus Sasaki, 1929: 45–47, pl. 27, figs. 6–9, pl. 24, figs. 4–5 (type locality: Pescadore Island=Peng-hu, Taiwan).

Octopus luteus: Norman and Hochberg, 1994: 151–152, fig. 2A; Nateewathana, 1997: 425–428, figs. 7A, B; Kubodera and Lu, 2002: 163.

Octopus macropus (non Risso): Voss and Williamson,



Fig. 4. *Amphiactopus* species collected from Vietnam. a, *A. aegina* (NSMT–Mo.75211, dorsal); b, *A. aegina* (NSMT–Mo.75211, ventral); c, *A. marginatus* (NSMT–Mo.75213, dorsal); d, *A. marginatus* (NSMT–Mo.75213, ventral); e, *A. cf. neglectus* (NSMT–Mo.74819, dorsal); f, *A. cf. ovulum* (NSMT–Mo.74820, dorsal); g, *A. cf. rex* (NSMT–Mo.75215, dorsal); h, *A. cf. rex* (NSMT–Mo. 75215, ventral). Scale bars: 50 mm.

1971: 86, 88, pl. 25, fig. 35.

Callistoctopus luteus: Norman and Hochberg, 2005: 138 (placed in a new genus).

Material examined. NSMT–Mo.74814: 1 male (mature), 96 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 14 January 2002.

Diagnosis. Body large (96 mm ML); ovoid (MWI: 61.5). Head narrow (HWI: 37.1). Arms

damaged in material examined; dorsal pair most robust (AWI: 13.7). Arm suckers in two rows, robust and enlarged at 18th sucker (ASiE: 14.1). Webs thick and shallow, web formula $A > B = C > D > E$. Right third arm of male hectocotylized; much shorter than the opposite arm (OAI: 50.3), with 75 suckers. Ligula of moderate size (LLI: 7.3); calamus of moderate size (CaLI: 28.6) (Fig. 5a). Gills with 11 lamellae per demi-branch. Funnel length moderate (FLI: 43.5); free

funnel length short (FFuI: 22.5). Funnel organ W-shaped. Male terminal organ short (TOLI: 8.4) straight; diverticulum small. Body of fresh specimen pale grey, fixed specimen reddish brown in

color. Skin sculptured with various size of flattened warts, which are partially aggregated (Fig. 5b). No primary papillae present on dorsal mantle. Several white spots on dorsal surface in fresh

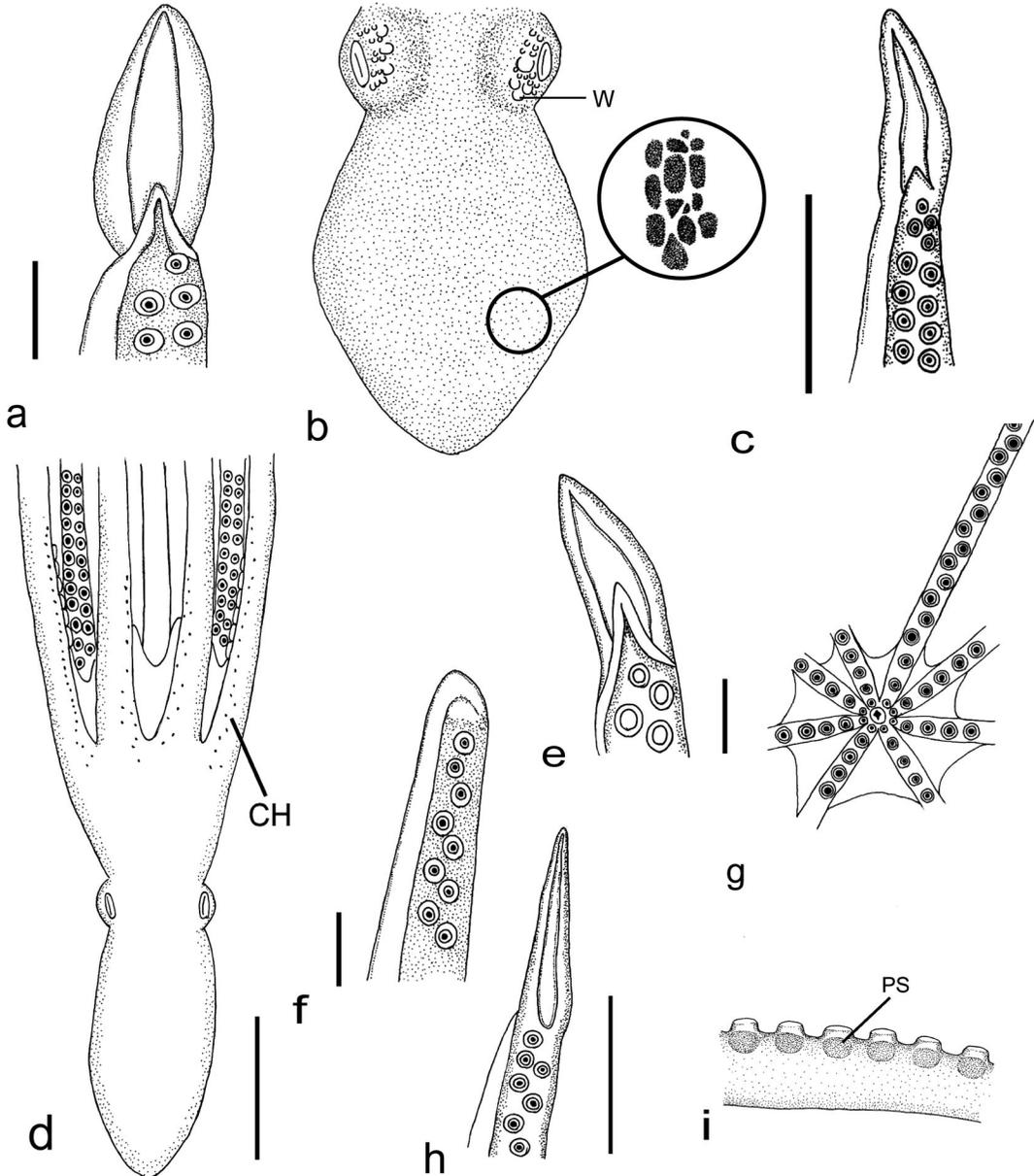


Fig. 5. a, Hectocotylus of *Callistoctopus luteus* (scale bar: 3 mm); b, Detailed skin sculpture of dorsal surface of *C. luteus*, showing warts above each eyes; c, Hectocotylus of *C. sp. 1* (scale bar: 3 mm); d, Dorsal view of *C. sp. 1* with chromatophores (CH) on dorsal surface of arms (scale bar: 30 mm); e, Hectocotylus of *C. sp. 2* (scale bar: 2 mm); f, Hectocotylus of *Cistopus cf. indicus* (scale bar: 1 mm); g, Sucker layout of *O. sp. 1* showing single row and zig-zag pattern; h, Hectocotylus of *O. sp. 1* (scale bar: 3 mm); i, Arm side of *O. sp. 1* showing purple spots (PS) at the base of suckers.

specimen, but unclear in fixed specimen. Relatively large rounded warts present around the eyes (Fig. 5b).

Remarks. *Callistoctopus luteus* is characterized by its distinct skin structure noted by Sasaki (1929): surface firm to the touch often finely wrinkled and thickly covered with pimple-like warts of various size, of which some above the eyes are enlarged into cirri; but these are by no means so marked as found in some octopi. Vietnam specimen in this study had badly damaged arms, but showed diagnostic skin structure of this species.

***Callistoctopus* sp. 1**

(Figs. 5c–d, 6c–d)

Material examined. NSMT–Mo.75219: 1 male (mature), 56 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 6 December 2003. NSMT–Mo.75220: 1 females (sub-mature) and 1 male (mature), 58, 60 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 8 December 2003.

Diagnosis. Body of moderate size (56–60 mm ML); ovoid (MWI: 44.9–49.8). Head slightly narrow (HWI: 37.9–40.8). Arms almost all damaged; dorsal pair likely to be longest and ventral pair shorter; dorsal pair most robust (AWI: 10.6–13.1). Arm suckers in two rows; 7–8th sucker slightly larger than the other suckers but not distinctly enlarged (ASIn: 9–12.6). Webs shallow, thin; web formula typically $A > B > C > D > E$. Right third arm of male hectocotyized; much shorter than the arms with 92 suckers. Ligula of moderate size (LLI: 7); calamus of moderate size (CaLI: 21.9) (Fig. 5c). Gills with 10–11 lamellae per demibranch. Funnel short (FLI: 37.1–42.9; FFuI: 16–24.5). Funnel organ W-shaped. Male terminal organ moderate in length (TOLI: 14.3–25.1), straight, diverticulum small. Body of fixed specimen pale reddish brown in color. Dorsal mantle surface smooth and no marks or textures. Numerous chromatophores scattered on dorsal surface of arms but not on webs (Fig. 5d).

Remarks. The genus *Callistoctopus* is characterized by long arms with the dorsal pair longest, moderate to high gill lamellae counts (10–14 per demibranch), a large copulatory organ on right arm 3 (Norman, 1992a). This species possess these general characters but is distinguished from the other member species of this genus by the numerous chromatophores on dorsal surface and the absence of distinct white spots. Thus we treat this species at this stage as an unknown species in the genus *Callistoctopus*.

***Callistoctopus* sp. 2**

(Figs. 5e, 6e–f)

Material examined. NSMT–Mo.75221: 1 male (mature) 61 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 6 December 2003.

Diagnosis. Body moderate size (61 mm ML); elongate (MWI: 28.2). Head very narrow (HWI: 23.1). Arms long but highly damaged; dorsal pair likely to be longest ($7 \times ML$) and ventral pair shorter; width very narrow (AWI: 8.2). Arm suckers in two rows; small (ASIn: 4.9). Enlarged suckers absent. Webs very shallow (WDI: to 3.5), web formula $B > C > A > D > E$. Right third arm of male hectocotyized; much shorter than opposite arm (OAI: 31.8); with 61 suckers. Ligula medium (LLI: 5.6); calamus large (CaLI: 48.9) (Fig. 5e). Gills 11 lamellae per demibranch. Funnel medium (FLI: 51.2); free funnel length very short (FFuI: 13.2). Funnel organ VV-shaped. Male terminal organ short (TOLI: 13); thick and straight; diverticulum muscular; very small. Body of fixed specimen dark reddish brown in color. Dorsal mantle surface smooth and no textures in fixed specimen, black marks supposed branchial heart visible through the mantle wall on both sides of the mantle in fresh dead specimens.

Remarks. *Callistoctopus* sp. 2 of this study possesses similar characteristics to those reported for the nominal species, *Octopus minor*, i.e. an elongate form, a long dorsal arm pair, a VV funnel organ and high gill lamellae counts. *Octopus*

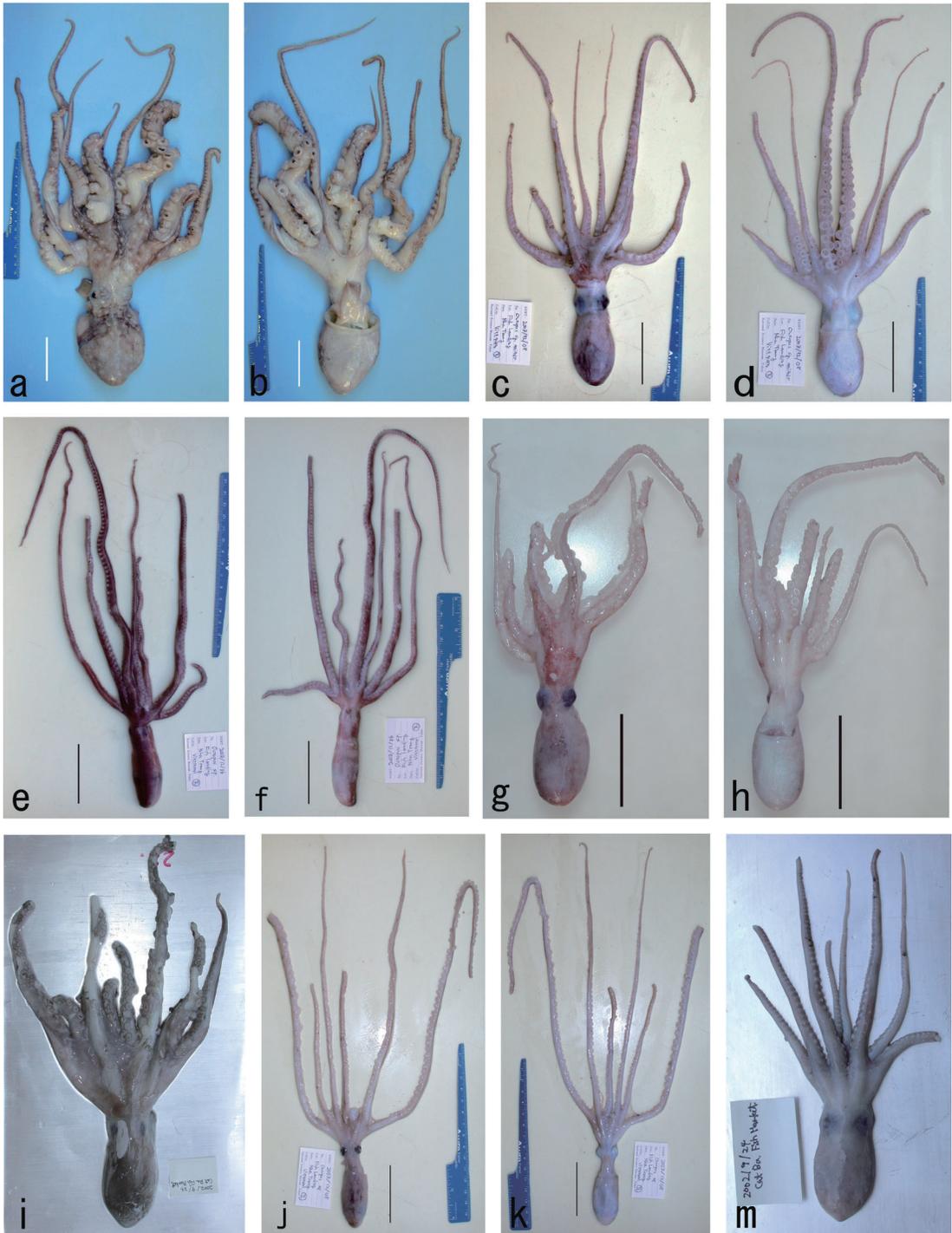


Fig. 6. *Callistoctopus*, *Cistopus* and *Octopus* species collected from Vietnam. a, *Callistoctopus luteus* (NSMT–Mo.74814, dorsal); b, *C. luteus* (NSMT–Mo.74814, ventral); c, *C. sp. 1* (NSMT–Mo.75220, dorsal); d, *C. sp. 1* (NSMT–Mo.75220, ventral); e, *C. sp. 2* (NSMT–Mo.75221, dorsal); f, *C. sp. 2* (NSMT–Mo.75221, ventral); g, *C. sp. 3* (NSMT–Mo.74815, dorsal); h, *C. sp. 3* (NSMT–Mo.74815, ventral); i, *Cistopus cf. indicus* (NSMT–Mo.74823, dorsal); j, *Octopus sp. 1* (NSMT–Mo.75218, dorsal); k, *O. sp. 1* (NSMT–Mo.75218, ventral); m, *O. sp. 2* (NSMT–Mo.74825, dorsal). Scale bars: 50 mm.

minor remains ill-defined and is in need of urgent revision. It has been widely reported from coastal waters throughout East Asia except for coral reef areas but may represent a suite of similar species (Norman pers. comm.). *Octopus minor* was first described by Sasaki (1920) as a variant of *O. macropus* Risso, 1826, under the name *Polypus macropus* var. *minor*. In 1929, Sasaki renamed this species as *P. variabilis* including three variation types: *P. var. minor*, *P. var. typicus* and *P. var. pardalis*. Toll and Voss (1998) proposed that the name *minor* become valid at the specific level in accordance with ICZN (International Code of Zoological Nomenclature), however the species remains poorly defined and the three variations reported by Sasaki remain unresolved.

Callistoctopus sp. 2 is distinguished from *O. minor* by its very narrow and elongate body and head, much smaller ligula and lack of transverse laminae, and absence of warts and cirri on dorsal mantle and around eyes. *Octopus* sp. B reported from Hong Kong by Voss and Williamson (1971) shows many similarities to this species and requires further resolution.

Callistoctopus sp. 3

(Fig. 6g–h)

Material examined. NSMT–Mo.74815: 1 female (sub-mature), 56 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 19 January 2002.

Diagnosis. Body of moderate size (56 mm ML); ovoid (MWI: 44.1). Head narrow (HWI: 31). Arms almost all damaged; dorsal pair robust (AWI: 10.5), likely to be longest and ventral pair shorter. Arm suckers in two rows; moderate in size (ASIn: 8.9). Enlarged suckers absent. Webs shallow, thin; web formula typically $A > B > C > D > E$. Gills with 8 lamellae per demibranch. Funnel short (FLI: 37.1–42.9; FFuI: 16–24.5). Funnel organ unclear. Body color of fresh specimen pale red; fixed specimen pale reddish brown. Dorsal mantle surface smooth and no marks, papillae or textures. Several chromatophores scattered on dorsal surface of arms but not on

webs.

Remarks. Although morphological character of this species is quite similar to *Callistoctopus* sp. 1, it is distinguished by the number of gill lamellae (10–11 for *C. sp. 1*; 8 for *C. sp. 3*). Although only single sub-mature female is known in this species and further study is required, we treat this species as distinct in this study.

Genus *Cistopus* Gray, 1849

Diagnosis. Possessing water pouches on the oral surface of the webs between arms; long arms with dorsal pair longest; conical tiny ligula on males without calamus.

Remarks. This genus includes only one species, *Cistopus indicus*. One species comparable to *Cistopus indicus* was reported from coastal water of Vietnam.

Cistopus cf. *indicus* (Rapp, 1835)

(Figs. 5f, 6i)

Material examined. NSMT–Mo.74821: 1 female (sub-mature) 38 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, fish market, 19 January 2002. NSMT–Mo.74822: 1 male (sub-mature), 53 mm ML; Cat Ba, north Vietnam; collected by T. Kubodera, fish market, 22 September 2002. NSMT–Mo.74823: 1 male (mature), 84 mm ML; Cat Ba, north Vietnam; collected by T. Kubodera, fish market, 24 September 2002. NSMT–Mo.75222: 2 females (sub-mature) and 2 males (sub-mature), 71, 64, 70, 50 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 3 December 2003.

Diagnosis. Body of moderate size (38–84 mm ML); ovoid to round (MWI: 56.4–77.1). Head narrow to moderate (HWI: 37.4–54.7). Arms long (4–7×ML); dorsal pair longer than ventral pairs in length (AF typically $1 > 2 > 3 > 4$); width moderate (AWI: 10.7–21.1). Arm suckers in two rows; normal arms with 130–162 suckers (ASIn: 8.6–12.4). Enlarged suckers absent. Webs shallow to moderate (WDI: to 19.6), depth vari-

able, sector A typically deepest. Water pouches present in oral surface of webs between bases of arms, visible in males, but often indistinct or difficult to find in females. Muscular pores of water pouches open to exterior at level of 3 to 4th proximal sucker. Right third arm of male hectocotylized; shorter than opposite arm (OAI: 87–88) with 116–119 suckers. Ligula tiny (LLI: <0.5); calamus absent (Fig. 5f). Gills with 9–10 lamellae per demibranch. Funnel short (FLI: 35.4–51.9; FFuI: 12.9–30.4). Funnel organ W-shaped. Male terminal organ short (TOLI: 9–10.3), straight, diverticulum small and rounded. Body of fixed specimen dark grey or purple in color. Dorsal surface generally smooth, with few scattered low papillae.

Remarks. It has been proposed that the genus *Cistopus* includes several distinct species, currently being treated under the single name *C. indicus* (Robson, 1929; Norman and Sweeney, 1997). Norman and Sweeney (1997) pointed out that the genuine *C. indicus* is characterized by water pouches, dorsal arms longer than ventral arms (AF 1>2>3>4), tiny ligula (~0.5% of hectocotylized arm length), absence of a calamus, 9–10 gill lamellae per demibranch, sucker counts of approximately 185 on normal arms and approximately 110–130 on hectocotylized arm, and the absence of enlarged suckers in either sex. Nanteewathana (1997) reported *C. indicus* from Andaman Sea, coast of Thailand, but he pointed that this species differs from the genuine *C. indicus* in possession of enlarged suckers in males. Liao (2003) also reported *Cistopus* sp. from Taiwan. He noted that sexual dimorphism of water pouches found in *Cistopus* sp. from Taiwan, female possess much smaller water pouches than that of males. This trend was also found in the *Cistopus* specimen from Vietnam. Specimens of present study showed high affinities to the genuine *C. indicus* except for the normal sucker counts. However, the most significant difference is the marked reduction of water pouches in the females. Robson (1929) suggested that function of water pouches may be used as reception of the spermatophore in female. This hypothesis is not

accepted now but it indicated at least that genuine *C. indicus* female possess well-developed water pouches. At this time, it is difficult to determine that these different characters are indicating difference between species. Thus, in this study, we identify the specimens of *Cistopus* species from Vietnam as *C. cf. indicus*.

Genus *Octopus* Cuvier, 1797

Diagnosis. Medium to large body; moderate to long arms (4–7×ML) with lateral and ventral pair longer than dorsal pair; small ligula (LLI: <3%); diamond pattern of primary papillae on dorsal mantle.

Remarks. By recent studies, the definition of *Octopus* sensu stricto has been reevaluated on the basis of original description of Cuvier, 1797, instead of the genus *Octopus* sensu lato by Lamarck, 1798 (Norman and Hochberg, 2005). In this study, two unidentified species were collected from Vietnam and placed into genus *Octopus*. These two species do not sufficient all generic characters mentioned above, and further investigation with fine condition materials are required.

Octopus sp. 1

(Figs. 5g–h, 6j–k)

Material examined. NSMT–Mo.75218: 1 male (sub-mature) 65 mm ML; Nha Trang, south Vietnam; collected by T. Kubodera, shrimp bottom trawl landing place, 8 December 2003.

Diagnosis. Body moderate (65 mm ML); elongate (MWI: 21.4). Head very narrow (HWI: 15.4). Arms almost all damaged; lateral pairs longer than dorsal and ventral pairs; width very narrow (AWI: 6.3). Arm suckers likely a single row, zig-zag pattern (Fig. 5g); small (ASIn: 4.8). Enlarged suckers absent. Webs very shallow, web formula D>C>B>E>A. Right third arm of male hectocotylized; with 106 suckers. Ligula medium (LLI: 1.7); calamus absent (Fig. 5h). Gills 9–10 lamellae per demibranch. Funnel short (FLI: 31.8); free funnel length very short (FFuI: 9.5). Funnel organ W-shaped. Male termi-

nal organ moderate (TOLI: 20.5); thin and straight; diverticulum very small. Body of fixed specimen pale grey in color. Dorsal mantle surface smooth and no marks or textures in fixed specimen; very small papillae scattered.

Remarks. This octopus possesses unique characters i.e. elongate narrow mantle and very thin arm with zig-zag pattern suckers that show no similarities with any named species of Octopodinae. We considered this species as undescribed species here, and further investigations based on more specimens are required.

Octopus sp. 2

(Figs. 5i, 6m)

Material examined. NSMT–Mo.74825; 1 female (mature) 46 mm ML; Cat Ba, north Vietnam; collected by T. Kubodera, fish market, 24 September 2002.

Diagnosis. Body moderate (46 mm ML); ovoid (MWI: 56.5). Head slightly narrow (HWI: 42.7). Arms almost all damaged; width narrow (AWI: 9). Arm suckers in two rows; small (ASIn: 4.8). Enlarged suckers absent. Webs shallow, web formula $C > A = B > D > E$. Funnel medium (FLI: 45.5); free funnel length short (FFuI: 18.2). Funnel organ W-shaped. Gills 8 lamellae per demi-branch. Eggs in ovary large (EgLI: 17.7 in 46 mm ML female). Body of fresh and fixed specimen pale red in color. Dorsal mantle surface smooth and no marks or textures. Iridescent pur-

ple spots on each sucker base (Fig. 5i). Six to seven low warts above each eye.

Remarks. Dorsal appearance of this species is similar to *Callistoctopus* sp. 1 collected in this study, but it is distinguished from *Callistoctopus* sp. 1 by absence of chromatophores, low gill lamellae, and presence of purple spots on each sucker base. Because this specimen lacks all arms and possesses low gill lamellae than member species of genus *Callistoctopus*, we placed this octopus as unknown species of genus *Octopus* in this study.

Nucleotide sequence analysis

Nucleotide sequence was analyzed for ten species out of twelve species. Two species (*Amphioctopus aegina* and *Octopus* sp. 2) were failed to keep a tissue sample. No insertion or deletion was observed in a total of 658 bp fragment of COI region. Our NJ tree with bootstrap values (based on 1000 replications) was shown in Fig. 7. Each genus made monophyletic clade and supported by high bootstrap values (>80%). The value of pairwise Kimura's two-parameter distance within a genus *Amphioctopus* ranged from 9.7% (*A. cf. neglectus* and *A. cf. rex*) to 14.8% (*A. cf. neglectus* and *A. cf. ovulum*), indicating three ocellate species are distinct species, and the monophyly of ocellate species was not supported. *A. marginatus* was observed to be more closely related to *A. cf. neglectus* and *A. cf. rex*. In genus *Callistoctopus*, *Callistoctopus* sp. 2 and

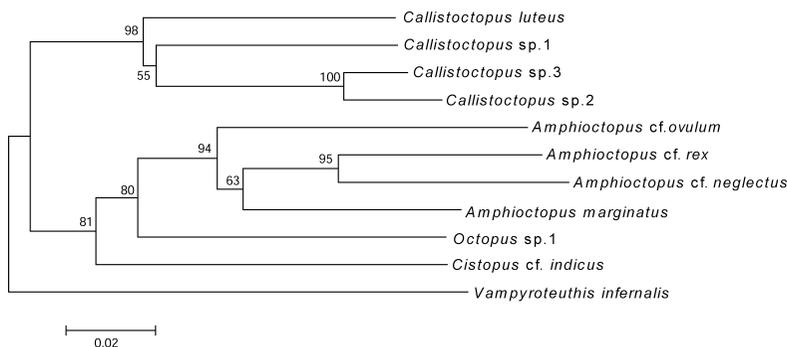


Fig. 7. Neighbor-joining tree derived from analysis of COI data. Numbers of nodes indicate >50% bootstrap support value (1000 replicates).

C. sp. 3 showed much lower value (3.6%) while the other combination ranged from 11.4 to 11.6%.

Discussion

In the present survey, we have recognized three species (*Amphioctopus aegina*, *A. marginatus*, and *Callistoctopus luteus*), four provisional identified species (*Amphioctopus cf. neglectus*, *A. cf. ovulum*, *A. cf. rex*, and *Cistopus cf. indicus*), three unidentified *Callistoctopus* species, and two unidentified *Octopus* species from shallow coastal waters in Vietnam. The octopus fauna of Vietnam shows affinities to that of adjacent areas of South East Asia. Nateewathana (1997) reported 11 benthic octopus species from the Gulf of Thailand. At least three of these species are shared with Vietnam: *A. aegina*, *A. marginatus*, and *Callistoctopus luteus*, and three species (*Amphioctopus cf. neglectus*, *A. cf. rex*, and *Cistopus cf. indicus*) showed strong affinities to the species reported from this region. Hasegawa *et al.* (2001) reported eight benthic octopuses from Hainan Island, the southernmost edge of mainland China. One of them is the common: *A. marginatus*, and five are considered to be the same or closely related species, although this required further confirmation: *A. ovulum*, *Cistopus indicus*, *A. cf. aegina*, *Abdopus cf. aculeatus*, and *Callistoctopus cf. luteus*. Some Vietnamese species are also reported from Hong Kong (Voss and Williamson, 1971; Norman and Hochberg, 1994): *A. marginatus*, *A. aegina*, *C. luteus*, and possibly *Octopus sp. B* [= *Octopus sp. 1* in Norman and Hochberg (1994); *Callistoctopus sp. 2* in present study]. Norman and Lu (2000) provided a preliminary list of cephalopod fauna from the South China Sea, reporting 18 species of shallow-water benthic octopuses. Kubodera and Lu (2002) then summarized the cephalopod fauna of western Japan, Taiwan, and the East and South China Seas. These authors listed 25 benthic octopus species from the South China Sea region including three species collected in this study.

Three ocellate *Amphioctopus* species, *A. cf.*

neglectus, *A. cf. rex*, and *A. cf. ovulum*, were added to the octopus fauna of Vietnam in this study; however, the identification of these species was complicated. They are all characterized by a pair of iridescent purple or pink ocelli on the arm web. *A. neglectus* and *A. rex* were reported from the Gulf of Thailand and are identified by the distinctive color patterns, arm sucker counts, and ligula length (Nateewathana and Norman, 1999). *Amphioctopus ovulum* was originally reported from the Tokyo market by Sasaki (1929), but Norman (1992b) pointed out that this specimen might be imported from outside Japanese waters. He also pointed out similarities between this species and the species of Thailand. According to the detailed description of Sasaki (1929), *Amphioctopus ovulum* is distinguished from *A. rex* by the number of suckers and size at maturity, and from *A. neglectus* by ligula length and dorsal mantle color pattern. Morphological characters of ocellate species of *Amphioctopus* collected from Vietnam were similar to the original description of these species. Phylogenetic relationships based on molecular analysis indicate that these three taxa are clearly separate species. However, the characters that discriminate each species are indecisive, particularly for female specimens. Comprehensive taxonomic re-examination is required for the genus *Amphioctopus* to fully characterize these species.

Remarkably, this study revealed a high diversity of *Callistoctopus* species in coastal waters of Vietnam. Three of four species with no similarity to any described species were still unnamed. *Callistoctopus sp. 2* and *C. sp. 3* are genetically closely related, but great differences of morphological characters indicate that these two species are distinct. *Callistoctopus sp. 3* is similar to *C. sp. 1* morphologically but is distinctly branched genetically; further investigation with materials in good condition is necessary to clarify the taxonomy of these species.

Most of the specimens in this study were collected at local bottom trawl landing places or fish markets; therefore, it should be biased toward the octopus species inhabiting the muddy bottoms

where fishery trawls are commonly conducted. This explains why *O. harmandi*, which was described from Poulo Condor (Con Dao Island), a small island located at the southeastern coast of Vietnam (Rochebrune, 1882) is absent in the present study. This species is considered to be a junior synonym of *Abdopus aculeatus* (Norman and Finn, 2001) and is a typical intertidal reef inhabitant. Along the coast of Vietnam, coral reefs and rocky shores are also distributed in some areas, and further surveys in these areas are required to gain a complete understanding of the rich shallow-water octopus fauna of this region.

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Appendix 1. Accession numbers and museum registration numbers of voucher specimens.

Species	Voucher specimen	Accession number
<i>Amphioctopus marginatus</i>	NSMT-Mo.74816	AB385870
<i>Amphioctopus</i> cf. <i>rex</i>	NSMT-Mo.75217	AB385871
<i>Amphioctopus</i> cf. <i>neglectus</i>	NSMT-Mo.74819	AB385872
<i>Amphioctopus</i> cf. <i>ovulum</i>	NSMT-Mo.74820	AB385873
<i>Callistoctopus luteus</i>	NSMT-Mo.74814	AB385874
<i>Callistoctopus</i> sp.1	NSMT-Mo.75219	AB385875
<i>Callistoctopus</i> sp.2	NSMT-Mo.75221	AB385876
<i>Callistoctopus</i> sp.3	NSMT-Mo.74815	AB385877
<i>Cistopus</i> cf. <i>indicus</i>	NSMT-Mo.74822	AB385878
<i>Octopus</i> sp.1	NSMT-Mo.75218	AB385879
<i>Vampiroteuthis infernalis</i>	NSMT-Mo.71614	AB385880