Vampyroteuthis southchinaseais sp. nov.—a second Recent widely distributed species of Vampyromorpha (Cephalopoda, Coleoidea)

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Running title: New species *Vampyroteuthis*

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Abstract

Until present, the Vampire squid *Vampyroteuthis infernalis* Chun, 1903 (Cephalopoda: Vampyromorpha) was the only accepted Recent species in the family Vampyroteuthidae Chun, 1903. However, morphological differences between specimens from the Gulf of Guinea, Africa, and California suggested the possibility that additional taxa existed. We report on a second species of *Vampyroteuthis* from the South China Sea, China which differs from *V. infernalis* in tail shape, lower beak morphology, and molecular characteristics, with phylogenetic trees based on large subunit ribosomal DNA and mitochondrial CO1 sequences supporting recognition of two species: *V. infernalis*, reserved for species lacking a tail, photophores near the fins, and a lower beak with a broad, short wing; and *Vampyroteuthis southchinaseais* sp. nov., which we apply to those species with a coracoid-shaped tail, a pair of photophores located between the fins and tail, and a lower beak with a broad, prolonged wing.

Keywords: *Vampyroteuthis southchinaseais* sp. nov., Vampyromorpha, Nuclear and mitochondrial gene, Phylogeny, Morphology, South China Sea

Introduction

Planktonic cephalopods and their larval and paralarval stages are widely distributed in the world's oceans from surface to abyssal depths, where they occupy epipelagic, mesopelagic and bathypelagic zones. One of these taxa, *Vampyroteuthis infernalis* Chun, 1903, is the only presently accepted Recent species in the Vampyroteuthidae Chun, 1903. The synonymy of this species is complex, and includes at least seven genera and 10 species (Sweeney and Young 1998).

The recognized distribution of *V. infernalis* includes temperate and tropical Pacific and Atlantic oceans (Yokobori et al. 2007, Hoving & Robison 2012) where it typically occurs between 600 and 900 m at oxygen concentrations of ~0.4 ml L^{-1} (Hoving & Robison 2012). However, morphological differences reported among specimens from the Gulf of Guinea, Africa, and California (Young 1972), and genetic differences or population structure analysis suggest that two or multiple Recent species may exist in this family (Braid and Bolstad 2019, Timm et al. 2020). In light of these differences, the Vampyroteuthidae requires revision.

Vampyroteuthis has not been earlier reported from the South China Sea, from which a recent faunal inventory cited 153 cephalopod species (Norman et al. 2016). We take this opportunity to describe a unique specimen from this region, for which we also report SSU and large

subunit (LSU) rDNA, mitochondrial CO1, and histone H3*a* sequences. Molecular phylogenies inferred from these data group this South China Sea specimen into one clade, separated from a second species which we nominally refer to *V. infernalis* in another. Both molecular phylogenies and morphological differences indicate the existence of two Recent species, one of which we herein describe as new species *Vampyroteuthis southchinaseais* sp. nov.

Materials and Methods

Sample collection, research ethics, and observations

A specimen of vampyromorph was collected during a Natural Science Foundation of China open cruise near Hainan Island, northwestern South China Sea (Stn 30: 17°59′28″N, 112°29′58″E) on 13 September 2016 (Figure 1) from a depth between 800 and 1000 m depth using a Hydro-Bios Multinet system (Type Max, mouth area 0.5m^2, 300 µm mesh; HydroBios Inc., Kiel, Germany) hauled vertically at approximately 0.5 m s^{-1}. Temperature at this station ranged 5.75–8.86°C and salinity 34.46–34.36 PSU. The net was equipped with internal and external flow meters and a depth transducer, all monitored and logged in real time; sampling of the water column otherwise occurred from 1000–800, 800–600, 600–400, 400–300, 300–200, 200–100, 100–50, 50–25 m and 25–0 m.

The specimen of vampyromorph was subsequently donated by the Chinese Ocean Conservation Association for research use in this study in accordance with local research guidelines and regulations on animal experimentation. All experimental protocols were reviewed and approved by the research ethics committee for animal experiments at the South China Sea Institute of Oceanology, Chinese Academy of Sciences. Immediately after sorting the specimen was photographed, and counts and measures were taken following Roper and Voss (1983). The specimen was then frozen at −20°C, and kept in darkness for subsequent molecular and morphological analyses.

DNA extraction, PCR, and gene sequencing

A tissue sample was resuspended in 0.5 mL of DNA lysis buffer (0.1 MEDTA pH 8.0, 1% SDS, 200 μg mL^{-1} proteinase K) in a 1.5 mL tube, then incubated for 48 hours at 55°C. DNA extraction and purification followed protocols of Qiu et al. (2011). After extraction, sample DNA was eluted in 50 μL Tris-HCl solution before 1 μL of DNA was used in PCR with pairs of SSU rRNA, rDNA LSU, mitochondrial CO1, and H3*a* primers to amplify SSU rDNA, LSU rDNA, mitochondrial CO1, and histone H3*a* genes, respectively. Primer sequences are detailed in Table 1. PCR amplifications were carried out in 25 μL reaction volumes under thermocycling conditions, including a denaturing step of 94°C for 4 min;

35 cycles of 94°C for 30 s, 56°C for 30 sec, 72°C for 45 sec, and a final extension step of 72°C for 10 min. PCR products were resolved by agarose gel electrophoresis with the DL2000 DNA Ladder (TaKaRa Bio, Dalian, China) before bands of expected sizes were excised to remove primer dimers. DNA was purified and directly sequenced as reported by Qiu et al. (2011).

Phylogenetic analyses

Sequences were trimmed with primers, and the two strands were merged. Assembled sequences were analyzed using the Basic Local Search Tool (BLAST) against the GenBank database. Sequences showing significant similarity in BLAST to our sequences were retrieved. Phylogenies based on partial SSU (1899 bp) and partial LSU (D1-D2, 408 bp), mitochondrial CO1 (702 bp), and histone H3*a* (311 bp) regions were used to investigate the phylogenetic position of *Vampyroteuthis*. Combined sequences were aligned with ClustalW. Alignment was run through ModelTest to select the most appropriate evolutionary model. The selected general time reversible model with gamma distribution was employed for maximum likelihood analysis using Mega 5.0. Categories of substitution rates were set at 4, and other parameters were estimated based on datasets. Sequences obtained in this study are deposited in GenBank with accession numbers MN044751, OP526744, OP531921,

OP549780.

Results

Our data strongly support the existence of two distinct *Vampyroteuthis* species that differ in tail shape, photophore position, lower beak morphology, and evolutionary distance between respective SSU and LSU rDNA, and mitochondrial CO1 sequences. We propose that one of these taxa, the South China Sea specimen, represents a new species, which we differentiate from the other which we refer to as *V. infernalis*.

Taxonomic Description

Vampyroteuthidae Chun, 1903,

***Vampyroteuthis southchinaseais* D. Qiu, B. Liu, & L. Huang sp. nov.**

Preliminary description

Fresh specimen (Figure 2) small, extensively gelatinous, of total length 9.5 cm, mantle length 5.2 cm, and mantle width 5 cm, with black and reddish-brown chromatophores interspersed over body surfaces. Mantle conical and rounded, with coracoid-shaped ‘tail’ (Figure 2). Head fused with mantle; brachial and nuchal constrictions poorly demarcated. Eyes brown (0.7 cm diameter), recessed into head tissues, covered by a crystalline membrane. Arms of comparable length to mantle, subequal, with tapering ends. Web depth ~2.5 cm, ~ 50% arm length. Suckers small,

in single series, on distal half of arms only, unstalked, without cuticular lining. Two rows of cirri extend along each arm for their length. Halfway between it and the web margin, threadlike arm ends naked. A pair of long, paddle-shaped fins (width ~5 cm, length ~0.9 cm) lacking a cartilaginous base protrude from the posterior mantle. A pair of large photophores (~0.3 cm diameter) lie between the fins and 'tail.'

Gladius

Gladius broad, chitinous, transparent, covering the dorsal mantle beneath the skin and a thick layer of loose connective tissue (Fig. 3). Gladius thin and flat anteriorly, gradually thickening and arching strongly posteriorly. Gladius formed of middle (ostracum), inner (hypostracum) and outer (periostracum) shell layers. The proostracum comprises five longitudinal elements: a middle plate (rachis), two lateral plates, and two wings (Fig. 3a).

Beaks

Upper beak rostrum long, curved, tip pointed. Jaw angle obtuse, lateral wall extends forwards of wing forming large, with distinct false angle. Hood long, ~0.8 times crest length. Posterior margin of hood/wing are convex. Wing extends to base of anterior margin of lateral wall. Crest straight, unthickened (Fig. 3b). Lower beak rostral tip pointed, have small hook. Wings are very broad, prolonged, spread parallel, with very high wing fold, highest opposite jaw angle, forming smooth cutting edge.

Crest short, wide, unthickened. Jaw angle obtuse. Shoulder tooth present (Fig. 3b). Beak measurements are presented in Table 2.

Holotype. Fig. 2B collected by Dajun Qiu. SCSMBC030991, Marine Biodiversity Collections of South China Sea, South China Sea Institution of Oceanology, Chinese Academy of Sciences. The specimen is barcoded in GenBank: SSU 1-1899 (MN044751), D1-D2 LSU 1-408 rDNA (OP526744), and CO1 1-702 (OP531921), H3a 1-311 (OP549780) sequences.

Etymology

The species name *southchinaseais* refers to the geographic locality, the South China Sea, where the species was collected.

Type locality

South China Sea, China (17°59′28″N, 112°29′58″E).

Distribution

From whole animals, the South China Sea species is known only from this locality. From molecular samples, this species appears to have a wider distribution. D1–D2 LSU rDNA sequences MG: 263928, 263931, 263941, 263942, 263948, 263949, 263954, 263969, 263987, and 263992 from GenBank identified as *V. infernalis* from the Gulf of Mexico, Atlantic Ocean, and an unknown locality, are identical to that of *V. southchinaseais*. Additionally, CO1 sequences AB266515; GU145060; MF432197; and (all MG) 591300, 591304, 591313, 591328, 591329,

591335, 591338, 591339, 591358, 591379, 591380, 591385, 591386, 591404, 591438, 591440, 490128; and NC009689 retrieved from GenBank as *V. infernalis* from the Gulf of Mexico, North Atlantic Ocean, Smith Caldera (North Pacific Ocean), and an unknown locality, are also identical to that of *V. southchinaseais*.

Phylogenetic position of *Vampyroteuthis* based on nuclear rRNA, mitochondrial CO1, and histone H3*a* genes

Nuclear-encoded partial SSU (MN044751) and LSU (OP526744) ribosomal RNA genes, mitochondrial CO1 (OP531921), and histone H3*a* gene sequences (OP549780), were obtained. The phylogenetic tree of SSU, LSU, CO1, and H3*a* contained 40, 29, 80, and 41 sequences, respectively, from GenBank, in addition to our sequences. LSU tree topologies inferred from the dataset using neighbor joining (NJ) and maximum likelihood (ML) revealed well-supported long distances and clear separation of *V. southchinaseais* from *V. infernalis* and other genera (Fig. 4). Because our LSU rRNA gene sequence is almost identical to 10 sequences (MG: 263928, 263931, 263941, 263942, 263948, 263949, 263954, 263969, 263987, 263992) from the Gulf of Mexico, and Atlantic Ocean, these specimens may be conspecific. The LSU phylogeny places *V. southchinaseais* sp. nov. in a distinct lineage, separate from 14 other sequences referred to *V. infernalis* from the Gulf of Mexico (MG: 263894, 263895, 263896, 263897, 263926, 263930, 263940, 263951, 263953,

263959, 263985, 263986) and two unknown locality (AH012197 and AY557548) (Fig. 4).

In the mitochondrial CO1 tree (Fig. 5), the South China Sea *V. southchinaseais* is identical to 21 sequences reported for *V. infernalis* from the Gulf of Mexico, northeast and northwest Atlantic Ocean, and North Pacific Ocean (Smith Caldera), and forms a strongly supported distinct lineage that is well-separated from 20 sequences referred to *V. infernalis* from the Gulf of Mexico, near New Zealand (South Pacific Ocean), Indian Ocean, and northwest Atlantic Ocean. Thus, the mitochondrial CO1 tree resolved these species into two groups. Sequences of AY557459 (SSU rDNA, Fig. 6), AY557548 (LSU rDNA, Fig. 4), AF000071 (CO1, Fig. 5), and AY557408 (histone H3 *a*, Fig. S1) were sourced from the same specimen referred to *V. infernalis* by Lindgren et al. (2004). In the SSU tree (Fig. 6), the 1899-bp SSU rDNA sequence from the South China Sea of *V. southchinaseais* (MN044751) differed by only 5 bp (0.003%) from that of *V. infernalis* (AY557459, AY145387). So SSU rDNA sequences of *V. southchinaseais* could be separated from the *V. infernalis* sequence (AY557459) based on sequence bases. LSU, SSU, and CO1 molecular phylogenies revealed *V. southchinaseais* to form strongly supported lineages (Fig. 4–6). However, in the H3*a* tree (Fig. S1), *V. southchinaseais* could not be separated from *V. infernalis* sequences (AY557408).

Discussion

Splitting *Vampyroteuthis infernalis* sensu lato into two species: *V. southchinaseais* sp. nov. and *V. infernalis* s.s.

SSU, LSU rDNA and CO1 phylogenies indicate that *V. southchinaseais* differs from what we refer to as *V. infernalis* (Fig. 4–6) with very strong bootstrap support. In addition to the contrast in general shape, *V. southchinaseais* and *V. infernalis* differ in 'tail' shape, lower beak morphology, and photophore position. In this study, *V. southchinaseais* has a broad and prolonged wing of lower beak, which differ from those of *V. infernalis* the southeast and northeast Pacific and central and eastern Atlantic (Clarke 1986, Schwarz et al. 2020). The ratio of lower beak CL to HL in this study is similar with previous studies, except for the eighth specimen (Table 2). The ratio of lower beak WL to RL in this study is significant different from the second, third and fourth specimens but similar with the seventh and eighth specimens (Table 2). *V. infernalis* has neither a 'tail' nor photophores near the fins, and the lower beak has a broad and short wing, while *V. southchinaseais* has a coracoid-shaped 'tail,' photophores between the fins and 'tail,' and a lower beak with a broad, prolonged wing (Table 3).

In previous molecular phylogenies, *V. southchinaseais* branched in a basal position to *V. infernali*s s.s.; these two species indisputably share

common ancestry (Figs. 4 and 5). Timm et al. (2020) reported that the evidence of two populations in *Vampyroteuthis* was found in the Gulf of Mexico and northwestern Atlantic Ocean based on population structure analysis..

Phylogenetic position of Vampyromorpha based on morphology and molecular evidence

Our analyses lend support to the idea that Vampyromorpha occupies an intermediate phylogenetic position between Decabrachia and Octobrachia (Fig. 6). Of surveyed taxa, Vampyromorpha appears to be more closely related to *Psychroteuthis* sp. (AY557513) than it is to *S. syrtensis*, differing from the former in 345 bp (15.92%) and the latter in 851 bp (32.78%). Our interpretation of these results is similar to those of Lindgren et al. (2004) using molecular data and Pickford (1939, 1940), Young (1977), Healy (1990), and Young et al. (1996, 1998) using morphological data, wherein Vampyromorpha was deemed to be more closely related to Decabrachia (or Teuthida) than to Octobrachia (particularly cirrate octopuses). However, this interpretation contrasts with that of Yokobori et al. (2007), wherein the Vampyromorpha was deemed to be more closely related to the Octobrachia based on mitochondrial gene sequences.

Morphological and molecular data support the position of the

Vampyromorpha as being intermediate between the Octobrachia and Decabrachia. However, our results and historical data (both morphological and molecular) have yet to unequivocally demonstrate the monophyly of Decabrachia and Vampyromorpha, or Octobrachia and Vampyromorpha. Further analyses are required to resolve phylogenetic relationships between these taxa.

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Author contributions

DQ designed the experiments. DQ, GL, and ZK collected the samples. DQ, BL, YG, WA conducted the experiments. DQ, LH, KL, and YT

contributed reagents, materials, analysis tools. DQ and BL carried out the data analysis. DQ and BL prepared the manuscript. All authors were involved in revision of the manuscript and approved its final version.

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Figure legends:

Figure 1. Map of study site. The *Vampyroteuthis* was collected from Station 30.

Figure 2. Fresh-collected *Vampyroteuthis southchinaseais* sp. nov.: A) dorsal, B) lateral views. Scale bar = 1 cm in Fig. 2 A–B.

Figure 3. Gladius (A), upper and lower beak (B) of *Vampyroteuthis southchinaseais* sp. nov. Scale bar = 1 cm in Fig. 3 A–B.

Figure 4. Cephalopod phylogeny inferred from LSU rDNA. Nodal support based on bootstrap values of ML/NJ with 1000 resamplings each; only values > 50 are shown (if a phylogenetic method yielded support < 50, it is denoted by ‘‘-’’). *Pyroteuthis margaritifera* was used as an outgroup to root the tree.

Figure 5. Cephalopod phylogeny inferred from mitochondrial CO1. Nodal support based on bootstrap values of ML/NJ with 1000 resamplings each; only values > 60 are shown (if a phylogenetic method yielded support < 60, it is denoted by ‘‘-’’). *Chaetoderma nitidulum* was used as an outgroup to root the tree.

Figure 6. Cephalopod phylogeny inferred from SSU rDNA. Nodal support based on bootstrap values of ML/NJ with 1000 resamplings each; only values > 60 are shown (if a phylogenetic method yielded support < 60, it is denoted by ‘‘-’’). *Chaetoderma nitidulum* was used as an outgroup to root the tree.

Figure S1. Cephalopod phylogeny inferred from histone H3 *a* gene. Nodal support based on bootstrap values of ML/NJ with 1000 resamplings each; only values > 60 are shown (if a phylogenetic method yielded support < 60, it is denoted by ‘‘-’’). *Chaetoderma nitidulum* was used as an outgroup to root the tree.

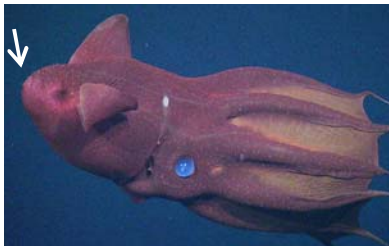
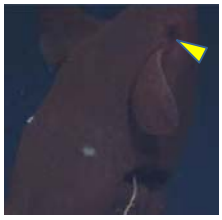
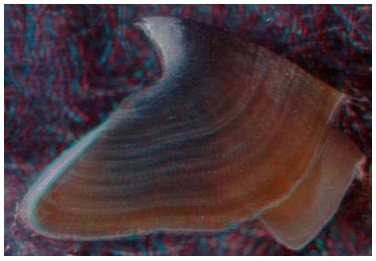
Table 1. Primers used in this study.

Primer name	Application	Sequences (5′-3′)	References
18SF	SSU for Vampyroteuthis	AAGGGTTGTGTTYATTAGNTACARAAC	This study
18ScomR	SSU for Vampyroteuthis	CACCTACGGAAACCTTGTTACGAC	This study
28Sa	LSU for Vampyroteuthis	GACCCGTCTTGAAACACGGA	Lindgren et al., 2004
28Sb	LSU for Vampyroteuthis	TCGGAAGGAACCAGCTAC	Lindgren et al., 2004
LCO1490	CO1 for Vampyroteuthis	GGTCAACAAATCATAAAGATATTGG	Lindgren et al., 2004
HCO2198	CO1 for Vampyroteuthis	TAAACTTCAGGGTGACCAAAAAATCA	Lindgren et al., 2004
H3*a* F	H3*a* for Vampyroteuthis	ATGGCTCGTACCAAGCAGAC(ACG)GC	Lindgren et al., 2004
H3*a* R	H3*a* for Vampyroteuthis	ATATCCTT(AG)GGCAT(AG) AT(AG)GTGAC	Lindgren et al., 2004

Table 2. Lengths of upper and lower beak characters of *Vampyroteuthis* in this study and histories data. HL, hood length; RL, rostral length; CL, crest length; LWL, lower wing length; WL, wing length

Specimen number	Break position	HL (mm)	RL (mm)	CL (mm)	LWL (mm)	WL (mm)	
1	Upper beak	8.718	2.249	11.590	8.005	2.924	This study
	Lower beak	5.436	2.077	6.559	6.905	4.630	
2	Upper beak	/	/	/	/	/	http://tolweb.org/Vamp
	Lower beak	3.41	1.00	3.78	3.91	2.81	yroteuthis_infernalis/20
3	Upper beak	/	/	/	/	/	084
	Lower beak	7.57	2.20	9.12	9.62	7.75	http://tolweb.org/notes/
4	Upper beak	/	/	/	/	/	?note_id=5249
	Lower beak	16.59	5.30	20.16	19.87	14.26	
5	Upper beak	13.23	3.67	16.13	13.49	4.31	Schwarz et al. 2020
	Lower beak	/	/	/	/	/	
6	Upper beak						Clarke 1986
	Lower beak	10.786	/	13.000	12.91	/	
7	Upper beak	7.10	2.61	9.45	5.84	1.58	Pickford 1949
	Lower beak	5.00	2.04	6.09	/	4.12	
8	Upper beak	5.04	/	7.02	4.80	/	
	Lower beak	3.66	0.91	5.21	4.84	1.95	

Table 3 Morphological characteristics and distributions of *Vampyroteuthis* species

	Vampyroteuthis infernalis s.s.	*Vampyroteuthis southchinaseais* nov. sp.
Tail	No tail  © 2007 MBARI	Coracoid shaped tail
Photophores	A pair of photophores are located on the dorsal mantle side near the fins.  Hoving and Robinson, 2012	A pair of photophores are located between the fins and tail.
Lower beak wing	Broad and short  http://tolweb.org/notes/?note_id=5249	Broad and prolonged
Distributions	The Gulf of Mexico,	South China Sea, Smith

	northwest Atlantic Ocean, near New Zealand (South Pacific Ocean), North Pacific Ocean, and Indian Ocean.	Caldera (North Pacific Ocean), the Gulf of Mexico, northeast and northwest Atlantic Ocean.
References	Young, 2019; Hoving and Robinson, 2012; http://tolweb.org/notes/?note_id=5249.	This study

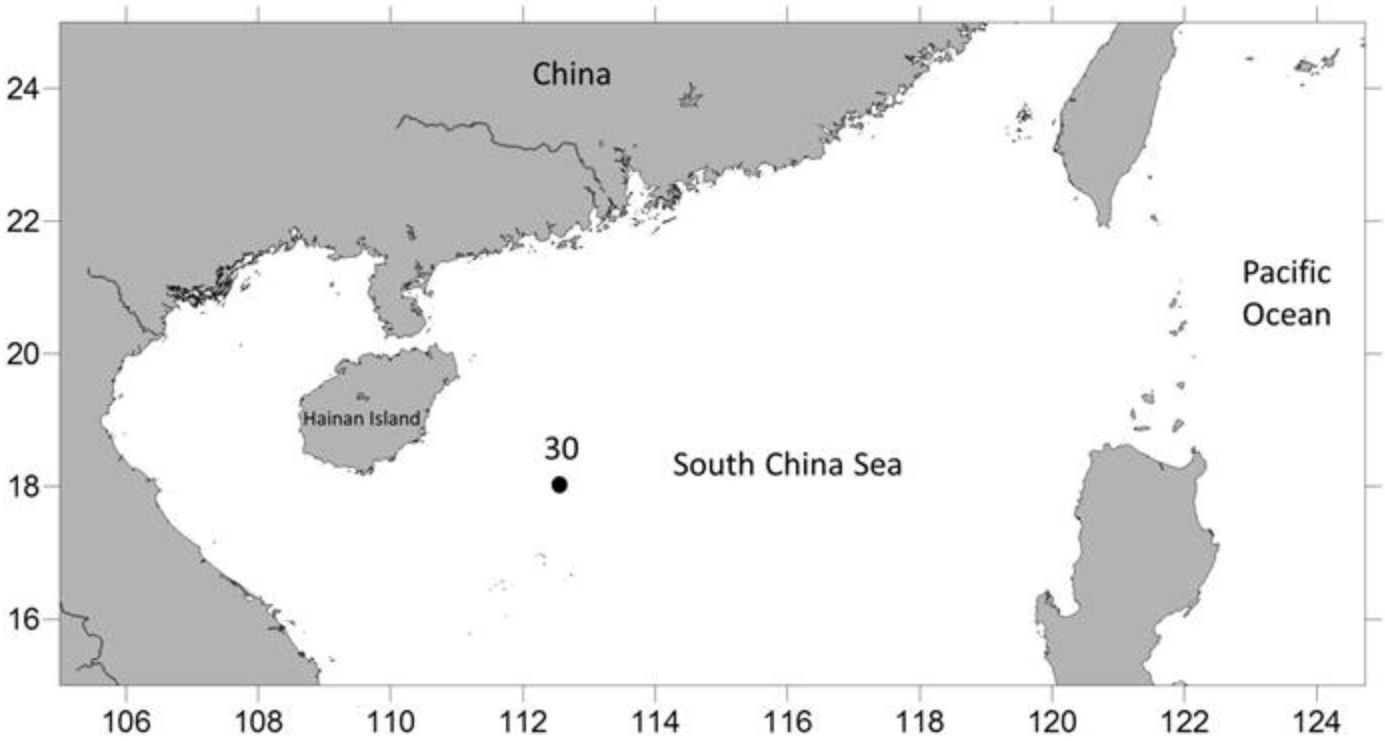

China
Pacific
Ocean
Hainan Island
30
South China Sea
24
22
20
18
16
106
108
110
112
114
116
118
120
122
124

Figure 1

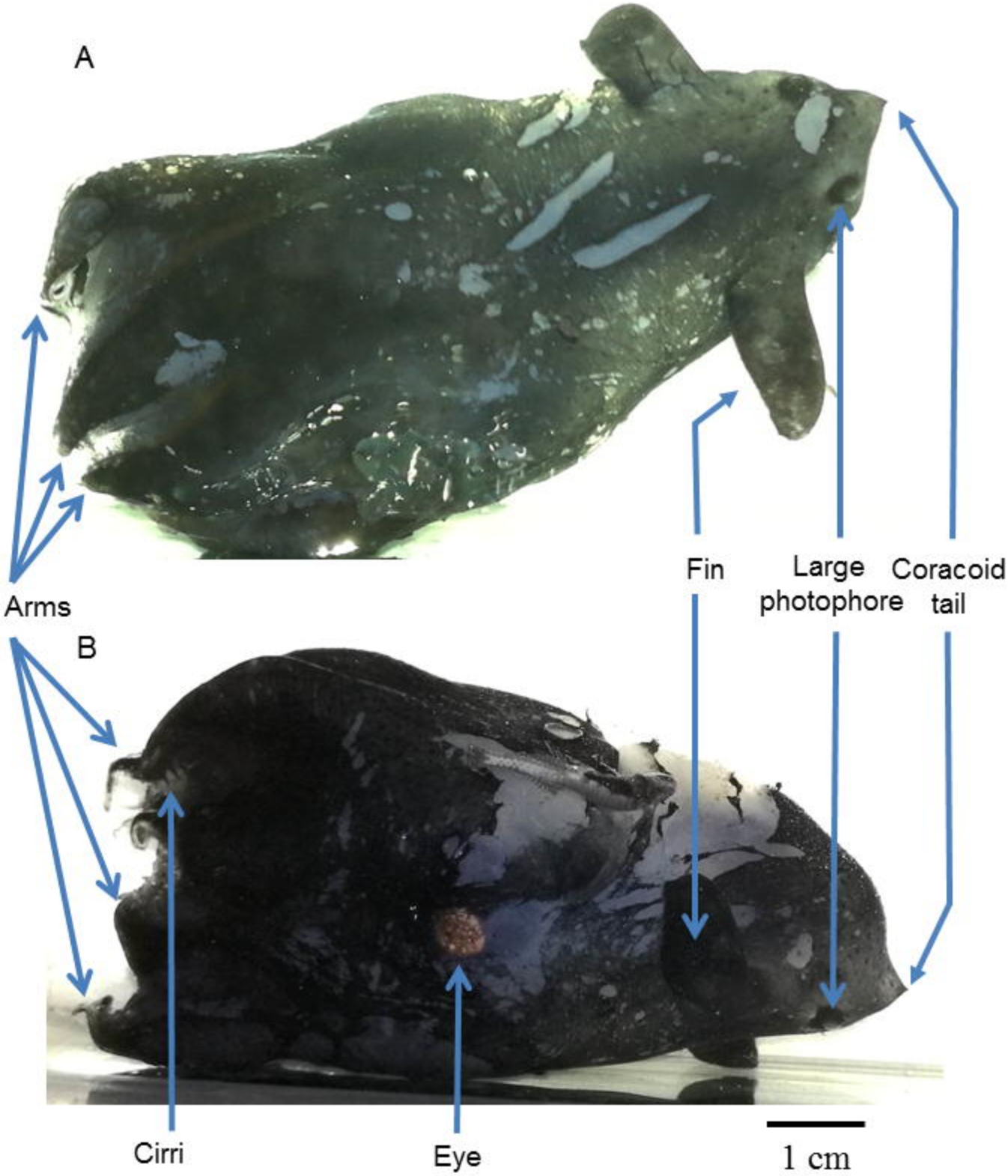
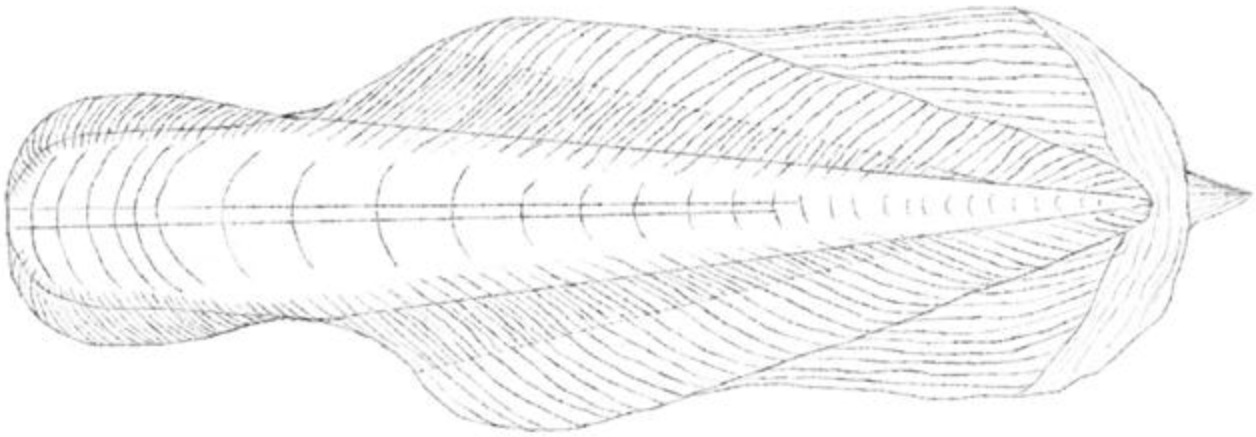


Figure 2

A

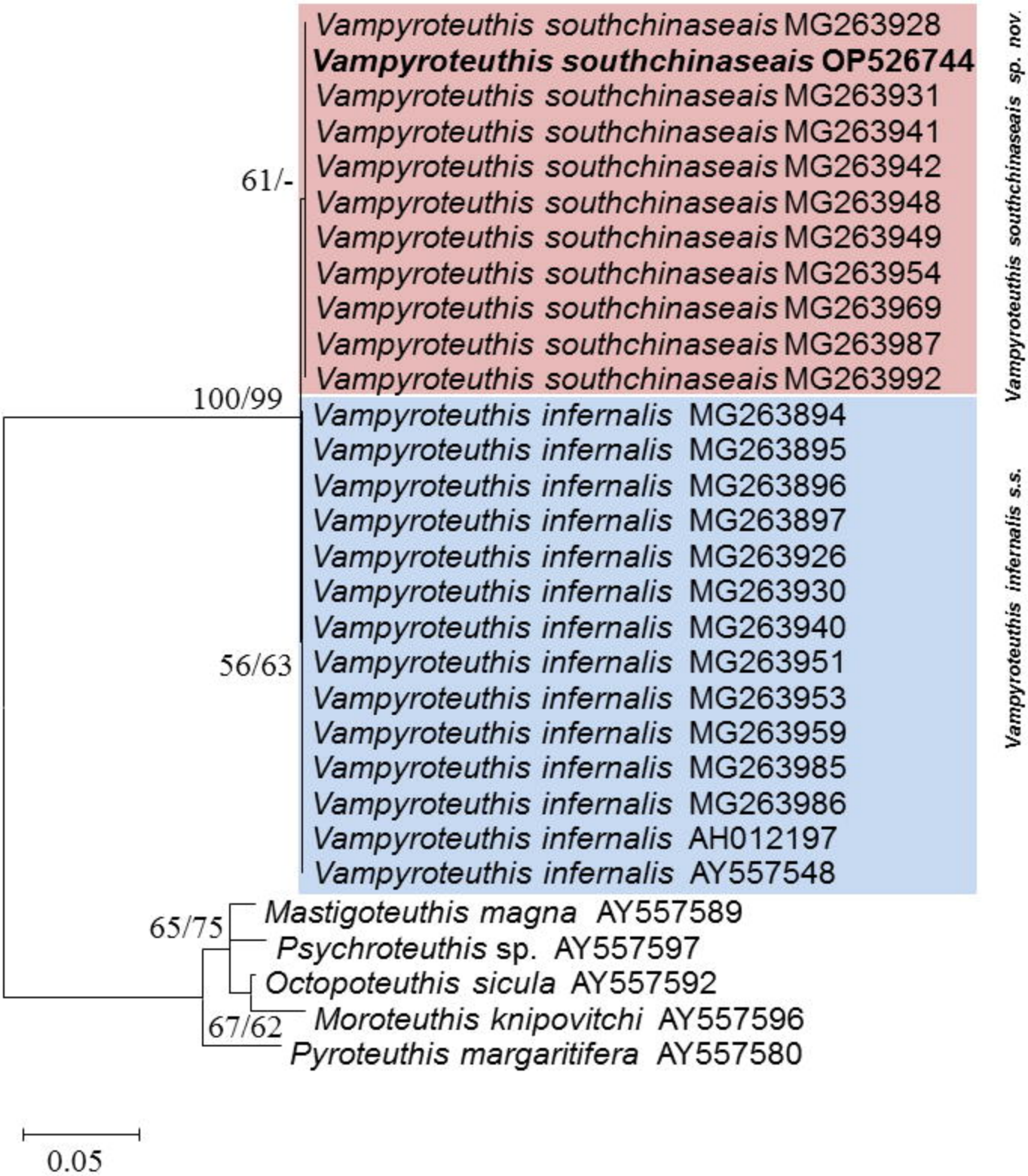


1 cm

B

1 cm

Figure 3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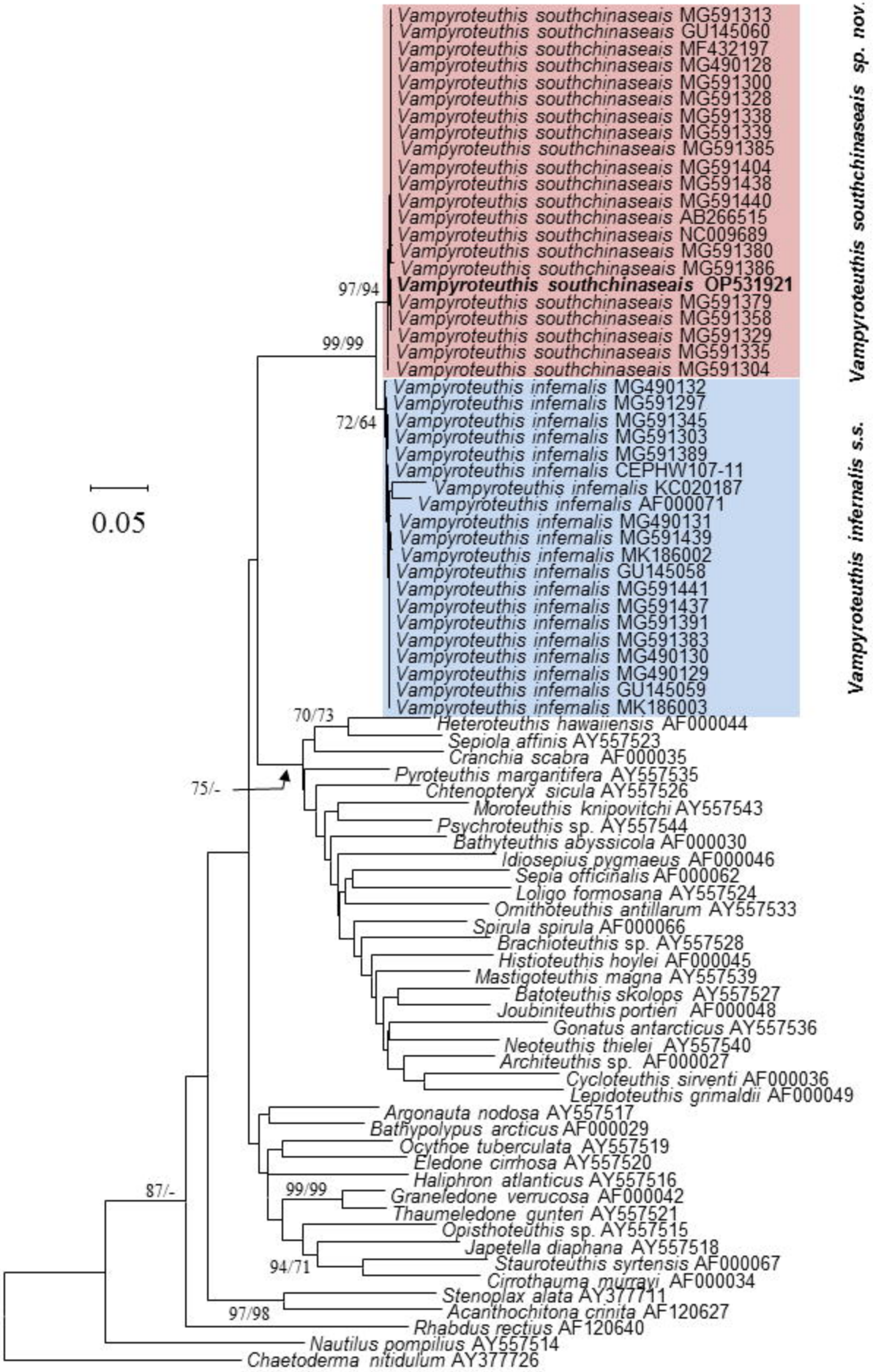
Vampyroteuthis southchinaseais OP526744
Vampyroteuthis southchinaseais MG263931
Vampyroteuthis southchinaseais MG263941
Vampyroteuthis southchinaseais MG263942
Vampyroteuthis southchinaseais MG263948
Vampyroteuthis southchinaseais MG263949
Vampyroteuthis southchinaseais MG263954
Vampyroteuthis southchinaseais MG263969
Vampyroteuthis southchinaseais MG263987
Vampyroteuthis southchinaseais MG263992
61/-
100/99
Vampyroteuthis infernalis MG263894
Vampyroteuthis infernalis MG263895
Vampyroteuthis infernalis MG263896
Vampyroteuthis infernalis MG263897
Vampyroteuthis infernalis MG263926
Vampyroteuthis infernalis MG263930
Vampyroteuthis infernalis MG263940
56/63
Vampyroteuthis infernalis MG263951
Vampyroteuthis infernalis MG263953
Vampyroteuthis infernalis MG263959
Vampyroteuthis infernalis MG263985
Vampyroteuthis infernalis MG263986
Vampyroteuthis infernalis AH012197
Vampyroteuthis infernalis AY557548
65/75
Mastigoteuthis magna AY557589
Psychroteuthis sp. AY557597
Octopoteuthis sicula AY557592
67/62
Moroteuthis knipovitchi AY557596
Pyroteuthis margaritifera AY557580
0.05
Vampyroteuthis southchinaseais sp. nov.
Vampyroteuthis infernalis s.s.

Figure 4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Vampyroteuthis southchinaseais GU145060
Vampyroteuthis southchinaseais MF432197
Vampyroteuthis southchinaseais MG490128
Vampyroteuthis southchinaseais MG591300
Vampyroteuthis southchinaseais MG591328
Vampyroteuthis southchinaseais MG591338
Vampyroteuthis southchinaseais MG591339
Vampyroteuthis southchinaseais MG591385
Vampyroteuthis southchinaseais MG591404
Vampyroteuthis southchinaseais MG591438
Vampyroteuthis southchinaseais MG591440
Vampyroteuthis southchinaseais AB266515
Vampyroteuthis southchinaseais NC009689
Vampyroteuthis southchinaseais MG591380
Vampyroteuthis southchinaseais MG591386
97/94
Vampyroteuthis southchinaseais OP531921
Vampyroteuthis southchinaseais MG591379
Vampyroteuthis southchinaseais MG591358
99/99
Vampyroteuthis southchinaseais MG591329
Vampyroteuthis southchinaseais MG591335
Vampyroteuthis southchinaseais MG591304
Vampyroteuthis southchinaseais sp. nov.
Vampyroteuthis infernalis MG490132
Vampyroteuthis infernalis MG591297
72/64
Vampyroteuthis infernalis MG591345
Vampyroteuthis infernalis MG591303
Vampyroteuthis infernalis MG591389
Vampyroteuthis infernalis CEPHW107-11
Vampyroteuthis infernalis KC020187
Vampyroteuthis infernalis AF000071
0.05
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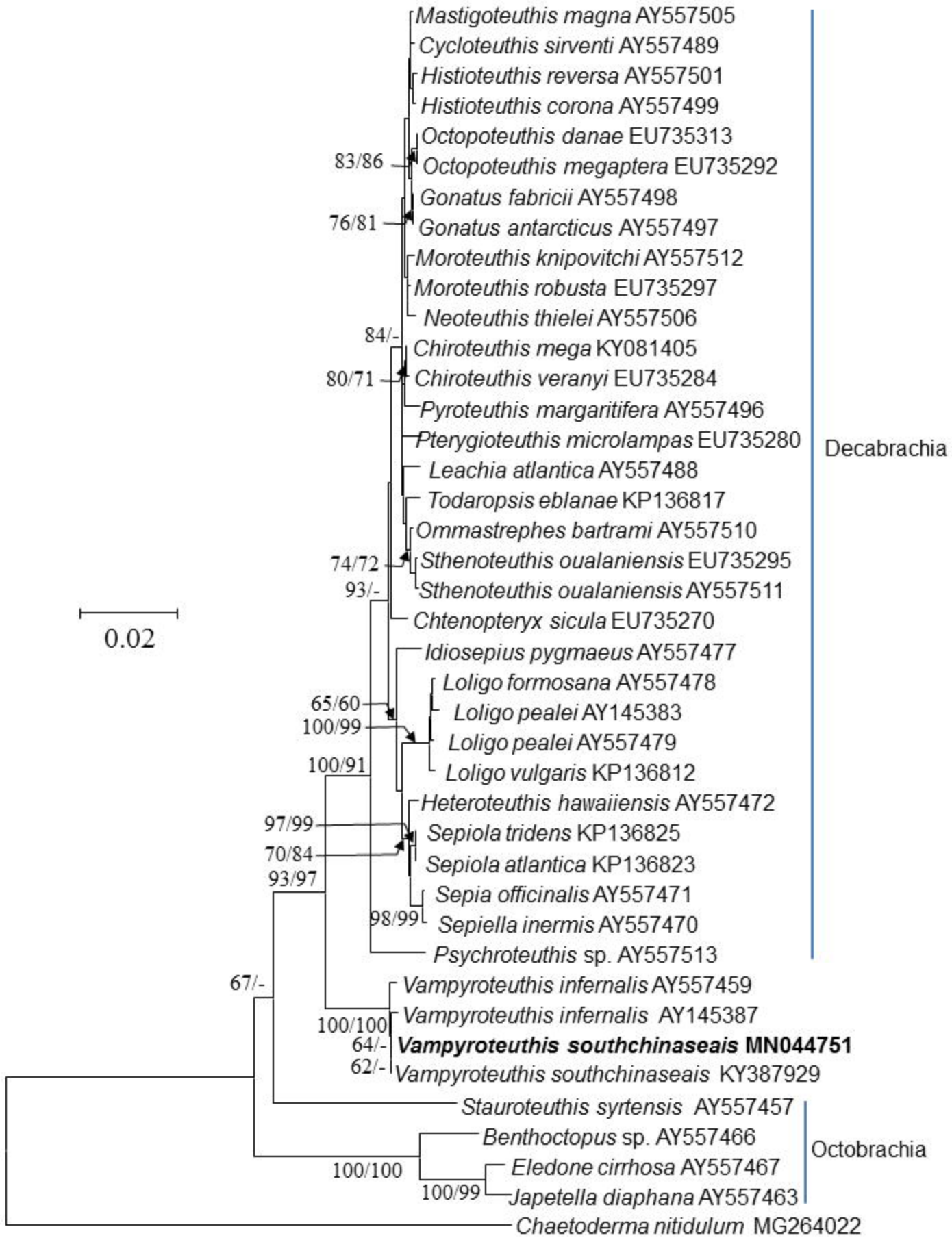
Vampyroteuthis infernalis MG591439
Vampyroteuthis infernalis MK186002
Vampyroteuthis infernalis GU145058
Vampyroteuthis infernalis MG591441
Vampyroteuthis infernalis MG591437
Vampyroteuthis infernalis MG591391
Vampyroteuthis infernalis MG591383
Vampyroteuthis infernalis MG490130
Vampyroteuthis infernalis MG490129
Vampyroteuthis infernalis GU145059
Vampyroteuthis infernalis MK186003
Vampyroteuthis infernalis s.s.
70/73
Heteroteuthis hawaiiensis AF000044
Sepiola affinis AY557523
Cranchia scabra AF000035
Pyroteuthis margantifera AY557535
75/-
Chtenopteryx sicula AY557526
Moroteuthis knipovitchi AY557543
Psychroteuthis sp. AY557544
Bathyteuthis abyssicola AF000030
Idiosepius pygmaeus AF000046
Sepia officinalis AF000062
Loligo formosana AY557524
Ornithoteuthis antillarum AY557533
Spirula spirula AF000066
Brachioteuthis sp. AY557528
Histioteuthis hoylei AF000045
Mastigoteuthis magna AY557539
Batoteuthis skolops AY557527
Joubiniteuthis portieri AF000048
Gonatus antarcticus AY557536
Neoteuthis thielei AY557540
Architeuthis sp. AF000027
Cycloteuthis sirventi AF000036
Lepidoteuthis grimaldii AF000049
Argonauta nodosa AY557517
Bathypolypus arcticus AF000029
Ocythoe tuberculata AY557519
Eledone cirrhosa AY557520
Haliphron atlanticus AY557516
87/-
99/99
Graneledone verrucosa AF000042
Thaumeledone gunteri AY557521
Opisthoteuthis sp. AY557515
Japetella diaphana AY557518
94/71
Stauroteuthis syrtensis AF000067
Cirrothauma murrayi AF000034
Stenoplax alata AY377711
97/98
Acanthochitona crinita AF120627
Rhabdus rectius AF120640
Nautilus pompilius AY557514
Chaetoderma nitidulum AY377726

Figure 5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Cycloteuthis sirventi AY557489
Histioteuthis reversa AY557501
Histioteuthis corona AY557499
Octopoteuthis danae EU735313
83/86
Octopoteuthis megaptera EU735292
Gonatus fabricii AY557498
76/81
Gonatus antarcticus AY557497
Moroteuthis knipovitchi AY557512
Moroteuthis robusta EU735297
Neoteuthis thielei AY557506
84/-
Chiroteuthis mega KY081405
80/71
Chiroteuthis veranyi EU735284
Pyroteuthis margaritifera AY557496
Pterygioteuthis microlampas EU735280
Leachia atlantica AY557488
Todaropsis eblanae KP136817
Ommastrephes bartrami AY557510
74/72
Sthenoteuthis oualaniensis EU735295
93/-
Sthenoteuthis oualaniensis AY557511
Chtenopteryx sicula EU735270
Idiosepius pygmaeus AY557477
Loligo formosana AY557478
65/60
Loligo pealei AY145383
100/99
Loligo pealei AY557479
100/91
Loligo vulgaris KP136812
Heteroteuthis hawaiiensis AY557472
97/99
Sepiola tridens KP136825
70/84
Sepiola atlantica KP136823
93/97
Sepia officinalis AY557471
98/99
Sepiella inermis AY557470
Psychroteuthis sp. AY557513
Decabrachia
67/-
Vampyroteuthis infernalis AY557459
Vampyroteuthis infernalis AY145387
100/100
64/-
Vampyroteuthis southchinaseais MN044751
62/-
Vampyroteuthis southchinaseais KY387929
Stauroteuthis syrtensis AY557457
Benthoctopus sp. AY557466
Octobrachia
100/100
Eledone cirrhosa AY557467
100/99
Japetella diaphana AY557463
Chaetoderma nitidulum MG264022
0.02

Figure 6

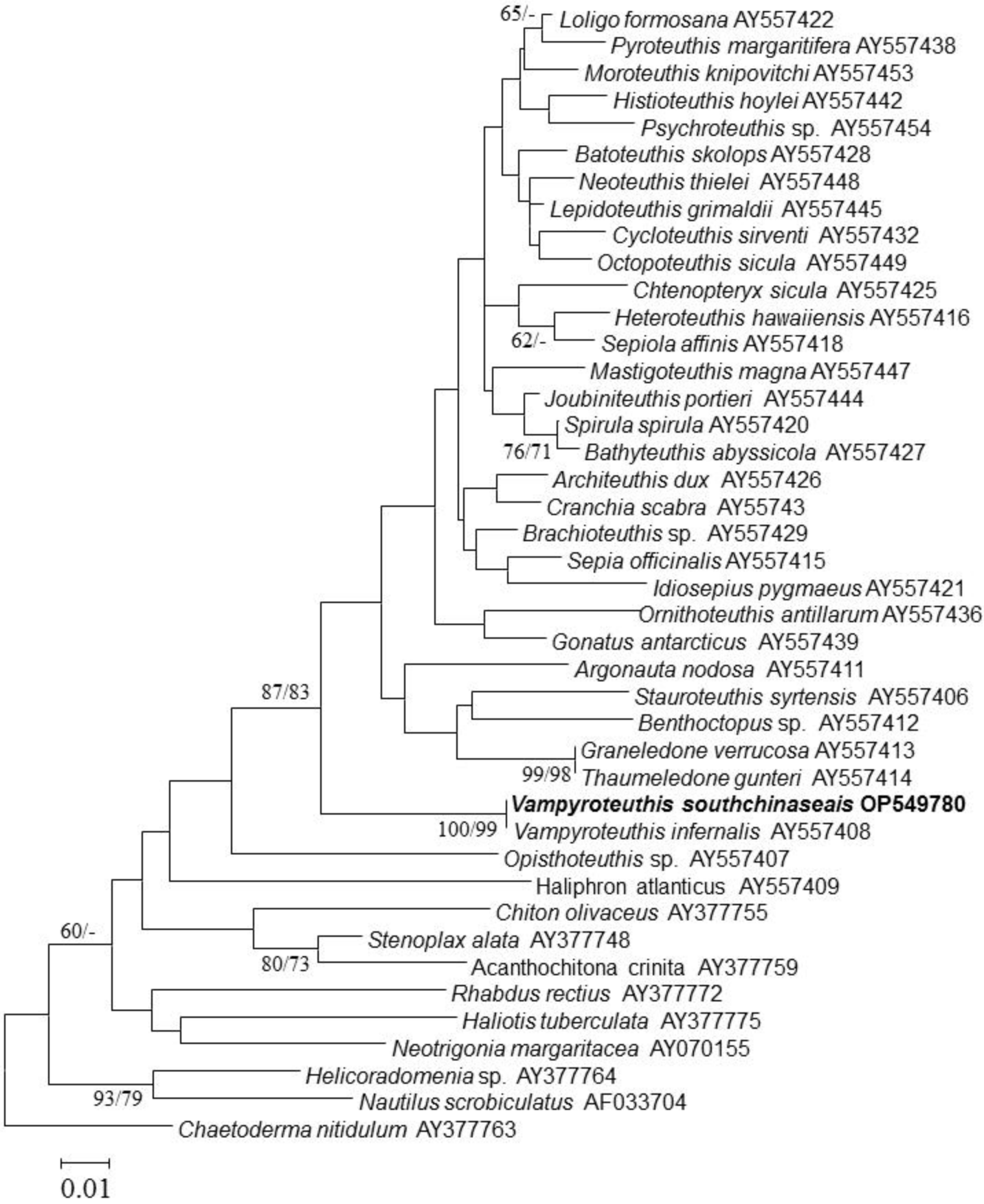
65/-
Loligo formosana AY557422
Pyroteuthis margaritifera AY557438
Moroteuthis knipovitchi AY557453
Histioteuthis hoylei AY557442
Psychroteuthis sp. AY557454
Batoteuthis skolops AY557428
Neoteuthis thielei AY557448
Lepidoteuthis grimaldii AY557445
Cycloteuthis sirventi AY557432
Octopoteuthis sicula AY557449
Chtenopteryx sicula AY557425
Heteroteuthis hawaiiensis AY557416
62/-
Sepiola affinis AY557418
Mastigoteuthis magna AY557447
Joubiniteuthis portieri AY557444
Spirula spirula AY557420
76/71
Bathyteuthis abyssicola AY557427
Architeuthis dux AY557426
Cranchia scabra AY55743
Brachioteuthis sp. AY557429
Sepia officinalis AY557415
Idiosepius pygmaeus AY557421
Ornithoteuthis antillarum AY557436
Gonatus antarcticus AY557439
Argonauta nodosa AY557411
87/83
Stauroteuthis syrtensis AY557406
Benthoctopus sp. AY557412
Graneledone verrucosa AY557413
99/98
Thaumeledone gunteri AY557414
Vampyroteuthis southchinaseais OP549780
100/99
Vampyroteuthis infernalis AY557408
Opisthoteuthis sp. AY557407
Haliphron atlanticus AY557409
Chiton olivaceus AY377755
60/-
Stenoplax alata AY377748
80/73
Acanthochitona crinita AY377759
Rhabdus rectius AY377772
Haliotis tuberculata AY377775
Neotrigonia margaritacea AY070155
Helicoradomenia sp. AY377764
93/79
Nautilus scrobiculatus AF033704
Chaetoderma nitidulum AY377763
0.01

Figure S1