DESCRIPTION AND PHYLOGENETIC RELATIONSHIPS OF A NEW GENUS OF OCTOPUS, *SASAKIOPUS* (CEPHALOPODA: OCTOPODIDAE), FROM THE BERING SEA, WITH A REDESCRIPTION OF *SASAKIOPUS SALEBROSUS* (SASAKI, 1920)

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ABSTRACT

A new genus of octopus, *Sasakiopus*, is erected for the species *S. salebrosus* (Sasaki, 1920) n. comb. *Sasakiopus salebrosus* is redescribed from the holotype and from new material recently collected in the eastern Bering Sea. Molecular phylogenetic analysis of one nuclear and three mitochondrial genes revealed that the new genus is the sister taxon of a clade containing the genera *Benthoctopus* and *Vulcanoctopus*. The clade containing *Sasakiopus*, *Benthoctopus* and *Vulcanoctopus* is the sister group of *Enteroctopus*. The genus *Bathypolypus* falls outside this clade. *Sasakiopus* differs from *Bathypolypus* and *Enteroctopus* by the shape of its ligula (simple in *Sasakiopus* and *Benthoctopus*, laminate in *Bathypolypus* and elongate in *Enteroctopus*), from *Enteroctopus* by the absence of enlarged suckers in mature male animals and from *Benthoctopus* by its skin sculpture and ability to ink.

INTRODUCTION

Great progress has been made in octopodid systematics in recent years and many new species and genera have been described. The work, however, has tended to focus on two groups of octopodids. A clade comprising benthic Antarctic and deep-sea octopodids with uniserial suckers has received extensive attention (Allcock *et al*., 2003, 2004; Allcock, 2005; Allcock, Strugnell & Johnson, 2008), as have the benthic shallow tropical octopodids with biserial suckers (Norman, 1992a, b, 1993; Norman & Finn, 2001; Guizik, Norman & Crozier, 2005). Molecular studies have shown that three benthic genera with biserial suckers, *Enteroctopus* Rochebrune & Mabille, 1889, *Bathypolypus* Grimpe, 1921 and *Benthoctopus* Grimpe, 1921, fall outside the group containing all other benthic biserially-suckered octopodids (Carlini, Young & Vecchione, 2001; Strugnell *et al*., 2005; Allcock *et al*., 2006). These three genera have received less attention. An exception is the excellent work by Muus (2002), which redefined *Bathypolypus*, narrowing its definition. As a result, Muus suggested that *B. salebrosus* (Sasaki, 1920) should be placed in *Benthoctopus*.

Originally described from the Sea of Okhotsk, *Benthoctopus salebrosus* was assigned by Sasaki (1920) to the genus *Polypus*. Robson (1932) reassigned it to *Bathypolypus* when he revised the family Octopodidae. His tentative placement of the species was based on its rough skin, short arms and deep web. At that time the species was only known from two females. The holotype was not available to Robson. He examined the paratype, an immature female; therefore the shape of the ligula of the male was unknown to him. Species of *Bathypolypus* have a distinctive large, laminate ligula. Robson did not include this character in his diagnosis of *Bathypolypus*, but he did supply a comparative figure of the ligulae of two species of *Bathypolypus* directly under the generic diagnosis (Robson, 1932: 286, fig. 53) and it is clear from his text that he felt a lack of knowledge of the hectocotylus of *B. salebrosus* hampered its generic placement.

Akimushkin (1965: 134, fig. 34) figured the ligula of *B. salebrosus* and described it as having distinct transverse striation. He recognized that his was the first description of the male but did not acknowledge that the absence of laminae in the hectocotylus suggested that the generic placement of this species was inappropriate.

Muus (2002) noted that the shape of the ligula excluded *B. salebrosus* from *Bathypolypus* and concluded that the slim, striated ligula best corresponded to the form seen in some species of *Benthoctopus*. He acknowledged that the warty skin of *B. salebrosus* was unusual for *Benthoctopus*. He did not examine specimens of *B. salebrosus* but relied on published descriptions.

Finally, Norman & Hochberg (2005), in their checklist of octopus species, removed *B. salebrosus* from any generic placement, terming it valid but unplaced. That publication gave an overview of octopodid systematics and did not deal with specific issues.

Two events initiated this revision. Firstly, groundfish surveys in the eastern Bering Sea have yielded a large number of specimens of *B. salebrosus* in good condition. Some of these specimens were seen inking, indicating the presence of a functional ink sac. Each previous account of this species noted that it lacked an ink sac, but detailed study of the holotype revealed a highly reduced sac. Presence of an ink sac is a character found neither in *Benthoctopus* nor *Bathypolypus*. Secondly, ongoing studies of the genus *Benthoctopus* (Allcock *et al*., 2006; Strugnell *et al*., 2009) meant that a molecular dataset for a variety of species in *Benthoctopus* and a small number of comparative species from *Enteroctopus* and *Bathypolypus* was available for analysis.

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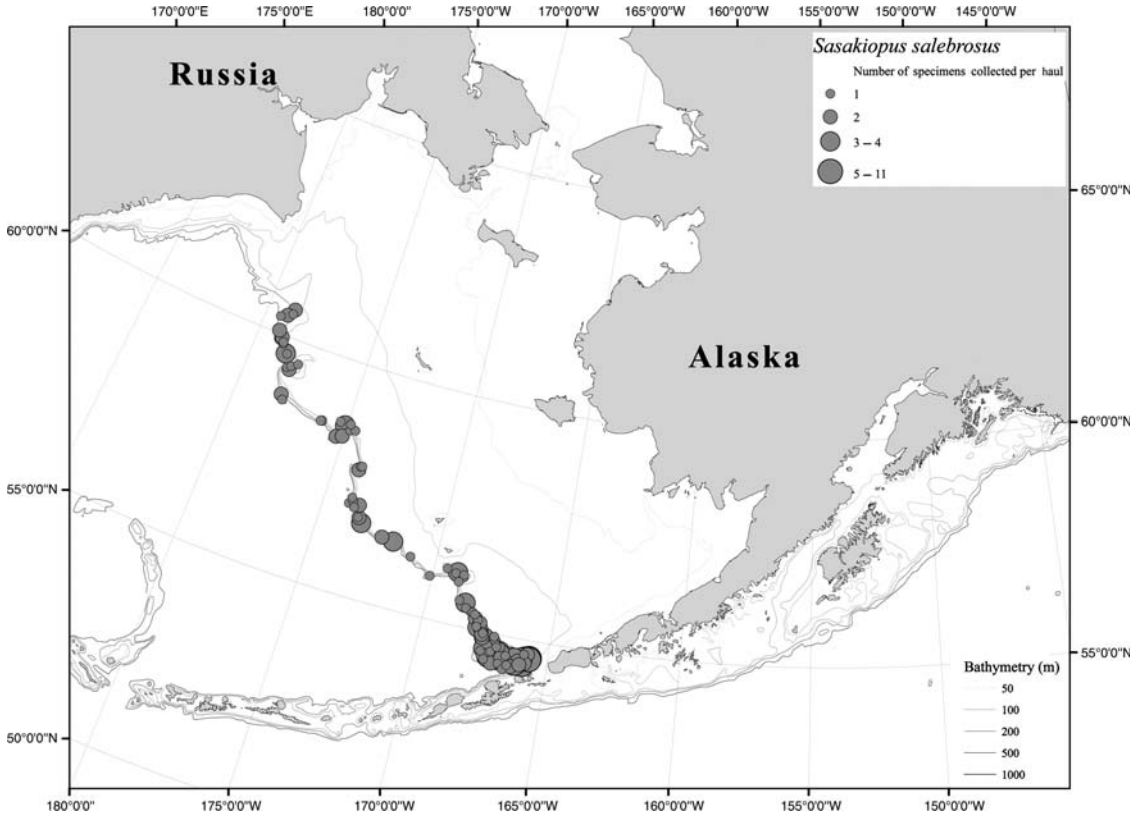

Figure 1. Distribution of *Sasakiopus salebrosus* (Sasaki, 1920) in the eastern Bering Sea. Symbol size indicates number of specimens collected per 30 min haul.

Table 1. GenBank accession numbers.

	12S rDNA	16S rDNA	COIII	*rhodopsin*
Octopus vulgaris Cuvier, 1797	EF016346	EF016336	EF016319	EF016312
Bathypolypus sponsalis (Fischer & Fischer, 1892)	EF016348	EF16338	FJ603530	GQ226024
Bathypolypus sp.	EF016347	EF016337	EF016320	EF16307
Bathypolypus bairdii (Verrill, 1873)	AY616944	AY616944	–	AY617041
Enteroctopus dofleini (Wulker, 1910)	AY545088	AY545109	FJ603531	AY545174
Enteroctopus megalocyathus (Gould, 1852)	GQ226030	GQ226032	GQ226027	GQ226026
Sasakiopus salebrosus (Sasaki, 1920)	GQ226029	GQ226031	GQ226028	GQ226025
Benthoctopus normani (Massy, 1907)	EF016352	EF016343	EF016324	EF016317
Benthoctopus yaquinae Voss & Pearcy, 1990	FJ603550	FJ603539	FJ603532	GQ226017
Benthoctopus eureka Robson, 1929	EF016349	EF016339	EF016321	EF016313
Benthoctopus johnsonianus Allcock *et al.*, 2006	EF016351	EF016342	EF016324	EF016316
Benthoctopus rigbyae Vecchione *et al.*, 2009	FJ428006	EF016341	EF016323	EF016315
Benthoctopus oregonensis Voss & Pearcy, 1990	FJ603545	FJ603543	FJ603538	GQ226016
Vulcanoctopus hydrothermalis Gonzalez *et al.*, 1998	FJ603547	FJ603544	FJ603533	GQ226020
Benthoctopus sp. B FMNH 309724	FJ603552	–	–	GQ226023
Benthoctopus cf. *profundorum*	FJ603549	FJ603542	FJ603537	GQ226021
Benthoctopus sp. A FMNH 278117	FJ603546	FJ603540	FJ603534	GQ226018
Benthoctopus sp. A FMNH 308674	FJ603551	FJ603541	FJ603535	GQ226019

In this paper, we erect a new genus, *Sasakiopus*, for *B. salebrosus*, and establish through molecular sequence analysis that this genus is, indeed, closely related to *Enteroctopus* and *Benthoctopus*, although its affinities with *Bathypolypus* are less clear. We also provide a redescription of *Sasakiopus salebrosus* new combination based on the holotype and new material.

MATERIAL AND METHODS

Sampling

Sampling was conducted from 6 June to 8 August 2004 during a groundfish survey of the eastern Bering Sea continental slope aboard the FV *Northwest Explorer*. Samples were collected using a bottom trawl; trawling duration was 30 min. On the slope,

sampling was conducted between 200 and 1,500 m water depth. Sampling yielded 232 specimens of *Sasakiopus salebrosus* at depths ranging from 220 to 1160 m (Fig. 1). Sampling was contiguous with a concurrent Bering Sea continental shelf survey; however, no *S. salebrosus* were collected on the shelf.

Specimens were examined live, when possible, as well as when freshly dead and after fixation. Approximately one-third of the specimens collected were fixed in 10% formalin and deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC (NMNH) or the Delaware Museum of Natural History, USA (DMNH). Before formalin fixation, muscle tissue samples were taken and preserved in 95% ethanol.

Morphological taxonomic analyses

Comparative material was made available by NMNH. Measurements were taken from formalin-fixed, ethanol-preserved material. Where indices were calculated, the values given are the mean ± standard deviation. Size descriptors (e.g. small, shallow), where given alongside indices, follow the unpublished guidelines proposed at the octopod taxonomy workshop at the Cephalopod International Advisory Council Symposium in Phuket, Thailand 2003 (Hochberg, Norman & Huffard, 2005).

Abbreviations used are those recommended in the published guidelines for octopus taxonomy by Roper & Voss (1983) and are as follows: dorsal mantle length (ML), total length (TL), mantle width index (MWI), head width index (HWI), web depth index (WDI), funnel length index (FuLI), mantle arm index (MAI), arm length index (ALI), arm sucker index (ASI), opposite arm index (OAI), ligula length index (LLI), calamus length index (CaLI) and spermatophore length index (SpLI).

Molecular analyses

DNA was extracted following the protocol given in Allcock *et al*. (2006). Primers for three mitochondrial genes [12S rDNA, 16S rDNA and cytochrome oxidase III (COIII)] were taken from Simon *et al*. (1990), Simon, Franke & Martin (1991) and Guizik *et al*. (2005). Primers for the nuclear gene, *rhodopsin*, are those of Allcock *et al*. (2008). The barcoding gene, cytochrome oxidase I (COI), was also targeted, but failed to amplify for most species used in this study and therefore it was not used.

Thermal cycling conditions consisted of a denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 40 s, at an optimized temperature (57°C for 12S rDNA, 55°C for 16S rDNA, 40°C for COIII and 55°C for *rhodopsin*) for 40 s, and 72°C for 90 s. A final extension step of 72°C for 10 min was added in each case.

Amplified products were purified using Qiagen PCR purification kits following the manufacturer's instructions. Purified PCR products were commercially sequenced by Macrogen in both directions using the same primers used for PCR amplification. Sequences for all species included in the analysis, except for *Enteroctopus megalocyathus* (Gould, 1852), *S. salebrosus* and *Bathypolypus* sp., were available from previous research (Allcock *et al*., 2006; Strugnell *et al*., 2009; Vecchione *et al*., 2009). GenBank accession numbers for all sequences are given in Table 1.

DNA sequences were compiled and aligned by eye in Se-Al v2.0a11 (Rambaut, 2002). Alignment of COIII required no insertion/deletion events (indels). Indels were introduced into aligned sequences of 12S rDNA, 16S rDNA and *rhodopsin*. Highly variable loop regions within 12S rDNA (37 bp in total) and 16S rDNA (118 bp in total) that were unalignable were removed prior to analysis. Of the 1,984 characters used in the analysis, 469 (23.6%) were found to be variable.

Congruence in the signal of each of the genes was evaluated with the partition-homogeneity test implemented in PAUP v4.0b10 (Swofford, 1998) using 1,000 random repartitions.

A substitution model was chosen on the basis of the Akaike Information Criterion (AIC; Akaike, 1974) implemented in ModelTest 3.7 (Posada & Crandall, 1998).

PAUP v4.0b10 was used to perform full heuristic searches. Starting trees were generated by neighbour joining (Saitou & Nei, 1987). A GTR ($\Gamma + I$) likelihood model incorporating rate heterogeneity (four rate categories) was used. Branch swapping was performed using TBR (tree-bisection-reconnection). Parameters were then reestimated and finally branch swapping was performed using NNI (nearest neighbour interchange). Substitution parameter values were $A = 0.32$, $C = 0.18$, $G = 0.16$, $T = 0.35$, $A \leftrightarrow C = 1.73$, $A \leftrightarrow G = 5.66$, $A \leftrightarrow T = 3.80$, $C \leftrightarrow G = 2.15$, $C \leftrightarrow T = 22.63$, $G \leftrightarrow T = 1.00$, $I = 0.44$, $\Gamma = 0.46$. ML bootstrap values of clade support were generated based on the parameters above, using 1,000 replicates.

MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) was used to calculate marginal posterior probabilities using the $GTR + I + \Gamma$ model of nucleotide substitution for each

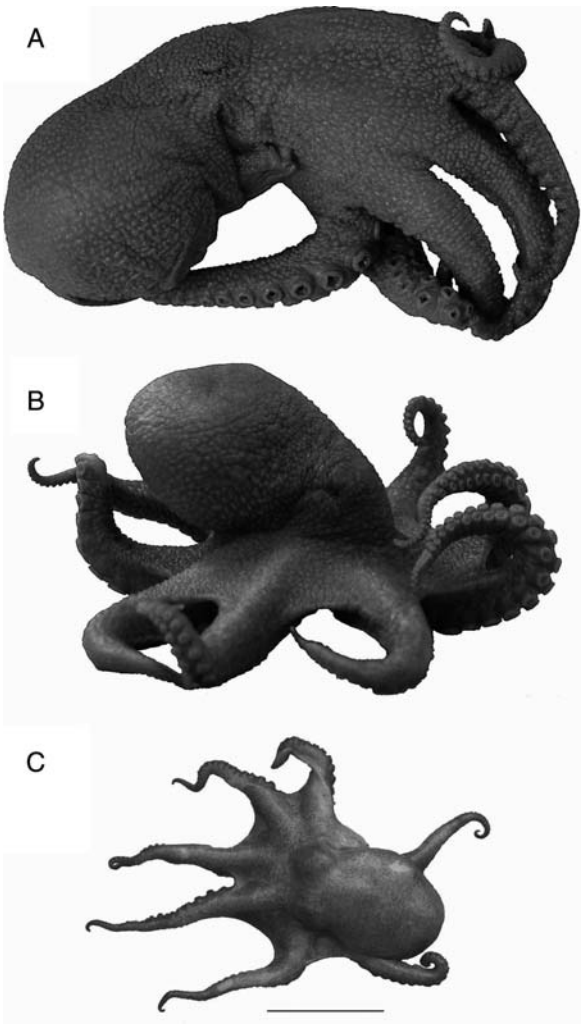

Figure 2. *Sasakiopus salebrosus* (Sasaki, 1920). **A.** Holotype (NMNH 332969). **B.** NMNH 1124206. **C.** Live animal (male). Scale bar = 2 cm.

partition. Model parameter values were treated as unknown and were estimated in each analysis. Random starting trees were used and analyses were run for 1 million generations, sampling the Markov chain every 100 generations. The analysis was performed twice, in each case starting from a different random tree to ensure the analyses were not trapped in local optima. Stationarity was deemed to have been reached when the average standard deviation of split frequencies, shown in MrBayes 3.1.2, was <0.01 (Ronquist & Huelsenbeck, 2003).

Phylogenetic trees were rooted using *Octopus vulgaris*, because previous phylogenetic studies using a wide range of octopodiform species have confirmed that this is a suitable outgroup to the clade containing *Enteroctopus*, *Benthoctopus* and *Bathypolypus* (Strugnell *et al*., 2005; Allcock *et al*., 2006).

SYSTEMATIC DESCRIPTIONS

Family Octopodidae Cuvier, 1797

Genus *Sasakiopus* new genus

Sasakiopus Jorgensen, 2009: 82. Nomen nudum.

Type species: *Polypus salebrosus* Sasaki, 1920.

Etymology: Named after Madoka Sasaki, the renowned cephalopod biologist who first described the type species of this genus.
Diagnosis: Benthic octopodid with biserial suckers. Arms approximately twice mantle length in mature animals. Arm autotomy absent. Enlarged suckers absent. Body sculpture of extensive, closely set, irregularly shaped, flat-topped papillae. Patch and groove sculpture absent. Dorsal white spots absent.

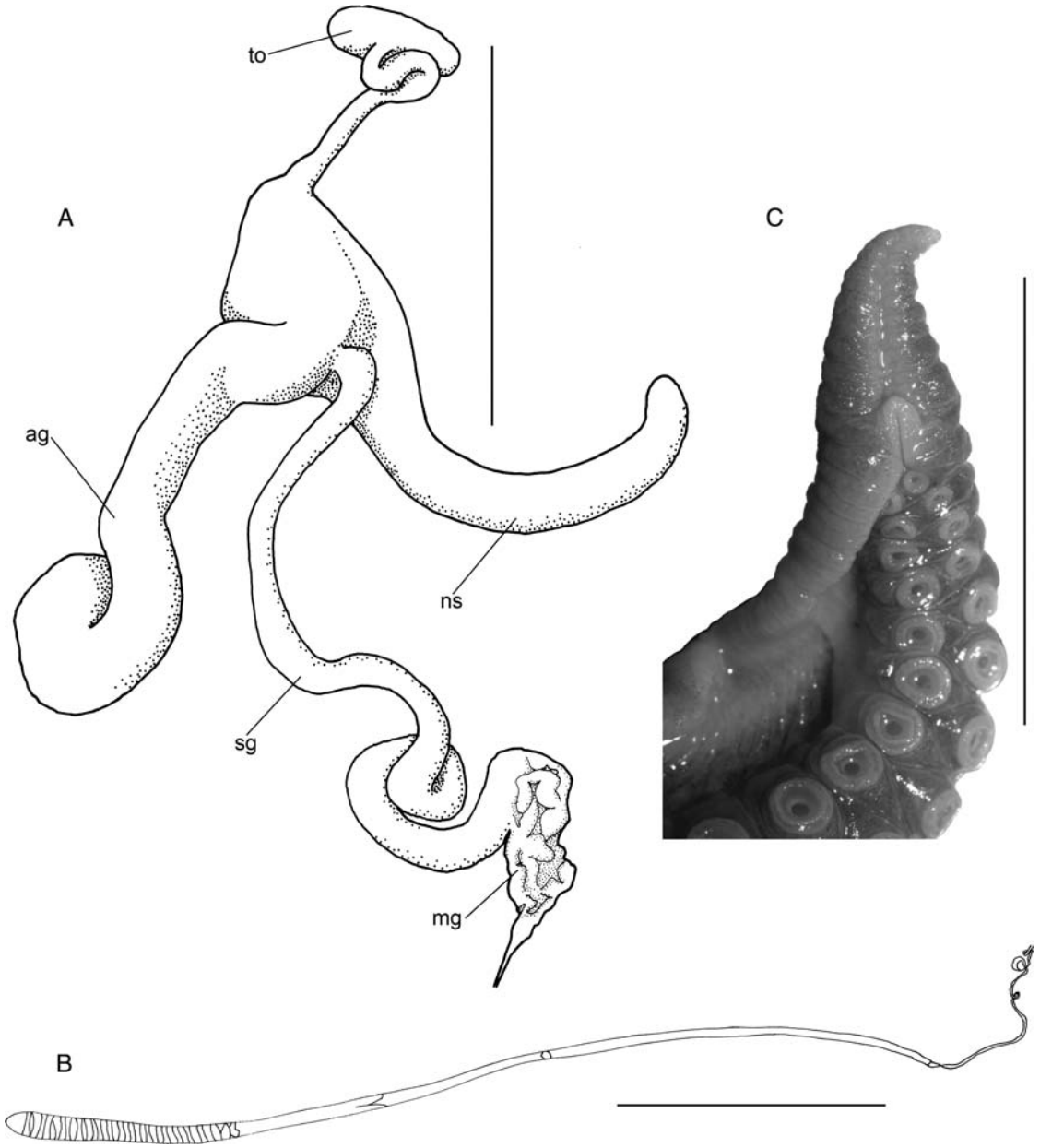

Figure 3. *Sasakiopus salebrosus* (Sasaki, 1920): male reproductive anatomy (NMNH 1125290). **A.** Male reproductive tract. **B.** Spermatophore. **C.** Ligula and calamus. Abbreviations: ag, accessory gland; mg, mucilaginous gland; ns, Needham's sac; sg, spermatophoric gland; to, terminal organ. Scale bars: **A** = 2 cm; **B** = 1 mm; **C** = 1 cm.

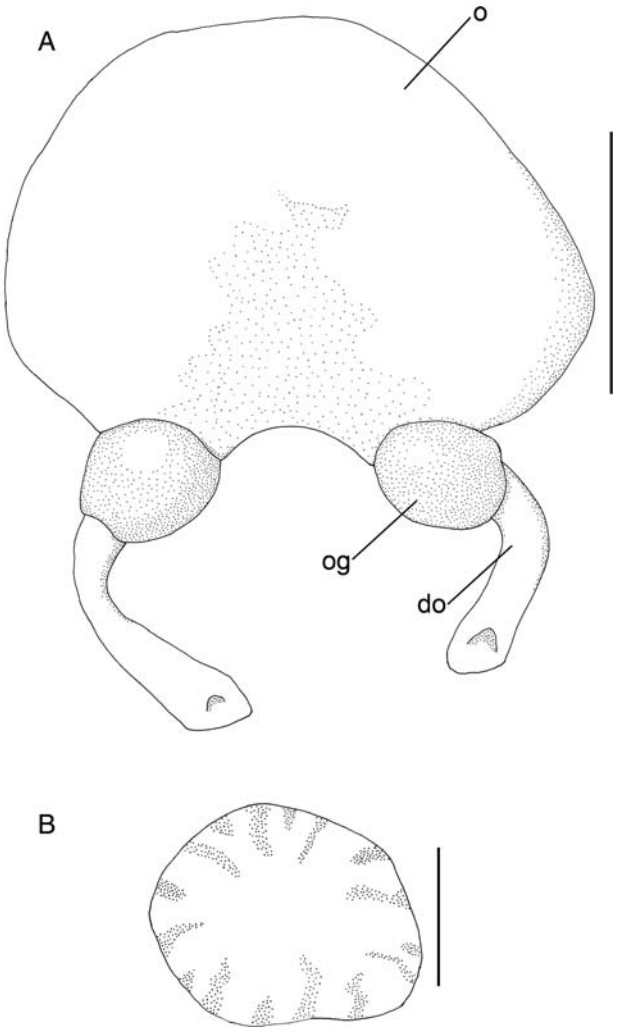

Figure 4. *Sasakiopus salebrosus* (Sasaki, 1920): female reproductive system (NMNH 1125287). **A.** Female reproductive tract. **B.** Cross-section through egg. Abbreviations: o, ovary; og, oviducal gland; do, distal oviduct. Scale bars: **A** = 2 cm; **B** = 2 mm.

Ocellae absent. No enlarged or supraocular papillae. R3 hectocotylized in male. Ligula large ($10 < \text{LLI} < 20$), not laminate. *In situ*, Needham's sac extends addextrally.

Remarks: This new generic name was inadvertently introduced as a *nomen nudum* by Jorgensen (2009), but is here validated, with authorship and date from the present publication.

***Sasakiopus salebrosus* (Sasaki, 1920)**

(Figs 2–6, Table 2)

Polypus salebrosus Sasaki, 1920: 182 (Japan; holotype NMNH 332969). Sasaki, 1929: 99, pl. 6, figs 5, 6, text-fig. 54 (first illustrations).

Bathypolypus salebrosus—Robson, 1932: 302. Akimushkin, 1965: 134, text-fig. 34 (description and illustration of male). Laptikhovsky, 1999: 342 (description of female reproduction).

Benthoctopus salebrosus—Muus, 2002: 204.

Sasakiopus salebrosus—Jorgensen, 2009: 82 (invalid use of generic name, a *nomen nudum*).

Types: Holotype NMNH 332969, Kinka San Pt., off Sendai, Honshu, Japan, 266 fathoms [486 m], Albatross station 5050, 38°11′30″N, 142°08′E, 10 October 1906, one female, submature, ML 42 mm. Paratype NMNH 332970, Sea of Okhotsk, 440 fathoms [805 m], Albatross station 5029, 48°22′30″N 145°43′30″E, 28 September 1906, one female, immature [not extant].

Material examined: Holotype: NMNH 332969 (Fig. 2A). Other material: NMNH 1124206, Bering Sea, FV *Northwest Explorer*, stn 62, 22 June 2004, 56.24°N, 171.02°W, 266 m, one female, submature, 45 mm ML; NMNH 1124208, Bering Sea, FV *Northwest Explorer*, stn 154, 19 July 2004, 55.94°N, 170.10°W, 655 m, one male, mature, 38 mm ML; NMNH 1124207, Bering Sea, FV *Northwest Explorer*, stn 161, 21 July 2004, 55.57°N, 168.75°W, 873 m, one female, submature, 49 mm ML; NMNH 1125289, Bering Sea, FV *Northwest Explorer*, stn 188, 29 July 2004, 55.26°N, 168.01°W, 627 m, one male, mature, 49 mm ML, one female, mature, 55 mm ML; NMNH 1125290, Bering Sea, FV *Northwest Explorer*, stn 194, 30 July 2004, 54.94°N, 167.64°W, 522 m, one male, mature, 38 mm ML, one female, immature, 34 mm ML; NMNH 1124204, Bering Sea, FV *Northwest Explorer*, stn 227, 6 August 2004, 54.49°N, 166.32°W, 495 m, one male, mature, 43 mm ML; NMNH 1125287, Bering Sea, FV *Northwest Explorer*, stn 227, 6 August 2004, 54.49°N, 166.32°W, 495 m, one female, mature, 65 mm ML. Additionally, 60 males and 40 females were collected.

Comparative material examined: see Supplementary material.

Diagnosis: With characters of genus. Maximum total length 200 mm. Funnel organ W-shaped, lateral limbs half length of medial limbs. Ink sac reduced but functional, anal flaps absent. Radula with nine elements, rachidian multicuspid.

Description: Based on holotype and other specimens listed in Table 2. Animals small, ML to 65 mm, TL to 200 mm (Fig. 2B). Body muscular. Mantle approximately spherical (MWI 97.7 ± 7.9), head narrower than mantle (HWI 79.9 ± 10.8). Web deep (WDI 35.5 ± 2.5), web formula approximately $C > D > B > A > E$. Funnel medium-sized (FuLI 40.4 ± 4.5), gently tapered, attached for majority of length; funnel organ W-shaped, lateral limbs about half length of medial limbs. Gills with 7–10 lamellae per outer demibranch. Arms short (MAI 43.7 ± 3.5), approximately twice length of mantle. Arm lengths subequal, arm order usually $III > IV > II > I$ (ALI LI 208.2 ± 25.8; L2 215.2 ± 24.5; L3 225.0 ± 15.3; L4 223.7 ± 14.5), arms taper abruptly in width. Suckers biserial, small- to medium-sized (ASI 8.5 ± 0.7), without sucker enlargement.

Third right arm of males hectocotylized, always shorter than opposite number (OAI 75.2 ± 6.4). Ligula large (LLI 14.4 ± 1.8); ligula groove long, well marked and shallow, without marked transverse ridges (Fig. 3C). Calamus distinct, medium to large (CaLI 31.2 ± 4.9). Hectocotylized arm with 38–43 suckers, opposite arm with up to 87 suckers. Male reproductive tract (Fig. 3A) with markedly long Needham's sac, storing up to 70 spermatophores. Penis small, penis diverticulum straight. Spermatophores (Fig. 3B) medium length, up to 32 mm long (SpLI 71.3 ± 8.7), slender, numerous (18–44). *In situ*, Needham's sac extends addextrally.

Female reproductive tract (Fig. 4A). Ovary containing up to 120 eggs, paired oviducts with large dark oviducal glands. Eggs with approximately 16 follicular folds (Fig. 4B). Mature ovarian eggs large, to 15 mm long, 4 mm wide.

Digestive system. Buccal mass approximately equal in length to posterior salivary glands (Fig. 5A). Anterior salivary glands small, closely associated with buccal mass. Short oesophagus leads into crop; crop with diverticulum. Stomach leads into caecum and caecum into intestine. Intestine located on right side of digestive gland. Connections between caecum and digestive gland are standard but obscured by the loop in the

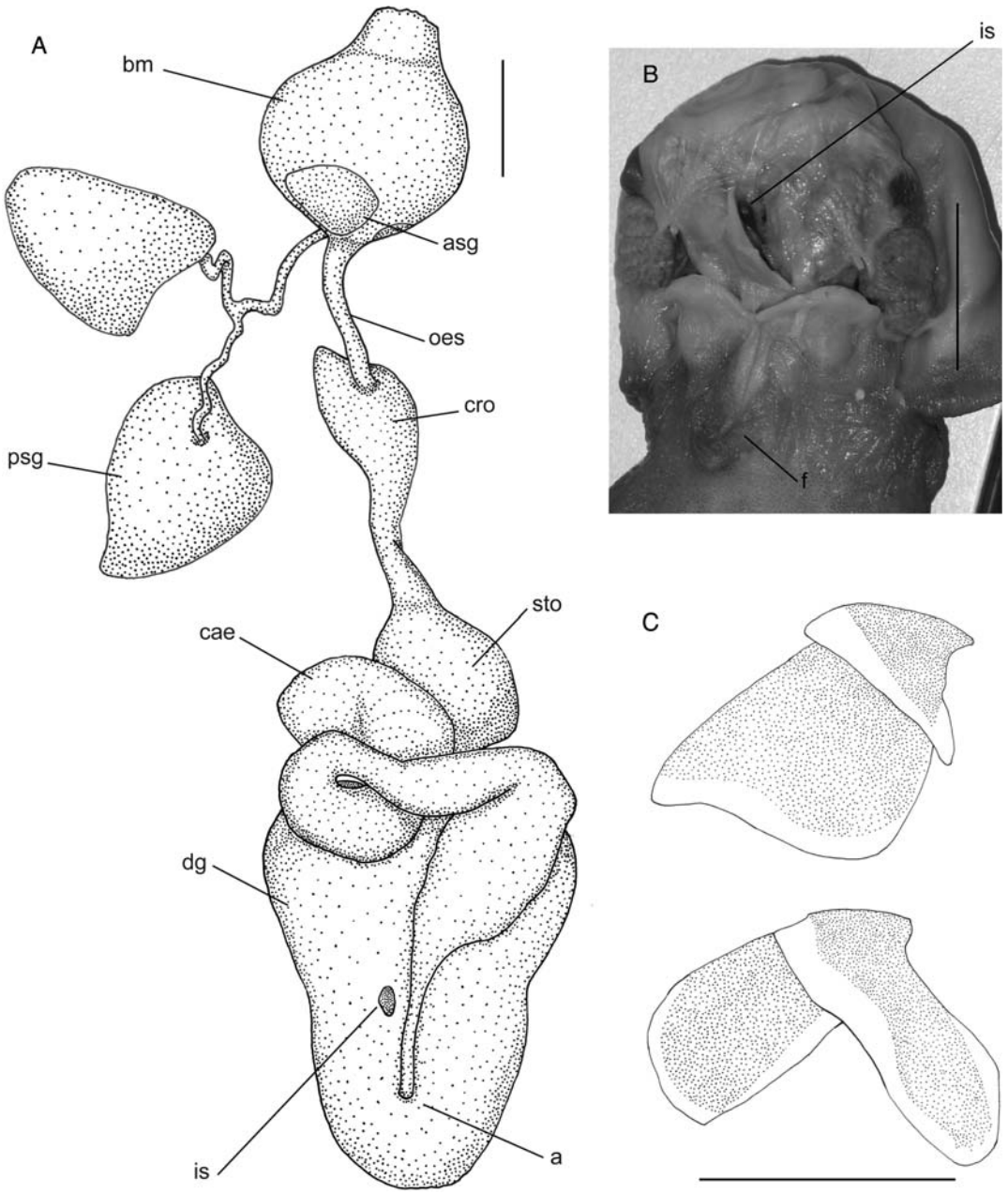

Figure 5. *Sasakiopus salebrosus* (Sasaki, 1920): digestive system. **A.** NMNH 1125287. Digestive tract. **B.** NMNH 1125289. Ink sac dissected from digestive gland but with duct still holding it *in situ*. Prior to dissection only small portion of sac visible as in (**A**). **C.** NMNH 1125287. Upper and lower beak. Abbreviations: a, anus; asg, anterior salivary gland; bm, buccal mass; cae, caecum; cro, crop; dg, digestive gland; f, funnel; is, ink sac; oes, oesphagus; psg, posterior salivary gland; sto, stomach. Scale bars: **A** = 5 mm; **B** = 1 cm; **C** = 1 cm.

intestine (Fig. 5A). Ink sac present, functional, extremely small, deeply embedded in digestive gland (Fig. 5B). Preserved ink in funnel of several fixed specimens. Live specimens observed inking. Anal flaps absent. Beak unremarkable, rostral tip of lower beak rounded (Fig. 5C). Radula with nine elements, rachidian multicuspid, seriation repeating every 4–5 teeth, lateral teeth unicuspid (Fig. 6).

Papillae compound, irregularly shaped, with raised flat top, cover entire dorsal and ventral surfaces. No distinct division between dorsal and ventral surfaces. No notably distinct papillae on dorsal mantle surface. Freshly dead specimens with dark pink hue. Live specimens deep red to purple (Fig. 2C). No colour change observed when disturbed, colour fades in injured/dying animals. Skin firm, texture of injured/dying animals smoother, papillae still visible (Fig. 2C). Colour in preservation brown, similar in holotype and newly preserved specimens.

Distribution: off Sendai, Honshu, Japan, 486 m, 38°11′30″N 142°08′E (holotype); Bering Sea, 54°16′–60°35′N, 165°41′–179°19′W, 220–1160 m (this study); Sea of Japan to Sea of Okhotsk, 41°47.49′N, 143°42.95′E, 359 m (National Science Museum, Tokyo, NSMT 71699), 44°58.2′N, 143°25.8′E, 685 m (NSMT 66688) (Kubodera & Tsuchiya, 1993); and 60°30′–61°50′N, 172°15′–179°40′E, 200–620 m (Laptikhovsky, 1999).

This study provides the first records of this species from the southeastern Bering Sea. Preserved specimens of *Sasakiopus*

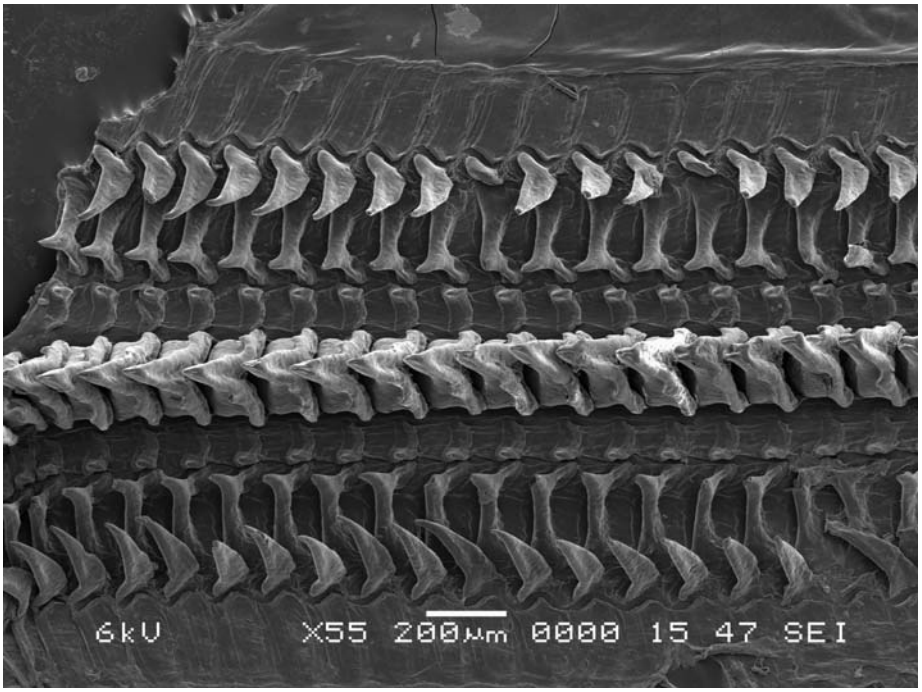

Figure 6. *Sasakiopus salebrosus* (Sasaki, 1920): radula. Scanning electron micrograph. Scale bar = 200 µm.

salebrosus have often been misidentified as immature *Enteroctopus dofleini* during fisheries surveys. On the Bering Sea slope, *S. salebrosus* was the most abundant octopod collected.

Molecular analyses: Results of the partition homogeneity test ($P = 0.697$) indicated that there was no significant conflict between partitions. Therefore each of the mitochondrial genes and the nuclear *rhodopsin* gene were concatenated into a single dataset for analysis.

All *Benthoctopus* species and *Vulcanoctopus hydrothermalis* fall in a highly supported clade (bootstrap, BS = 76; posterior probability, PP = 100). *Sasakiopus salebrosus* is the sister taxon to this clade. The monophyly of the clade containing *Sasakiopus*, *Benthoctopus* and *Vulcanoctopus* is also highly supported (BS = 98, PP = 98). A sister relationship between the two species of *Enteroctopus* is retrieved, but without significant support (BS = 61, PP = 58), and this *Enteroctopus* clade is the sister group to the clade containing *Sasakiopus*, *Benthoctopus* and *Vulcanoctopus*. *Enteroctopus*, *Sasakiopus*, *Benthoctopus* and *Vulcanoctopus* form a highly supported clade (BS = 99, PP = 100) to the exclusion of *Bathypolypus* (Fig. 7).

Remarks: The molecular analysis shows that *Sasakiopus* is most closely related to species of the genus *Benthoctopus*; in this analysis these genera form a clade with *Enteroctopus*. *Sasakiopus* can be distinguished from *Enteroctopus* by the size and shape of the ligula (elongate in *Enteroctopus* with LLI > 20) and from *Benthoctopus* by its ability to ink. Its papillae distinguish it from both genera: *Enteroctopus* has paddle-shaped papillae, which can be relaxed or raised, whilst *Benthoctopus* species are generally smooth-skinned and never have extensive papillae. The molecular data suggest that *Sasakiopus* does not have close affinities with *Bathypolypus*. Morphologically, male specimens can easily be separated from those of *Bathypolypus* by the ligula (laminate in *Bathypolypus*).

Due to an absence of recognized synapomorphies that define subfamilies such as the Octopodinae, it is necessary to distinguish *Sasakiopus* from other more distantly related genera. It can be distinguished from *Abdopus* Norman & Finn, 2001, *Ameloctopus* Norman, 1992, *Euaxoctopus* Voss, 1971, *Macrotritopus* Grimpe, 1922, *Thaumoctopus* Norman & Hochberg, 2005 and *Wunderpus* Hochberg *et al*., 2006 by the presence of arm autotomy in these genera (Voss, 1971; Hochberg, Norman & Finn, 2006). It can be distinguished from *Octopus s. s.* Cuvier 1797, *Amphioctopus* Fischer, 1880–1887 and *Callistoctopus* Taki, 1964 by the patch-and-groove skin sculpture in these genera. The shape of the ligula distinguishes *Sasakiopus* from *Cistopus* Gray, 1849 (tiny ligula), *Galeoctopus* Norman *et al*., 2004 (club-shaped ligula) and *Scaeurgus* Troschel, 1854 (edges of ligula rolled inward). *Grimpella* Robson, 1928 has much longer arms (4–5 times mantle length). *Danoctopus* Joubin, 1933 and *Pteroctopus* Fischer, 1880–1887 both have flared web membranes. *Hapalochlaena* Robson, 1929 is covered with iridescent rings and *Teretoctopus* Robson, 1929 lacks skin sculpture. Other genera have uniserial suckers.

As molecular work begins to identify well-supported clades below family level (e.g. Strugnell *et al*., 2008) it becomes increasing important to identify characters that are synapomorphic for these clades. To this end, we include a new descriptor of the position of Needham's sac *in situ* (Fig. 8). Male reproductive tracts are normally only examined after removal from the mantle cavity but we have noted variation in its placement. In *Sasakiopus*, the proximal end of Needham's sac (containing the heads of spermatophores) is positioned to the left of the midline. It extends towards the right and also adorally, curling around the testis just dorsal to it (Fig. 8B). The distal end of Needham's sac is therefore situated to the right of the midline. We refer to this configuration as 'addextral'. Initial work to investigate this character in other genera shows that at least one other configuration is present. In this case, the proximal end of Needham's sac is positioned close to the midline, but it extends towards the left (Fig. 8A). This 'adsinistral' form appears to be more prevalent in genera from shallow tropical waters. We hypothesize that this character may be phylogenetically important; it is the subject of ongoing research.

Table 2. Raw measurements from specimens of *Sasakiopus salebrosus*.

Status	Holotype	None	None	None	None	None	None	None	None	None
Repository	NMNH	NMNH	NMNH	NMNH	NMNH	NMNH	NMNH	NMNH	NMNH	NMNH
Catalogue number	332969	1124208	1125290	1124204	1125289	1125290	1124206	1124207	1125289	1125287
Sex	♀	♂	♂	♂	♂	♀	♀	♀	♀	♀
Maturity	Submature	Mature	Mature	Mature	Mature	Immature	Submature	Submature	Mature	Mature
Total length	125	130	132	152	165	120	130	170	180	200
Mantle length (dorsal)	42	38	38	43	49	34	45	49	55	65
Mantle length (ventral)	38	30	35	35	38	28	35	37	54	60
Mantle width	44	35	37	39	46	33	43	53	61	56
Head width	34	32	38	33	38	31	36	34	39	43
Pallial aperture	45	20	23	24	32	22	27	29	34	35
Full funnel length	20	15	16	15	20	14	16	21	25	22
Free funnel length	8	7	4	10	8	5	6	4	7	8
Funnel organ length (m/l)	9/7	10/7	8/5			7/4	9/8	9/5		12/9
Web depth sector A	30	27	32	36	27	25	29	32	33	42
Web depth sector B (l/r)	30/30	27/29	28/33	38/33	38/36	24/29	30/33	36/39	50/41	43/45
Web depth sector C (l/r)	27/30	28/30	30/30	32/29	39/33	24/30	31/33	37/42	48/43	43/49
Web depth sector D (l/r)	30/30	d/30	27/27	30/23	34/36	24/30	34/35	36/35	43/41	43/47
Web depth sector E	23	23	18	26	29	21	30	31	29	33
Arm length L1	70	81	87	105	116	66	81	108	117	120
Arm length L2	80	80	89	103	127	73	82	108	117	122
Arm length L3	d	86	90	98	118	75	86	114	123	d
Arm length Hc		70	70	65	89					
Arm length L4	90	86	86	102	114	75	d	d	131	126
Sucker count Hc		43	40	38	43					
Sucker count L3	72	74	82	75	78	82	76	85	87	d
Sucker diameter	4	3	3	4	4	3	4	4	5	5
Arm width	8	7	8	8	7	7	10	11	10	12
Ligula length		9	10	11	12					
Calamus length		3	3	4	3					
Gill lamellae: inner (l/r)	9/9	9/8	7/9	9/9	9/9	8/8	10/10	8/9	9/9	9/9
Gill lamellae: outer (l/r)	10/9	8/9	9/9	8/8	9/9	9/9	9/9	8/8	9/9	9/9
Gill length (l/r)	13/13	10/11	9/9	12/10	11/13	10/10	11/11	19/18	17/13	20/14
Gamete length	6	26	32	29	32		9	13	15	15
Gamete count	80-100	40	44	18	28		119	94	34	100

All measurements are in mm. Abbreviations: m/l, medial/lateral; l/r, left/right; d, damaged.

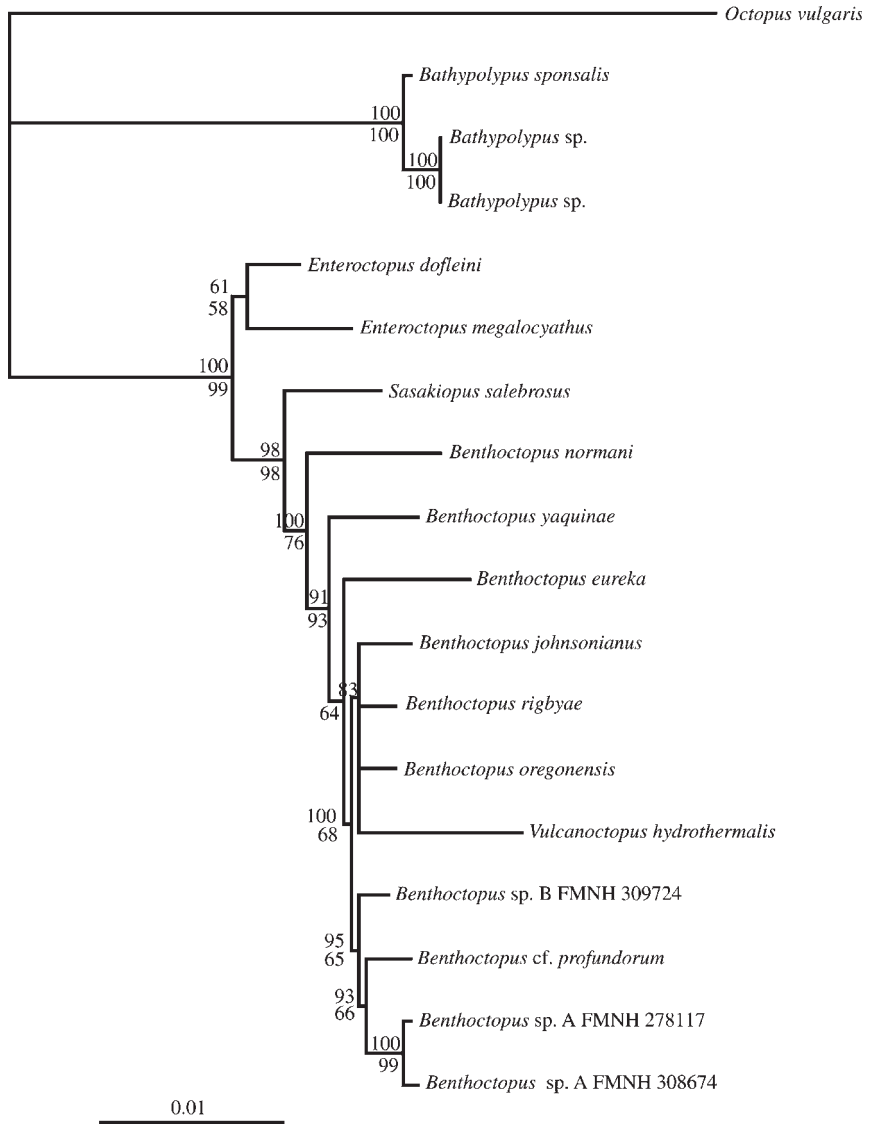

Figure 7. Maximum likelihood tree depicting the phylogenetic relationship of 17 species of Octopoda. The analysis employed three mitochondrial (12S rDNA, 16S rDNA, COIII) genes and the nuclear *rhodopsin* gene. Bayesian posterior probabilities are indicated above nodes, maximum likelihood bootstrap values are indicated below nodes. Only posterior probability and bootstrap values with 50% support or greater are shown. Scale bar indicates 0.01 substitutions per site.

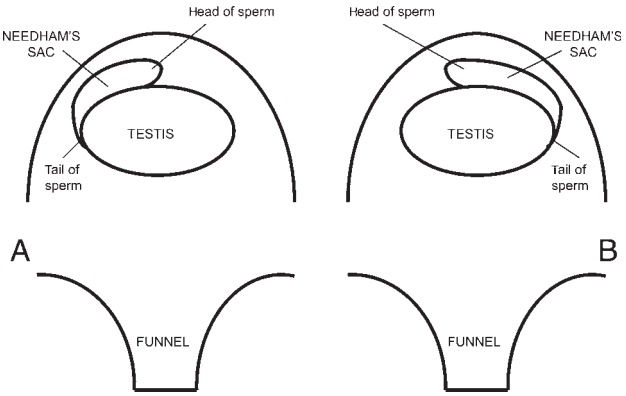

Figure 8. Ventral schematic illustration of *in situ* position of Needham's sac relative to testis. **A.** Adsinistral. **B.** Addextral.

Of all the comparative specimens examined, that which resembled *S. salebrosus* most closely was the holotype and only reported specimen of *Polypus validus* from 31°31′N, 129°25′30″E. Of particular note was the structure of the papillae. Papillae were simple or compound and formed 'rosettes' of up to 6 papillae per rosette in *P. validus*. In some cases these rosettes comprised a single compound papilla. The arms were slightly longer (*c.* 3× mantle length). The funnel organ was not discernible. The stylets were similar in shape to those of *S. salebrosus* (Bizikov, 2004). Unfortunately most of the internal organs of this specimen were missing, and in the absence of additional specimens we hesitate to include *P. validus* in *Sasakiopus*. Following Norman & Hochberg (2005) *P. validus* remains unplaced.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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