Population structure of *Sepioteuthis lessoniana* in the northwestern Pacific: inference by genetic structure, life-history traits and statolith morphology

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Abstract

Sepioteuthis lessoniana is widely distributed in coastal waters in the Indo-Pacific region and is an important fisheries resource along its distributional ranges. At least three taxa of the squid have been identified in the northwestern Pacific region; namely *Sepioteuthis* sp. 1, sp. 2, and sp. 3. However, geographic distribution and basic biological information of these taxa is limited. In this study, taxa composition of *Sepioteuthis* spp. in the waters off southwestern Japan and off northern Taiwan was examined using molecular evidences, and the life-history traits and statolith morphology of taxa were analyzed to illustrate potential population structure of the species. The objectives of this study are, (1) taxa composition of *Sepioteuthis* spp. in the waters off southwestern Japan and off northern Taiwan; (2) life-history traits for the taxa in the two areas; (3) statolith morphology for the taxa in the two areas. The results may provide better understandings for the population structure of the squid in the northwestern Pacific region.

In the first part of this study, *Sepioteuthis* spp. off southwestern Japan were examined. A total of 142 squid samples were collected from six sites in the waters off southwestern Japan. Three taxa (*Sepioteuthis* sp. 1, sp. 2 and sp. 2A) were identified based on the mitochondrial cytochrome oxidase c subunit I (COI) gene, among which *Sepioteuthis* sp. 2A was defined for the first time. *Sepioteuthis* sp. 2 was predominant in the region (average occurrence rate 84%), whereas *Sepioteuhtis* sp. 1 was rare, with only two individuals found near Goto Islands. The mantle length (ML) composition, ML-body weight (BW) relationship, age structure, and average growth rates were similar for *Sepioteuthis* sp. 2 and sp. 2A. However, *Sepioteuthis* sp. 2 mainly hatched during summer (June to July), whereas *Sepioteuhtis* sp. 2A hatched during winter (December to February). The statolith shapes of the squids were similar for both taxa, although slight differences were noted in the wing regions. The three taxa largely overlapped in a principal component analysis bi-plot based on variations extracted from five life-history traits.

In the second part of the study, the squid samples off southwestern Japan and off northern Taiwan were analyzed. A total of 189 squid samples were collected from 2 sites in the waters off northern Taiwan. Three taxa (*Sepioteuthis* sp. 1, sp. 2 and sp. 2A) were identified off northern Taiwan. *Sepioteuthis* sp. 1 was predominant in Taiwan (average occurrence rate 70%), while *Sepioteuthis* sp. 2 was predominant in Japan (81%). For the same taxa, the ML composition, ML-BW relationship, age structure, and average growth rates were different between Japan and Taiwan. *Sepioteuthis* sp. 2 in Japan mainly hatched in summer (June to July), whereas that Taiwan hatched in early season (April to July). The statolith shapes of the squids for the same taxa were different between Japan and Taiwan, particularly in lateral dome and rostrum. Results of a principal component analysis and cluster analysis varied in life-history traits between two geographic locations (Japan and Taiwan) and in statolith morphology between three taxa. These results suggested that life-history traits of squid were mainly influenced by the experienced environments, while the statolith morphology were influenced by synergistic effects of genetic and environmental factors. The results of this study could provide better understanding in population structure and connectivity of *Sepioteuthis* spp. in the northwestern Pacific region, which is crucial information for future conservation and management measures in this region.

Keywords: *Sepioteuthis lessoniana*, population structure, genetic structure, life-history traits, statolith morphology, northwestern Pacific

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1. Introduction

1.1 Fishery importance

The bigfin reef squid *Sepioteuthis lessoniana*, also as oval squid in Japan, is widely distributed along the coastal waters from Japan to Australia in the Indo-west Pacific regions (Jereb and Roper, 2010). The commercial value of this squid was the highest in loligindae species, and were harvested in many fishing methods, e.g. set nets (mainly in Japan), purse seines, jigs, etc. throughout its distributional area. The unit price of this squid reaches the highest among other squids both in Japan (~US\$30 kg^{-1}) and Taiwan (~US\$17 kg^{-1}; Anonymous, 2017). This squid is often targeted by small scale fisheries (hand jigging) in Japan and Taiwan, especially there are competition hosted annually in Japan for this species, and as a by-catch species, occurred in set nets (mainly in Japan) and neritic trawls fisheries. For the landing of loligindea squid had decreased over the past 30 years, to maintain the biology diversity for a stable fishery there is a need to investigate the population structure. It is important to understand the relationship between environmental factors and life-history span, morphology, and genetic structure among populations (Forsythe et al., 2001; Pecl, 2001; Jackson and Moltschaniwskyj, 2002; Ikeda and Kobayashi, 2010).

1.2 Population structure

The species composition and population structure of commercially exploited marine resources are essential and critical information when developing appropriate conservation and management measures (Cadrin et al. 2005). Population structures of exploited marine resources have been examined using several techniques, such as life-history traits, body morphometrics, parasites, and genetic marker analysis (Cadrin et al. 2005; Abaunza et al. 2008). There are many methods to identify different populations, covered by morphometrics (Elliott et al., 1995) and parasite loading (Braicovichand and Timi, 2008) over conventional tagging (Lear and Green, 1984), otolith microstructure analysis (Mosegaard and Madsen, 1996) and otolith microchemistry (Campana et al., 2000) to molecular tools (Nielsen et al., 2009) and otolith shape analyses (Campana and Cassleman, 1993). Recently, molecular tools applications on the populations managements are higher expense, and the morphology of otolith and body were called to gain interest among fisheries biologist. Otolith shape is known to depend on a support of genetic and environmental factors (Cardinale et al., 2004) and identification of populations in both temporal and spatial patterns (Lombarte and Lleonart, 1993). The application methods of population identification were also operated on squids (Green et al., 2015; Olmos-Pérez et al., 2018), as they tried to follow the studies of fish to describe the population structure combing two different methods and to support the evidence between complex factors.

1.3 Population studies of *S. lessoniana*

The population structure and potential cryptic species of bigfin reef squid in the Northwest Pacific have been studied. The squid occurring around Japan are divided into three distinct types by fishermen based on seasonal occurrence and body appearance (Okutani, 1984). These three types of squid were confirmed through allozyme analysis and named *aka-ika* (red squid), *shiro-ika* (white squid), and *kua-ika* (small squid; Izuka et al., 1994; 1996) or *Sepioteuthis* sp. 1, sp. 2, and sp. 3, respectively (Aoki et al., 2008). Using mitochondrial and nuclear genes, three cryptic lineages (A, B, and C) of the squid in the Indo-West Pacific region were evident (Cheng et al., 2014), of which lineages B and C could correspond to *Sepioteuthis* sp. 1 and sp. 2, respectively (Tomano et al., 2015a). *Sepioteuthis* sp. 2 was commonly found around Japan and could be a single stock (Tomano et al., 2015b), whereas *Sepioteuthis* sp. 1 was found on the Pacific side of Japan (Tomano et al., 2016). However, a highly variable taxa composition of the squid in the waters off southwestern Japan was noted, and the relevant life-history information of these cryptic species is limited. Furthermore, recent developments in the analysis of hard tissues, such as otolith in fish (Campana and Thorrold 2001; Campana 2005) and statolith in squid (Arkhipkin 2005; Xavier et al. 2015), provide potential applications for determining the population structure. The most adequate population identification method for fish involves a holistic approach (Begg and Waldman 1999), which may provide various types of information from ecological, evolution, and operational perspectives (Cadrin et al. 2005). Evidently, combining various types of information from different techniques, such as genetic and otolith data, may enable more accurate descriptions of potential population structures of species; for example, Patagonian Hoki *Macruronus magellanicus* in the South Atlantic and Pacific regions (Mckeown et al. 2015) and arrow squid *Nototodarus gouldi* in southern Australia (Green et al. 2015); for management applications.

1.4 Applications of statolith

Similar to otoliths in fish, squid statoliths form by accumulating alternating layers of calcium carbonate and organic matter (Bettencourt and Guerra 2000). Following the development of studies for fish otolith, microstructure in statolith are examined in recent years (Arkhipkin et al. 2004) and apply to explore life-history events and population structure of squids (Arkhipkin et al., 2004; 2005; Gillanders, 2005; Arkhipkin and Shcherbich, 2012). In addition, shape of otolith and statolith can be a composite effects of genetic and ontogenetic environmental variations (Castonguay et al., 1991; Lombarte and Lleonart, 1993; Begg and Brown, 2000). The shape of otolith is variable between species and even between populations of interspecies, although otolith growth showed less variable effect than the body growth of fish (L'Abee-Lund, 1988). By contrast, the body growth of squid is influenced more easily by oceanographic conditions during their life history, and the body shape of squid has rarely been examined because of their soft texture and vulnerability to fishing gear (Sweeney et al. 1992; Arkhipkin et al. 2015). Thus, the hard structures of squid, such as beaks and statoliths, are adequate for investigating their population structure (Arkhipkin et al. 2004; Swearer et al. 2003). Among statolith features, to analyze the morphology is particularly relevant in taxonomic, biological and ecological investigations. While the statolith is a similar structure as otolith, the shape is sufficiently conservative to be regarded as specific (L'Abée-Lund, 1988; Stransky and MacLellan, 2005; Tuset et al., 2006), their morphological variability is considerable and mainly influenced by sex, age and year class (Castonguay et al., 1991; Lombarte and Castellón, 1991;

Lombarte and Lleonart, 1993; Begg and Brown, 2000; Monteiro et al., 2005; Reichenbacher et al., 2009). Thus, the growth considered to be a factor to influence the shape of statolith in squid. It is currently recognized that otolith growth and shape occur under dual regulation: overall shape is regulated genetically, but there is a strong variability related to environmental factors. A number of authors have suggested that otolith shape is genetically constrained, species specific and reflects phylogenetic relationships (Lombarte and Castellón, 1991; Lombarte and Lleonart, 1993; Reichenbacher et al., 2009). Studies were based on theoretical considerations and indirect evidence, implying that otolith shape variation is determined to some degree by genetics. Only a limited number of studies have directly investigated the determinants of otolith shape. A strong variability related to environmental factors such as depth, water temperature and substrate type (Lombarte and Lleonart, 1993; Gagliano and McCormick, 2004; Mérigot et al., 2007; Hüssy, 2008). As a consequence, otoliths have been used for decades as a popular tool for the discrimination of species, population and stock differences related to geographic locations and to chemical and physical properties of the environment (Campana and Casselman, 1993; DeVries et al., 2002; Cardinale et al., 2004; Tracey et al., 2006; Burke et al., 2008). So far, only Vignon and Morat (2010) investigate the factors that influence the morphology of otolith, but related studies of statolith morphology were scare until now.

1.5 Oceanographic environments in the East China Sea

The seas between south of Japan and Taiwan were the northwestern Pacific mainly part by the China Sea. The China Seas are seas bounded by China, Taiwan the Nansei Islands, Kyushu, and the Korean Peninsula (Ichikawa and Beardsley, 2002). Ryukyu Islands consist of many islands between south of Kyushu and east of Taiwan. The East China Seas was influenced by three main currents, i.e., the Kuroshio Current, the Tsushima Warm Current (TSWC), and the Yellow Sea Warm Current (YSWC) and also flew by China coastal current and Taiwan warm current (Nitani, 1972).

Nagasaki locates on the western side of Kyushu, Japan. The Tsushima Strait, to the west of Nagasaki, connects the East China Sea and the Japan Sea (Morimoto et al., 2009). The Tsushima Warm Current flows from the East China Sea to the Japan Sea through the Tsushima Strait and affects physical and biological processes of the Japan Sea (Onitsuka et al., 2007; Morimoto et al., 2009). Such material transport and impacts are complicated and have been shown with seasonal and inter-annual variations (Morimoto et al., 2009).

1.6 Objectives of this study

Sepioteuthis lessoniana is a critical squid species in fisheries and marine ecosystems in the northwestern Pacific. Population structure of the species have been studied locally in the previous studies, while essential biological information are limited. In this study, taxa composition of *Sepioteuthis* spp. in the waters off southwestern Japan and off northern Taiwan was examined using molecular evidences, and the life-history traits and statolith morphology of taxa were analyzed to illustrate potential population structure of the species. The objectives of this study are, (1) taxa composition of *Sepioteuthis* spp. in the waters off southwestern Japan and off northern Taiwan; (2) life-history traits for the taxa in the two areas; (3) statolith morphology for the taxa in the two areas. These results may provide better understandings for the population structure and connectivity of the squid in the northwestern Pacific region.

2. Material and methods

2.1 Squid samples

The squid samples in Japan were collected from six sites in Nagasaki, southwestern Japan, between June 2016 and April 2017. The six locations were as follows: (1) Sasebo (SSB); (2) Omura Bay (OMB); (3) Tameshi (TMS); (4) western Nomozaki (wNMZ); (5) eastern Nomozaki (eNMZ); and (6) Goto Islands (GTI; Figure 1). Squid samples were caught using set-net fisheries in neritic waters. Squid samples were stored in a cooler immediately after collection and transported to the laboratory at Nagasaki University. The number of squid samples depended on the catches of the fishermen.

The squid samples in Taiwan were collected from two sites, Keelung (KL) and Penghu (PH) off northern Taiwan (Figure 1c). Squid samples were harvested by jigging and trawl in the neritic waters. Squid samples were stored in a cooler immediately after collection and transported to the laboratory at the National Taiwan Ocean University.

Basic biological information of the squid samples was measured after thawed. The dorsal mantle length (ML), total body weight (BW) and Gonad weight (GW) were measured to the nearest unit of the instrument, with 1 mm (calipers) and 1 g (scale), respectively. The sexual maturity stage (I: Juvenile; II: Immature; III: Maturing; IV: Mature; V: Spent) of the squid was determined according to the maturity scale of Boyle and Ngoile (1993). Statoliths were dissected from the statocysts and washed with Milli-Q water and H2O2, and then stored in a 0.2 ml microtube with cap for further age determination or shape analyses.

2.2 DNA analysis

Total DNA of the squid was extracted from mantle muscle tissue using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) based on the manufacturer's protocol. Multiplex cytochrome oxidase c subunit I (COI) haplotype-specific polymerase chain reaction (MHS-PCR) was performed to identify the potential taxa of the squid. The haplotype-specific primers with known DNA barcoding had been designed in previous studies (*Sepioteuthis* sp. 1, sp. 2, and sp. 3; Hsiao et al. 2016; Shen et al. 2016) and were applied in this study. One more taxa, defined as *Sepioteuthis* sp. 2A, was found in a preliminary experiment, whose haplotype-specific primer (Annex 1) was also applied to the MHS-PCR analysis.

The PCRs were conducted using a PCR Thermal Cycler (Gene Atlas), each with 9.8 μL of reaction solution containing 0.2 ng of template DNA, 0.3 μM of each primer, 0.2 mM of the dNTP mixture, 10× PCR buffer (20 mM Tris–HCl pH 8.0, 15 mM KCl, and 15 mM $MgCl_2$), and 5 U/μL of DNA polymerase (Fermentas, Thermo Fisher Scientific Inc.). The reaction conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Amplified products were confirmed using 1.5% agarose gel electrophoresis. The amplified size of COI sequence was about 630 bp. The PCR products (1 μl) were mixed with dye (3 μl), and the gel electrophoresis, under 100V for 30 min, were performed in the Agarose gel (2%) with TAE buffer (0.5X) using 100 bp marker. The bands in the gel were examined under the UV light after dyeing with Ethidum Bormide (EtBr). The bands for *Sepioteuthis* sp. 1, sp. 2, sp. 2A, and sp. 3 were 122, 281, 608, and 207 bp, respectively. We amplified approximately 700 base pairs (bp) of the mtCOI by polymerase chain reaction (PCR), using the following primers: jgLCO1490 (5′-GGTCAACAAATCATAAAGATATTGG -3′) and jgHCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA -3′) (Geller et al. 2013). The PCRs were performed using a PCR Thermal Cycler Dice Gradient (TaKaRa Bio, Shiga, Japan), each with 10 μL of reaction solution containing 10 ng template DNA, 0.5 μM of each primer, 0.2 mM dNTP mixture, 1 × PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), and 0.5 U *Ex Taq* DNA polymerase (TaKaRa Bio). The reaction conditions were as follows: an initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The PCR products were purified by illustra ExoStar (GE Healthcare Bio-Sciences, Uppsala, Sweden) and sequenced on an ABI Prism 3130 xl DNA sequencer (Applied Biosystems, Waltham, MA, USA), using the BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems) and each primer used in the PCR.

DNA sequences were checked and aligned (630 bp) by CustalW with deleting the missing genes using BioEdit. The sequence of partial mtDNA COI (583 bp) of each individual was analyzed using the MEGA X and DnaSP 6 software. The haplotypes of *Sepioteuthis* sp. 2A were first detected in this study, which comprised 8 haplotypes with 15 nucleotide substitutions in the COI region. The genetic distances (Nei 1978) were calculated in MEGA X, and neighbor-joining (NJ) tree of haplotypes between *Septioteuthis* taxa were calculated by the Tamura 3-parameter with discrete Gamma distribution (T92+G) model, which was selected as the best-fit substitution model whose BIC (Bayesian Information Criterion) value is the lowest one (BIC = 5437.1) (MEGA-X). We also performed with 1000x bootstrap to check the reliability (Felsenstein, 1985). The haplotype network was placed in a frame by Network 10.1. Genetic diversity of the squid taxa was estimated using haplotype (*h*) and nucleotide diversity (π) by DnaSP vs. 5.10.1. Fu's *F*s (Fu 1997) and Tajima's *D* (Tajima 1989) were applied to test for deviations from the mutation-drift equilibrium, and the genetic differentiation between taxa and locations were evaluated by the gene flow value (F_{ST}) also in the DnaSP vs 5.10.1. Analysis of mismatch distribution was conduct by the constant population size model using the DnaSP vs 5.10.1, which can check the population demographic history. GenBank accession numbers for CO1 sequences generated in this study showed in Annex 2.

2.3 Statolith processing and age determination

The left statolith (wing facing up and lateral dome on left side) was selected for age determination. After cleaning with xylene and alcohol for two minutes, the statolith was mounted in resin with a hardener (EpoFix kit, Struers, Danmark). After the resin had fully hardened, the statolith was ground on the ventral side to the nucleus with wet waterproof sandpaper (of 1200, 4000, and 6000 grit), and then polished with a polishing suspension (0.05 µm, Buehler, Lake Bluff, IL, USA) on a polishing cloth. The prepared statoliths were examined under a compound microscope (400×, Nikon ECLIPSE, E800) and photographed with a digital camera (DS-Ri1, Nikon). Each frame of images along the longest axis, from the focus to the tip of the lateral dome (statolith radius, SR, Annex 3), was taken and stitched together using e-Tiling (Mitani Corporation, Japan). Growth increments of the statolith were determined using Image J software (National Institutes of Health, USA). For regions where growth increments were unclear, an extrapolation method (Jackson, 1990) was applied to estimate the number of increments. The daily deposition of growth increments in statoliths was previously validated for *S. lessoniana* (Jackson, 1990). The hatching date was estimated by subtracting the number of increments from the date of collection.

2.4 Statolith morphology analysis

Elliptical Fourier functions represent a precise method for describing and characterizing outlines, efficiently capturing outline information in a quantifiable manner (Kuhl and Giardina, 1982, Lestrel, 1997). The method does not require equal intervals along the outline and therefore can accommodate significantly more complex shapes than polar Fourier functions, the approach that has traditionally been used in stock discrimination studies based on fish otoliths (Bird et al., 1986, Castonguay et al., 1991, Campana and Casselman, 1993, Torres et al., 2000) and also squid statolith. Polar Fourier functions are constrained to a series of radii originating from a barycentre of the study specimen, each radius can only intersect the perimeter once. In the case of statolith that are particularly convoluted, or where the rostrum has significant curvature it would be possible for radii to intersect the perimeter at more than one point. Elliptical Fourier functions alleviate this problem by not relying on a radiating centroid, instead generating harmonics by calculating x and y co-ordinates as a function of a third variable (t) (Tracy et al. 2006). Compare to traditional methods of measuring statolith shape index (length and width) with additional indices (roundness, rectangularity, and ellipticity etc.), the Elliptical Fourier analysis can described more details of the shape as the statolith of squids changes in a fine distinction than otoltih.

Statolith morphology was defined following the method of Clarke (1978), and statoliths comprised the dorsal dome, lateral dome, wing, and rostrum regions (Annex 4). For the body size effected the statolith size, we choose mature individuals and near body length of the samples. Considering the consistency of the morphology analysis, the angle and direction of all statolith should place in the same condition. The statoliths (right) were placed (convex side facing up) on a black clay within a Petri dish, which allowed to fine-tune focal plane of the statoliths as consistent as possible. The statolith was photographed using a digital camera (DS-Fi1; Nikon) connected to a dissecting microscope (40×, SMZ-800; Nikon). The statolith images were saved in digital files for further analyses.

The outlines of statoliths were extracted and analyzed using Elliptic Fourier Analysis (EFA), which can delineate the outline of an object in a two-dimension closed curve. The depicted outline can be decomposed into sine and cosine functions harmonically (descriptors), that four parameters (Fourier coefficients, FCs) are estimated for each function (Crampton 1995). The EFA was performed using the software SHAPE 1.3 (Iwata and Ukai 2002). Following the steps of chain coding algorithm in the software, approximately 99% of the cumulative power can be achieved for the first 21 harmonics, while the statolith outline was described by 84 FCs (Crampton 1995). The FCs was normalized by the first harmonics, whereas the orientation of statolith outlines was calibrated by the fixed scale and direction. We used the smallest variation tip of the dorsal dome to align the final mean shape of three taxa in two locations.

2.5 Statistical analysis

Differences in life-history parameters (ML, number of increment, and growth rate) between *Sepioteuthis* taxa were examined using analysis of variance. The similarity of distribution (hatching month distibution) between *Sepioteuthis* taxa was examined using non-parametric Kolmogorov-Smirnov goodness-of-fit test. Principal component analysis (PCA) was performed to examine the variations of life-history parameters (BW, ML, number of increments (NI), average growth rate (GR), and gonadosomatic index (GSI)) between *Sepioteuthis* taxa. The scores of the first two principal components (PCs) of each taxa were applied to analyze the similarity of life-history traits between taxa by cluster analysis with Euclidean distance and unweighted pari-group average method. The statistical analyses were performed using SPSS Statistics v. 20.0 (SPSS, Chicago, IL, USA). The statistical significance for the tests is at 5% level.

3. Results

3.1 *Sepioteuthis* spp. off southwestern Japan

A total of 142 squid samples were collected from six locations near Nagasaki, of which 134 individuals were successfully aged based on the statolith microstructure (Table 1). Male individuals were larger in size than were female individuals in all locations. The smallest individuals occurred in OMB for both sexes (95 mm and 94 mm for female and male individuals, respectively) with the maturity stage from 2-3, whereas the largest individuals occurred in GTI for both sexes (295 mm and 407 mm for female and male individuals, respectively) within Japan.

3.1.1 Taxa composition

A total of 138 squids from six locations around Nagasaki were examined using MHS-PCR. At least three taxa were identified: *Sepioteuthis* sp. 1 (n = 2), *Sepioteuthis* sp. 2 (n = 115), and *Sepioteuthis* sp. 2A (n = 21), among which *Sepioteuthis* sp. 2A was first defined with 15 nucleotide substitution from sp. 2 in this region. *Sepioteuthis* sp. 2 was dominant in all locations (proportion between 68% and 95% with an average of 84%). *Sepioteuthis* sp. 2A was also found in six locations, although it was less abundant (proportion between 4% and 32% with an average of 15%). *Sepioteuthis* sp. 1 was rare in this region, occurring only in GTI (Figure 2). A total of 21 haplotypes were found based on comparisons of partial mtDNA COI sequences (583 bp) across 138 individuals, of which all 21 haplotypes were found in *Sepioteuthis* sp. 2, whereas only 8 haplotypes were found in *Sepioteuthis* sp. 2A (Table 2). The genetic diversity (h and π) were higher than 0.5 in haplotype diversity (h) for both taxa, and the nucleotide diversity (π) were higher than 0.005 for sp. 2 but lower than 0.005 for sp. 2A (Table 2) The genetic distance between *Sepioteuthis* sp. 2 and sp. 2A (0.0252) was smaller than that between *Sepioteuthis* sp. 1 and sp. 2 (0.1166) (Table 3).

3.1.2 Life-history traits for taxa

No significant differences existed in ML composition between *Sepioteuthis* sp. 2 and sp. 2A for both sexes ($F_{1,138} = 1.027$, $p = 0.313$; Figure 3), although a wider range of ML for was noted *Sepioteuhtis* sp. 2. No significant differences were found in the relationships between ML and BW between *Sepioteuthis* sp. 2 and sp. 2A for both sexes ($F_{1,138} = 7.084$, $p = 0.227$; Figure 4). Age composition of *Sepioteuthis* sp. 2 and sp. 2A exhibited a similar pattern, peaking at 90–109 days, followed by 110–129 days (Figure 5). However, there areold-age (170–189 days) mature individuals of *Sepioteuthis* sp. 2A was noted. Hatching months of *Sepioteuhtis* sp. 2 were mainly in early summer (June to July), followed by winter (December to February). Hatching months for *Sepioteuthis* sp. 2A mainly occurred in winter (December to February; Figure 6). The ML of squids increased with age for *Sepioteuthis* sp. 2 and sp. 2A and for both sexes (nonparametric statistics: Female: $P < 0.05$, Male: $P = 0.398$, locations: $p=0.189$, Figure 7). Variations of ML-at-age data in male individuals were higher compared with those in female individuals. Furthermore, no significant differences were found in the average growth rate (ML per days) between *Sepioteuthis* sp. 2 and sp. 2A (Figure 8; $F_{1,1290} = 0.439$, $p = 0.509$). Few mature squid were found in this study. The average ML and age for mature *Sepioteuthis* sp. 2 and sp. 2A individuals were calculated for comparison (Figure 9; sp. 2: f [41], m [63]; sp.2A: f [4], m [12]). In general, mature individuals of *Sepioteuthis* sp. 2A had higher ML and were older compared with those of *Sepioteuthis* sp. 2.

3.1.3 Statolith morphology for taxa

A total of 142 squid statoliths (right) were examined, and their outlines were extracted. No significant differences were found in statolith shape between *Sepioteuthis* sp. 2 and sp. 2A, although slight differences in the wings of statoliths were noted (Figure 10; $F_{20,142} = 1.298$, $p = 0.176$).

PCA was performed to extract potential variations in five life-history traits (BW, ML, number of increments [NI], average growth rate [GR], and gonadosomatic index [GSI]) between *Sepioteuthis* spp. The first two factors (PC1 and PC2) explained 82.3% of the total variance in life-history traits (Table 4). The first factor (PC1) was positively correlated with BW, ML, and GR, and the second factor (PC2) was positively correlated with GSI. *Sepioteuthis* sp. 2 and sp. 2A overlapped in the PC1 and PC2 bi-plot (Figure 11), although *Sepioteuthis* sp. 2 tended to be in the negative direction along PC1. *Sepioteuthis* sp. 1 also overlapped with *Sepioteuthis* sp. 2 and sp. 2A along PC1 but tended to be in the negative direction along PC2.

3.2 *Sepioteuthis* spp. in the Northwest Pacific

The squid samples in Japan (SSB, OMB, TMS, eNMZ, wNMZ and GTI) and in Taiwan (KL and PH) were used for further analysis. The total collection of 347 samples (142 in Japan, 205 in Taiwan) were analyzed, of which 293 individuals for DNA sequencing, 313 individuals for life-history traits analysis, and 302 individuals for statolith morphology analysis. The smallest sample was *Septioteuthis* sp. 2A in Taiwan (58.3 mm) and the largest one was *Sepioteuthis* sp. 1 in Taiwan (463.4 mm). The size range of squid in Taiwan were wider than those in Japan (Table 5). The maturity stage were similar in the two locations ranging from 2-4 (Table 5).

3.2.1 Taxa composition in two areas

A total of 293 squids from two locations from southwestern Japan (141) and northern Taiwan (187) were examined using MHS-PCR. At least three taxa were identified: *Sepioteuthis* sp. 1 (2%), *Sepioteuthis* sp. 2 (81%), and *Sepioteuthis* sp. 2A (17%). *Sepioteuthis* sp. 2 was dominant in Japan, while *Sepioteuthis* sp. 1 (70%) was dominated in Taiwan. The *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A were less in northern Taiwan (25% and 5% for sp. 2 and sp. 2A, respectively) than in southwestern Japan (84% and 15% for sp. 2 and sp. 2A, respectively; Table 6; Figure 12).

The nearest genetic distance were between two locations of *Sepioteuthis* sp. 2 (0.0069), and the farthest were between *Sepioteuthis* sp. 1 and sp. 2A in Taiwan (0.1414) (Table 7). The genetic diversity (*h* and π) were higher than 0.5 in haplotype diversity (*h)* for all taxa in Japan and Taiwan expect the sp. 2 from Taiwan, and the nucleotide diversity (π) were all lower than 0.005 except the sp. 2 from Japan (Table 8).

Neighbor joining tree showed the separation of *Sepioteuthis* sp. 1 and sp. 2 with the sp. 3 as the outer group, and a clear branch from sp. 2 as sp. 2A were found (Figure 13). The haplotype relationships were place in a network frame, and showed the three taxa (*Sepioteithis* sp. 1, sp. 2 and sp. 2A) were in star-like type, and did not share the haplotype in two locations (Japan and Taiwan; Figure 14). The genetic differentiation between locations in the same taxa were significant different (sp. 2, $F_{ST} = 0.044$; sp. 2A, $F_{ST} = 0.015$, $P < 0.05$). A mismatch distribution were analyzed in Japan and Taiwan for both taxa sp. 2 and sp. 2A, and the unimodal distribution appeared in each taxa of both sites (Figure 15).

3.2.2 Life-history traits for taxa in two areas

Mantle length (mm) of the squids in Japan were significantly larger than those in Taiwan ($F_{1,313} = 32.113$, $P < 0.05$). The females are usually larger than males ($F_{1,313} = 14.329$, $P < 0.05$; Figure 16). The relationship of mantle length and body weight of squid were significantly different between two areas ($F_{1,313} = 5.184$, $P < 0.05$), while no different between sex ($F_{1,313} = 0.144$, $P = 0.704$; Figure 17). Growth increments, which represents the daily age of squid, were determined in 268 squids (103 individuals in Japan and 165 individuals in Taiwan) by the statolith microstructure (Figure 18). Age ranges of the squid were wider in Taiwan (64-202 days for females and 52-170 days for males) than in those Japan (76-158 days for females and 78-172 days for males; Figure 18). The peak of squid age composition in Taiwan (110-129 days) were elder than those in Japan (90-109 days; Figure 18).

Hatching month of squid were estimated by back-calculating the daily age from the date of collection. Squids in Taiwan hatched almost year-round and peaks in April for females and March and July for males. Squid in Japan were lack of hatching individuals in August and September and peaks in June and July for females and February for males (Figure 19).

The squids in Japan seemed to grow faster than those in Taiwan; the males appear to be faster than females (different between taxa: $F_{1, 264} = 31.692$, $P < 0.05$; sex: $F_{1, 264} = 17.992$, $P < 0.05$; Figure 20). Growth rate of squids were higher in Japan than those in Taiwan, and the males grow faster than females ($F_{1, 262} = 51.249$, $P < 0.05$; sex: $F_{1, 262} = 16.218$, $P < 0.05$; Figure 21). The squids mature (mature stage 3 and 4) at around 120 days, but squids in Taiwan were mature at elder age than those in Japan (Figure 22). The ML at maturity for male squids in Japan were greater than the others (Figure 22).

Variations in life-history traits, including BW, ML, number of increment (NI), growth rate (GR), and Gonadosomatic index (GSI), were analyzed using Principal components analysis (PCA). The first two factors, PC 1 and PC 2, respectively explained 56.7% and 26.1% of the total variance (Table 9). PC 1 was positively correlated with four variables (ML, BW, GR and Age), while PC 2 was positively correlated with the GSI. The squid in two areas (Japan and Taiwan) were identified in the PC 1 and PC 2 bi-plot. The squid in Japan located in the positive direction of PC 1, while squid in Taiwan located in the opposite direction of PC 1 (Figure 23). The dendromgram from the cluster analysis, based on the PCA scores, illustrated the variation in life-history traits for the three taxa in two areas (Figure 23). The squid in Taiwan were grouped firstly, while the squid in Japan showed high variations in life-history traits. In general, variations in life-history traits of squid between geographic locations were greater than those of squid between taxa.

3.2.3 Satolith morphology for taxa in two areas

Twenty harmonics (excluded the first harmonic), each with four coefficients, were estimated by the Elliptic Fourier analysis and were used to illustrate the statolith shape of squids. The mean value of each harmonics of the squids were calculated by taxa in two areas. There were slight differences between squids in two areas: squid in Taiwan were wider in lateral dome than those in Japan. Besides, the dorsal dome of squid in Taiwan were smoother than those in Japan. The statolith shapes of squids were significantly different between two areas ($F_{20, 189} = 2.077$, $P < 0.05$; Figure 24).

Variations in 80 coefficients for three taxa in two areas were analyzed using PCA. The first two factors, PC 1 and PC 2, explained 40.1% and 29.6% of the total variance, respectively (Table 10). The dendromgram from the cluster analysis, based on the 76 FCs, illustrated the variation in statolith shape for the three taxa in two areas (Figure 25). The squid of the same taxa were grouped firstly (*Sepioteuthis* sp. 2 and sp. 2A) in Japan, while the *Sepioteuthis* sp. 1 in Taiwan and Japan showed high variations in statolith shape. The taxa and locations were confused in the clustering result, but showed obvious different in statolith morphology in different locations. In general, variations in statolith shape of squid between geographic locations were greater than those of squid between taxa.

4. Discussion

Sepioteithis lessoniana is a commercially important marine resource and is targeted by various fisheries across its distribution range (Arkhipkin et al., 2015). However, the taxonomic conditions of this species remain questionable because of its wide geographic distribution range and flexible life-history traits (Pecl, 2001; Jackson and Moltschaniwsky, 2002). The potential cryptic species and population structure of bigfin reef squid in the Northwest Pacific have been studied (Cheng et al., 2014; Tomano et al., 2016). A diverse taxa composition of the squid was noted in the waters off southwestern Japan, although relevant life-history information for these taxa is limited. This study deliberately identified the taxa composition of *Sepioteuthis* spp. using molecular analysis and examined variations in life-history traits and statolith shapes by taxa in the region. These results may provide essential information on the population structure of these squid in the Northwest Pacific.

4.1 Taxa composition of *Sepioteuthis* spp.

Application of molecular data for exploring the subtle population structures of exploited marine resources is an emerging approach in fisheries ecology, which may provide essential population information for conservation and management (Thorpe et al., 2000; Ward, 2000). There have been several studies and applications in various commercially exploited fish and squid (Kumar and Kocour, 2015; Sanchez et al., 2016). Based on body appearance, three types of *S. lessoniana*, namely *aka-ika* (red squid), *shiro-ika* (white squid), and *kua-ika* (small squid), were proposed by local fishermen in Japan and were recognized later using isozyme analysis (Izuka et al., 1994; 1996). Using mitochondrial DNA (noncoding region 2) analysis, Aoki et al. (2008) suggested three taxa, namely *Sepioteuthis* sp. 1, sp. 2, and sp. 3, in the waters around Japan. Additionally, three lineages of *Sepioteuthis* (A, B, and C) in the Indo-West Pacific region were evident using mitochondrial and nuclear genes (Cheng et al., 2014), of which lineages B and C could correspond to *Sepioteuthis* sp. 1 and sp. 2, respectively (Tomano et al., 2015a). At least three taxa of *Sepioteuthis* spp. were identified using MHS-PCR in this study, of which *Sepioteuthis* sp. 2A was first defined. On the basis of confirmed species (populations) and barcode information, MHS-PCR with specifically designed primers can rapidly identify taxa through the PCR products of mtDNA (Rocha-Olivares, 1998), and it has been applied by taxonomy and population studies in various fish (Rocha-Olivares and Chávez-González, 2008; Matsui et al., 2012). The genetic distance between *Sepioteuthis* sp. 2 and sp. 2A (J: 0.0252; T: 0.0270) was smaller than that between *Sepioteuthis* sp. 1 and sp. 2 (J: 0.1166; T: 0.1223). Previous reports the genetic divergence in mtDNA of *Sepioteuthis* sp. 1 and sp. 2 were more than 10% and considered as different species (Tomano et al., 2015a; Cheng et al., 2014) which is similar to the current study, while the genetic divergence between *Sepioteuthis* sp. 2 and sp. 2A was 2%, which suggests that Sepioteuthis sp. 2A could be a “distinct lineage”. The genetic diversity (*h* and π) revealed that *Sepioteuthis* sp. 2 in Japan may be a stable large population for a long time; by contrast, *Sepioteuthis* sp. 2A, which suffered a population bottle-neck effect, may be a small population, but it may play a crucial role in maintaining haplotype diversity for survival. The combination of low h and low π suggest "Recent population bottleneck or founder event by single of a few mtDNA lineages" (Grant and Bowen 1998), were the situation of sp. 2 in Taiwan. In addition, a number of squid specimens from Japan, Indonesia, and the Philippines in previous studies showed a high similarity to the defined *Sepioteuthis* sp. 2A in the sequence of specific sites, for example 100% in sites 36–489 for GenBank accession numbers LC152011, LC152044, and LC15201 (Tomono et al. 2016), and 99% in sites 1–617 for GenBank accession numbers LC553387, LC553369 and LC553356 (Cheng et al. 2014). The genetic diversity and Tajima’s *D* and Fu’s *F*s neutrality test for *Sepioteuthis* sp. 2 in the present study confirmed the results of the previous studies, i.e. a recent population expansion (Aoki et al. 2008; Tomano et al. 2015a, b). The values of genetic diversity and Tajima’s *D* and Fu’s *F*s neutrality test for *Sepioteuthis* sp. 2A were similar to those of *Sepioteuthis* sp. 2. Nevertheless, combined a bimodal distribution of the mismatch distribution analysis suggested a taxa complex in

Sepioteuthis sp. 2 and sp. 2A. From data, it suggest that *Sepioteuthis* sp. 2 and sp. 2A off southwestern Japan were both a recent expanded population, while *Sepioteuthis* sp. 2A might experience further population expansion. The genetic differentiation of the same taxa between locations were significant different (sp2: $F_{ST} = 0.044$; sp2A: $F_{ST} = 0.015$ ($P < 0.05$), which means the genetic isolations might exist in geographic distribution. However, more squid samples (temporal and spatial scale) and analyses (molecular and ecological data) are needed, which could elaborately illustrate the situation and relationship between *Sepioteuthis* sp. 2 and sp. 2A.

In this study, *Sepioteuthis* sp. 2 were dominant (average proportion of 84%) in the waters off southwestern Japan, followed by *Sepioteuthis* sp. 2A (average proportion of 15%). No definite geographic boundaries exist for the distribution of the three lineages of *Sepioteuthis* in the Indo-West Pacific region, although various local oceanographic conditions (environmental factors and currents) were noted (Cheng et al., 2014). By contrast, *Sepioteuthis* sp. 2 was commonly found around Japan, whereas *Sepioteuthis* sp. 1 was found on the Pacific side of Japan (Tomano et al., 2016). Distinct distribution depths for these two taxa have been suggested: less than 30 m for *Sepioteuthis* sp. 2 and approximately 30–60 m for *Sepioteuthis* sp. 1 (Tomano et al., 2015). Furthermore, the distribution boundary in southwestern Japan was suggested to be around Goto and Koshiki for these two taxa (Tomano et al., 2016). Nevertheless, two individuals of *Sepioteuthis* sp. 1 were found in GTI in this study, which suggested variable distribution boundaries in the waters off southwestern Japan. Different water depths were also noted at distinct sampling locations: the GTI sampling location was over the continental shelf at an approximate depth of 140 m, whereas the other five sampling locations were in coastal waters at depths of less than 60 m. Thus, according to the results of relevant studies and the present one, a distribution pattern for *Sepiotuthis* sp. 1 was proposed: *Sepioteuthis* sp. 1 is distributed in southern regions of the Northwest Pacific and can be transported to the waters off southern Japan by northward oceanic currents, such as the Kuroshio Current. The distribution boundaries and depths for these squid in the waters off southwestern Japan may be potentially influenced by temporal variations in oceanographic conditions, particularly currents. According to the genetic results, we suggests the hypothesis for spawning ground of *Sepioteuthis* sp. 1, sp. 2 and sp. 2A. It might locate at the coastal waters of Taiwan for sp. 1 and result in squids abundant in north Taiwan but few in south Japan, on the other hand spawning ground of sp. 2 and sp. 2A were considered at southern East China Sea shelf and transport northward to Japan and southward to Taiwan by seasonal currents, also considered the ones in locally spawning grounds with some isolations. However, more studies on the population structure and distribution of *Sepioteuthis* spp. and the influences of relevant oceanographic condition are necessary.

4.2 Variations in life-history traits

Although no statistically significant differences were found, this study noted slight differences in ML composition, age composition, hatching date distribution, and size of mature individuals between *Sepioteuthis* sp. 2 and sp. 2A. The differences in growth and maturation parameters between these two taxa could be the result of distinct hatching months; that is, *Sepioteuthis* sp. 2 mainly hatch during summer, whereas *Sepioteuhtis* sp. 2A mainly hatch during winter (Figure 6). Compare to the hatch month of squids in Taiwan, is the individuals of *Sepioteuthis* sp. 2 and sp. 2A mainly hatch during spring (Figure 19), showed difference between locations (Annex 5). Growth and maturation parameters of squid have been demonstrated to be significantly influenced by ambient oceanographic conditions such as water temperature during their life cycle, particularly in their early life history (Arkhipkin et al., 2000; Hatfield, 2000; Jackson and Moltschaniwskyj, 2001; Forsythe, 2004). A cold cohort of *Loligo vulgaris* in Portuguese waters was shown to mature later and to larger sizes compared with a warm cohort (Moreno et al., 2005). Water temperature during hatching may have a substantial impact on the size of squid; however, environmental conditions throughout their life could continue to play a critical role in their growth rate, leading to distinct growth strategies for squid hatching in different oceanographic environments or seasons (Moreno et al., 2007; Wang et al., 2013; Chen et al., 2015; Yamaguchi et al. 2020). However, overlapping of hatching months between these two taxa were also found, which implied that individuals of *Sepioteuthis* sp. 2 and sp. 2A likely experience similar oceanographic environments, but seasonal variations exist for each sampling location, where the life-history traits of squid could be largely influenced by environmental conditions rather than genetic factors.

Furthermore, great variations in life-history traits within each taxa of *Sepioteuthis* could mask variations of life-history traits between these taxa. The individuals of these two taxa occurred across all sampling locations, where the oceanographic environments were highly variable and showed seasonal variations. Although no statistically significant differences in life-history traits were found, two taxa of *Sepioteuthis* hatched in distinct seasons, which might be a crucial hint for the genetic significance of the squid in the region. More data are necessary to explicitly elucidate the characteristics in life-history traits for various *Sepioteuthis* taxa and their possible growth strategies in the Northwest Pacific region.

This study examined the life-history parameters, shape of statolith and genetic structure of squids in Japan and Taiwan populations, and supported the holistic knowledge for the *Sepioteuthis* spp. in the northwestern Pacific region. We found the mantle length and growth rate of squids in Japan (227.1 mm, 2.0 mm/day) were greater than those in Taiwan (185.4 mm, 1.6 mm/day). The squid in Taiwan (age peak in 110-129 days) are elder than those in Japan (90-109 days). The hatching month of *Sepioteuthis* sp. 2 in Taiwan peaked in April for female and in May and July for male, while the taxa in Japan peaked in June to July for female and February for male. After selecting the mature individuals (maturity stage 3 and 4), we found squids in Japan (235.5 mm, 123.3 day) were greater than those in Taiwan (204.0 mm, 115.9 day). Life-history traits of squids strongly effects by environment conditions. The current system of Japan and Taiwan waters mainly influenced by Kuroshio Current, but located in different straits (Tsushima Strait in Japan and Taiwan Strait in Taiwan). The oceanographic conditions around Taiwan also influenced by the China Coastal Current, while those in Japan influence by Tsushima Current. Squid growth patterns are influenced by ambient water temperatures

(Forsythe, 1993, 2004), as well as food availability (Jackson and Moltschaniwiskyj, 2001). Squid inhabited cold waters could reach a large ML and show fast growth rate. Ching et al. (2019) found the ML of *Sepioteuthis lessoniana* in cold waters (off northern Taiwan) were larger those in warm waters (Taiwan Strait). In this study, the average water temperature in waters off northern Taiwan were higher than those off southwestern Japan, which might explain the large individuals occurring off southwestern Japan.

We compared among taxa and also between locations for *Sepioteuthis* spp. and the Mantle length at maturity and growth rate were bigger and faster in Japan, but the age at maturity were elder in Taiwan individuals. The life-history traits showed the variations between locations greater than those among taxa in the *Sepioteuthis* spp (Annex 6).

4.3 Statolith morphology

The shape of statoliths in a squid is a composite function of genetics and accumulated effects of the environment inhabited by the squid (Campana and Casselman, 1993; Arkhipkin, 2003). Thus, shape analysis of statoliths can identify populations (or stocks) of squid inhabiting different environments (Semmens et al., 2007), particularly highly migratory squid (Barcellos and Gasalla, 2015; Fang et al., 2018). Oceanographic factors in the ambient environment, such as temperature, salinity, depth, and water composition, can be responsible for inter-stock differences in otolith shape (Campana and Casselman, 1993; Cañás et al., 2012; Sea et al., 2008; Tuset et al., 2003). In this study, no significant differences were found in statolith shapes between *Sepioteuthis* sp. 2 and sp. 2A, whereas slight differences were noted among geographic groups (GTI and OMB). The results suggested that variations among geographic groups have greater influences on statolith shape than do those between seasonal and/or genetic groups. However, more detailed analyses of the statolith shape of squid between taxa and locations are necessary to clearly illustrate the potential effects. Additionally, a holistic approach, such as combining statolith element composition and shape analyses, may be more effective for discriminating subtle populations (or stocks), which can provide substantial information for fishery management (Semmens et al., 2007; Green et al., 2015).

Slight differences exist in the wings (The outline points: harmonics 14-15, 12 FCs) of statoliths between *Sepioteuthis* sp. 2 and sp. 2A. The morphology of statoliths presents considerable differences between two ecological groups of decapods, which indicate different movement patterns (Arkhipkin and Bizikov, 2000). Among the other differences, a narrow and short wing for demersal squid and a wide and long wing for pelagic squid were distinguished (Arkhipkin and Bizikov, 2000). Furthermore, the size of the wing and volume of the endolymph stream are closely related to the sizes of statoliths and statocysts, which determine the movement of squid (Arkhipkin and Bizikov, 2000). A distinct distribution range and depth for *Sepioteuthis* sp. 1 and sp. 2 were suggested (Tomano et al., 2016). By contrast, a great overlap in geographic distribution between *Sepioteuthis* sp. 2 and sp. 2A was found in this study, although distinct hatching seasons were noted for these two taxa. The life-history traits, such as growth and maturation parameters, of squid have been shown to be strongly influenced by the ambient oceanographic conditions during their early life history (Arkhipkin et al., 2000; Jackson and Moltschaniwskyj, 2001; Moreno et al., 2005). However, the potential effects of temporal variations of oceanographic conditions on the statolith shape of seasonal cohorts of squid have been scarcely studied. Further analysis on the differences in statolith shape of seasonal cohorts of squids and substantial influence of ambient environments should be addressed, which may provide substantial implications for the potential effects.

Morphology of the *Sepioteuthis lessoniana* were confused between taxa and due to the freshness of individuals, the body color were easily mistakenly classified. The body of squids are also rashly damaged by fishery gears, and result in the morphometric of soft tissue hard to compare. Statolith, as a hard structure, the shape can change between populations and relate to growth and genetic regulation, and act as a useful reference for population identification. The mean shape of mature samples in Taiwan developed a wider lateral dome, while shape of Japan samples had a prominence wing of the statolith. Ikeda and Kobayashi (2010) investigated the growth of statolith as bigfin reef squid was observed in juveniles up to 45 days post hatching and raised in controlling water temperatures (20°C, 25°C, and 30°C). They found the statolith grew in a circular manner from the central and the statolith size were biggest under 30°C condition. Interestingly, the ontogeny of *Sepioteuthis lessoniana* statolith were obviously wider in the lateral dome raised in warmer water (30°C; Ikeda and Kobayashi, 2010). The circumstances of wider lateral dome of statolith in squids of Taiwan as a warmer environment than Japan happened to hold the same view of the study above. In the other hand, evidence were reported the otolith shape in fish have a clear genetic basis and confirms the genetic regulation of otolith shape (Berg et al., 2018). The genetic structure of squids in Japan and Taiwan were different, i.e. *Sepioteuthis* sp. 1 dominated in Taiwan while *Sepioteuthis* sp. 2 dominated in Japan. Statolith shape in squid could also be genetic regulated as a similar microstructure and organ to the otolith in fish (Castonguay et al., 1991; Lombarte and Lleonart, 1993; Begg and Brown, 2000). However, it is worth noting that squids as short-lived species are strongly influence by environmental condition than fish, and the level of effects by genetic factors or environment factors are not clearly studied. According to the results of previous study, shape of statolith were no difference between the *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan. For squid, environment factors seems to have a major effect on statolith morphology and growth instead of the genetic factors (Annex 7, Annex 8).

4.4 *Sepioteuthis* spp. in the northwestern Pacific

In this study, at least three taxa of *Sepioteuthis* were identified in the waters off southwestern Japan. However, the differences in life-history traits between *Sepioteuthis* sp. 2 and sp. 2A were slight. PCA was performed to extract variations in five life-history traits (BW, ML, NI, GR, and GSI) of *Sepioteuthis* spp. among six locations (Figure 11). Furthermore, cluster analysis based on the PC scores of life-history traits was performed to group the locations. Three clusters were found: the first comprised SSB and wNMZ; the second comprised GTI, eNMZ, and TMS; and the third cluster only included OMB (Figure 11. The resulting clusters of locations based on life-history traits of the squid are likely to be explained by the oceanographic environments and currents. The oceanographic environments for the first cluster (SSB and wNMZ) and second cluster (GTI, eNMZ, and TMS) are influenced by the warm Kuroshio Current (Morimoto et al., 2009). By contrast, the environments in OMB differ from other locations because of their special upwelling effects (Takahashi et al., 2009), where the squid may inhabit during their life cycles. However, the potential influence of oceanographic conditions on the life-history traits of squid cannot be addressed until more data on local oceanographic environments and population structure of the squid are examined.

In this study, the life-history traits between the taxa of *Sepioteuthis* spp. were similar, whereas those among locations were different. These results imply that the variations in life-history traits are strongly influenced by the environmental conditions experienced by the squid during their life cycles. Furthermore, distinct taxa of the squid might experience similar oceanographic conditions, such as by inhabiting the same locations or distinct locations while being influenced by the same current and thus display similar life-history traits (Anderson and Rodhouse, 2001). Crucially, unlike molecular markers, phenotypic variation in body parts is influenced by environmental factors (Carvalho and Nigmatullin, 1998) and does not always result from genetic divergence (Cadrin, 2000). The results of this study suggest that the genetic structure of the squid can be obtained from molecular evidence, and the life-history traits and statolith shape might reveal the accumulated effects of the environments experienced by the squid. A holistic approach for population and/or stock identification, such as combining molecular and morphometric analyses, could reveal the subtle population structure of the squid; the structure is difficult to find using a single stock identification method, and such a method may provide essential information for conservation and management measures for fisheries (Semmens et al., 2007). The taxa composition and population structure of *Sepioteuthis* spp. in the Northwest Pacific require more studies to elucidate potential connectivity among them, which is crucial for achieving sustainability goals for the squid and relevant fisheries in the region.

The genetic structure of *S. lessoniana* were obviously different between Japan and Taiwan.

Sepioteuthis sp. 1 were dominated in waters off northern Taiwan, while *Sepioteuthis* sp. 2 were dominated in waters off southwestern Japan. Tomano et al. (2015) pointed out the genetic structure of squid around Japan were mainly composed of *Sepioteuthis* sp. 2, but some individuals of *Sepioteuthis* sp. 1 were found in the waters off southern Japan. We suggested a complex population structure occurs in the waters off southwestern Japan, including the seas around Nagasaki prefecture. In our result, the population structure of squid composed of *Sepioteuthis* sp. 2 (81%), *Sepioteuthis* sp. 2A (17%) and *Sepioteuthis* sp. 1 (2%) off southwestern Japan. The samples of *Sepioteuthis* sp. 1 appeared in the seas around Goto Island where locate close to the open seas, and were more directly influenced by the Kuroshio Current. In contrast, the squid in Taiwan, which composed of *Sepioteuthis* sp. 1 (70%), *Sepioteuthis* sp. 2 (25%) and *Sepioteuthis* sp. 2A (5%). The Kuroshio Current origins from North Equatorial Counter Current, moves towards north between Taiwan and Yonagunijima, through Tokara Strait and flows into southern coast of main islands Japan. The Kuroshio Current might bring the *Sepioteuthis* sp. 1 individuals from Taiwan to Japan seas. According the results of genetic structure of bigfin reef squid, the population structure around northern Taiwan seems to be more complex than off southwestern Japan, and such situation was similar to the study of Aoki et al. (2008). The low gene flow between squid in Japan and Taiwan might relate to the geographic isolation and distance. Geographical distance was about 2,000 km far between southern Japan and northern Taiwan, and it seems hard to swim from Japan to Taiwan. Geographical distribution of *Panulirus longipes* is another example in which Kuroshio Current act as a barrier between Japan and Taiwan (Sekiguchi and Inoue, 2010). Accordingly, several marine organisms, *Uca arcuata* and *Siganus guttatus* shows different genetic structure between Japan (Ryukyu Archipelago) and Taiwan, showing no gene flow (Aoki et al., 2008b; Iwamoto et al., 2012). These genetic structure patterns may be affected by Kuroshio Current. Especially squid possess short life-history, some of the species has only one spawning season in their lifetime. Drastic environmental changes and other accidental events may destroy population. In order to raise the fitness of squids, water temperature and appropriate environment of growth phase are essential (O'Dor and Coelho, 1993). Kuroshio Current may supply appropriate temperature and abundant feed resources for *Sepioteuthis* spp. habitats. The comparative study of *Sepioteuthis* sp. 2 reported by Aoki et al. (2008) and *Sepioteuthis* sp. 1 showed these two species can belong to each clade of Japan and Taiwan. In each taxa, it was suggested that Nagasaki and Taiwan are genetically different, but the genetic composition of the regional population between Nagasaki and Taiwan are still unclear and how much spawning in which region were not evaluated, some further research is needed. However, to steady the hypothesis of spawning ground we plan to combine the analysis of trace elements of statolith from each taxa, and to collect more data of squids from north and east of Japan and south of Taiwan might complete the geographic distribution of taxa composition in the future.

5. Conclusion

5.1 Study 1

5.1.1 Taxa composition: First defined *Sepioteuthis* sp. 2A as a distinct lineage and Sp. 2 were dominate in southwestern Japan.

5.1.2 Comparison between sp. 2 and sp. 2A, the Hatching month were different (sp. 2 in July and sp. 2A in February and December), and there is no difference in statolith shape.

5.2 Study 2

5.2.1 Taxa composition: Sp. 1 dominate in Taiwan, and Sp. 2 dominate in southwestern Japan.

5.2.2 The Variation in life-history traits showed the environmental effects greater than genetic effects.

5.2.3 Variation in statolith shape also showed the environmental effects greater than genetic effects.

5.3 Hypothesis

5.3.1 Spawning grounds for *Sepioteuthis* sp. 1 were considered locate at coastal waters of Taiwan which the squid abundant in North Taiwan and few in South Japan.

5.3.2 Spawning ground for *Sepioteuthis* sp. 2 and sp. 2A might locate at southern East China Sea shelf and transport northward (Japan) and southward (Taiwan) by seasonal currents. The second possible spawning ground were locate in locally (in Japan and Taiwan).

6. References

Abaunza P, Murta AG, Campbell N, Cimmaruta R, Comesaña AS, Dahle G, Gallo E, García Santamaría MT, Gordo LS, Iversen SA, MacKenzie K, Magoulas A, Mattiucci S, Molloy J, Nascetti G, Pinto AL, Quinta R, Ramos P, Ruggi A, Sanjuan A, Santos AT, Stransky C, Zimmermann C (2008) Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: integrating the results from different stock identification approaches. Fish Res 89: 196–209

Anderson CIH, Rodhouse PG (2001) Life cycles, oceanography and variability: ommastrephid squid in variable oceanographic environments. Fish Res 54: 133-143

Aoki M, Imai H, Naruse T, Ikeda Y (2008) Low genetic diversity of oval squid, *Sepioteuthis* cf. *lessoniana* (Cephalopoda: Loliginidae), in Japanese waters inferred from a mitochondrial DNA non-coding region. Paci Sci 62: 403-411

Arkhipkin AI, Jereb P, Ragonese S (2000) Growth and maturation in two successive seasonal groups of the short-finned squid, *Illex coindetii* from the Strait of Sicily (central Mediterranean) ICES J Mar Sci 57: 31-41

Arkhipkin AI (2003) Towards identification of the ecological lifestyle in nektonic squid using statolith morphometry. J Moll Stu 69: 171-178

Arkhipkin AI (2005) Statoliths as 'black boxes' (life recorders) in squid. Mar Freshw Res 56: 573–583

Arkhipkin AI, Campana SE, FitzGerald J, Thorrold SR (2004) Spatial and temporal variation in elemental signatures of statoliths from the Patagonian longfin squid (*Loligo gahi*). Can J Fish Aquat Sci 61: 1212–1224

Arkhipkin AI, Shcherbich ZN (2012) Thirty year's progress in age determination of squid using statoliths. J Mar Bio Ass UK 92: 1389–1398

Arkhipkin AI, Rodhouse PGK, Pierce GJ, Sauer W, Sakai M, Allcock L, Arguelles J, Bower JR, Castillo G, Ceriola L, Chen CS, Chen XJ, Diaz-Santana M, Downey N, González AF, Amores JG, Green CP, Guerra A, Hendrickson LC, Ibáñez C, Ito K, Jereb P, Kato Y, Katugin ON, Kawano M, Kidokoro H, Kulik VV, Laptikhovsky V V, Lipinski M R, Liu B L, Mariátegui L, Marin W, Medina A, Miki K, Miyahara K, Moltschaniwskyj N, Moustahfi H, Nabhitabhata J, Nanjo N, Nigmatullin CM, Ohtani T, Pecl G, Perez JAA, Piatkowski U, Saikliang P, Salinas-Zavala CA, Steer M, Tian YJ, Ueta Y, Vijai D, Wakabayashi T, Yamaguchi T, Yamashiro C, Yamashita N, Zeidberg LD (2015) World squid fisheries. Rev Fish Sci Aquac 23: 92-252

Barcellos DD, Gasalla MA (2015) Morphology and morphometry of *Doryteuthis plei* (Cephalopoda: Loliginidae) statoliths from the northern shelf off São Paulo, southeastern Brazil. J Natu His 49: 1305-1317

Begg GA, Waldman JR (1999) An holistic approach to fish stock identification. Fish Res 43:35–44

Begg G, Brown R (2000) Stock identification of haddock *Melanogramus aeglefinus* on Georges Bank based on otolith shape analysis. Trans Am Fish Soc 129: 935–945

Begg G, Overholtz WJ, Munroe NJ (2000) The use of internal otolith morphometrics for identification of haddock (*Melanogram aelefinus*) stocks on Georges Bank. Fish Bull 99: 1–14

Bettencourt V, Guerra A (2000) Growth increments and biomineralization process in cephalopod statoliths. J Exp Mar Bio Ecol 248: 191–205

Berg F, Almeland OW, Skadal J, Slotte A, Andersson L, Folkvord A (2018) Genetic factors have a major effect on growth, number of vertebrae and otolith shape in Atlantic herring (*Clupea harengus*) PLoS One, 13: p. e0190995

Bird JL, Eppler DT, Checkley DM Jr (1986). Comparisons of herring otoliths using fourier series shape analysis. Canadian Journal of Fisheries and Aquatic Sci.43:1228–34.

Braicovichand PE, Timi JT (2008) Parasites as biological tags for stock discrimination of the Brazilian flathead, *Percophis brasiliensis* Quoy & Gaimard, 1824, in the southwest Atlantic. J Fish Biol 73: 557–571

Boyle PR, Ngoile MAK (1993) Assessment of maturity state and seasonality of reproduction in *Loligo forbesi* (Cephalopoda: Loliginidae) from Scottish waters. In: Okutani T et al (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 37－48

Burke N, Brophy D, King PA. (2008) Shape analysis of otolith annuli in Atlantic herring (*Clupea harengus*); a new method for tracking fish populations. Fish Res. 91:133–43.

Cardinale M, Doering-Arjes P, Kastowsky M, Mosegaard H. (2004) Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. Canadian Journal of Fisheries and Aquatic Sci. 61:158–67

Cadrin SX (2005) Morphometric landmarks. In: Cadrin SX et al (eds) Stock identification methods. Elsevier, San Diego, pp 153–172

Carvalho GR, Nigmatullin CM (1998) Species identification and stock struckture analysis within the genus *Illex*. In: Rodhouse PG, Dawe EG, O’Dor RK (eds) Squid Recruitment Dynamics. The genus *Illex* as a model. The commercial species and influences on variability. 376: 199–232

Campana SE, Casselman JM (1993) Stock discrimination using otolith shape analysis. Can J Fish Aquat Sci 50: 1062–1083

Campana SE, Chouinard GA, Hanson JM, Fréchet A, Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res., 46, pp. 343-357

Campana SE, Thorrold SR (2001). Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58: 30–38

Campana SE (2005) Otolith science entering the 21st century. Mar Freshw Res 56: 485–495

Castonguay M, Simard P, Gagnon P (1991) Usefulness of Fourier analysis of otolith shape for Atlantic mackerel (*Scomber scombrus*) stock discrimination. Can J Fish Aquat Sci 48: 296–302

Cañás L, Stransky C, Schlickeisen J, Sampedro MP, and Fariña AC (2012) Use of the otolith shape analysis in stock identification of anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. ICES J Mar Sci 69: 250–256

Chen C-S, Chen J-Y, Lin, C-W (2015) Variation in life-history traits for micro-cohorts of *Sepioteuthis lessoniana* in the waters off northern Taiwan. Fish Sci 81: 53–64

Cheng SH, Anderson FE, Bergman A, Mahardika GN, Muchlisin ZA, Dang BT, Calumpong HP, Mohamed KS, Sasikumar G, Venkatesan V, Barber PH (2014) Molecular evidence for co-occurring cryptic lineages within the *Sepioteuthis* cf. *lessoniana* species complex in the Indian and Indo-West Pacific Oceans. Hydrobiologia 725: 165–188

Ching T-Y, Chen C-S, Wang C-H (2019) Spatiotemporal variations in life-history traits and statolith trace elements of *Sepioteuthis lessoniana* populations around northern Taiwan. J Mar Biol Ass UK 99: 203–213

Clarke MR (1978). The cephalopod statolith – an introduction to its form. J Mar Biol Ass UK 58: 701–712

Crampton JS (1995) Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia 28: 179–186

DeVries DA, Grimes CB, Prager MH (2002) Using otolithshape analysis to distinguish eastern Gulf of Mexico and Atlantic Ocean stocks of king mackerel. Fish Res 57: 51–62

Elliott N, Haskard KA, Koslow JA (1995) Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of Southern Australia. J Fish Biol 46: 202-220

Fang Z, Chen XJ, Su H, Staples K, Chen Y (2018) Exploration of statolith shape variation in jumbo flying squid, *Dosidicus gigas*, based on wavelet analysis and machine learning methods for stock classification. Bull Mar Sci 19: 1465–1482

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evol 39: 783-791.

Forsythe JW (1993) A working hypothesis on how seasonal temperature change may impact the filed growth of young cephalopods. In: Okutani T et al (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 133–143

Forsythe J, Walsh L, Turk P (2001) Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Mar Biol 138: 103–112

Forsythe JW (2004) Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. Mar Freshw Res 55: 331–339

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Gene 147: 915–925

Gagliano M, Mccormik ML (2004) Feeding history influences otolith shape in tropical fish. Mar Ecol Prog Ser 278: 291-296

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. J Hered 89: 415-426

Geller J, Meyer C, Parker M, Hawk H. (2013) Redesign of PCR primers for mitochondrial cytochrome *c* oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol Ecol Resour 13:851–861

Gillanders BM (2005) Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuar Coast Shelf Sci 64: 47–57

Green CP, Robertson SG, Hamer PA, Virtue P, Jackson GD (2015) Combining statolith element composition and Fourier shape data allows discrimination of spatial and temporal stock structure of arrow squid (*Nototodarus gouldi*). Can J Fish Aquat Sci 72: 1609–1618

Hatfield MCE (2000) Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae) Fish Res 47: 27–40

Hüssy K (2008) Otolith shape in juvenile cod (*Gadus morhua*): ontogenetic and environmental effects. J Exp Mar Biol Ecol 364:35–41

Hsiao C-D, Shen K-N, Ching T-Y, Wang Y-H, Ye J-J, Tsai S-Y, Wu S-C, Chen C-H, Wang C-H (2016) The complete mitochondrial genome of the cryptic “lineage A” big-fin reef squid, *Sepioteuthis lessonaina* (Cephalopoda: Loliginidae) in Indo-West Pacific. Mitochondrial DNA Part A 27: 2433–2434

Ichikawa H, Beardsley RC (2002) The Current System in the Yellow and East China Seas. J Ocean 58: 77-92

Ikeda Y, Kobayashi M (2010) Statolith growth of juvenile oval squid Sepioteuthis lessoniana (Cephalopoda: Loliginidae) with special reference to ambient thermal condition. Mar Biol Res 6: 485-495

Imai H, Aoki M (2012) Genetic diversity and genetic heterogeneity of bigfn reef squid "Sepioteuthis lessoniana" species complex in northwestern Pacifc Ocean. In: Caliskan M (ed) Analysis of genetic variation in animals. InTech, Croatia, pp 151–166

Iwata H, Ukai Y (2002) SHAPE: A computer program package for quantitative evaluation of biological shape based on elliptic Fourier descriptors. J Hered 93: 384–385

Izuka T, Segawa S, Okutani T (1996a) Biochemical study of the population heterogeneity and distribution of the oval squid *Sepioteuthis lessoniana* complex in southwestern Japan. Am Malacol Bull 12: 129–135

Izuka T, Segawa S, Okutani T (1996b) Identification of three species in oval squid, *Sepioteuthis lessoniana* complex by chromatophore arrangements on the funnel. Jap J Malacol 55: 139–142

Izuka T, Segawa S, Okutani T, Numachi K (1994) Evidence on the existence of three species in the oval squids *Sepioteuthis lessoniana* complex in Ishigaki Island, Okinawa, Southwestern Japan, by isozyme analysis. Jap J Malacol 53: 217–228

Jackson GD (1990) Age and growth of the tropical nearshore loliginid squid *Sepioteuthis lessoniana* determined from statolith growth-ring analysis. Fish Bull 88: 113–118

Jackson GD, Moltschaniwskyj NA (2001) Temporal variation in growth rates and reproductive parameters in the small near-shore tropical squid *Loliolus noctiluca*; is cooler better? Mar Ecol Porg Ser 218: 167–177

Jackson GD, Moltschaniwskyj NA (2002) Spatial and temporal variation in growth rates and maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae). Mar Biol 140: 747–754

Jereb P, Roper CFE (eds) (2010) Cephalopods of the world: an annotated and illustrated catalogue of cephalopod species known to date, Volume 2. Myopsid and Oegopsid Squids. FAO Species Catalogue for Fishery Purposes No. 4. FAO, Rome

Kuhl F Giardina C (1982) Elliptical Fourier Features of a Closed Contour. Computer Graphics Image Processing 18: 236-258

Kumar G, Kocour (2015) Population genetic structure of tunas inferred from molecular markers: A review. Rev Fish Sci Aquac 23: 72–89

L’Abée-Lund JH (1988) Otolith shape discriminates between juvenile Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. J Fish Biol 33: 899–903

Lear WH, Green, JM 1984. Migration of the “northern” Atlantic cod and the mechanisms involved. In Mechanisms of Migration in Fishes, pp. 309–315. (eds) by J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill. Plenum Press, New York. 574 pp

Lestrel P (eds) (1997) Fourier descriptors and their applications in biology. Cambridge: Cambridge University Press.

Lombarte A, Castelln A (1991) Interspecific and intraspecific otolith variability in the genus Merluccius as determined by image analysis. Can J Zool 69: 2442-2449

Lombarte A, Lleonart J (1993) Otolith size changes related with body growth, habitat depth and temperature. Environ Biol Fish 37: 297–306

Matsui S, Nakayama K, Kai Y, Yanashita Y (2012) Genetic divergence among three morphs of *Acentrogobius pflaumii* (Gobiidae) around Japan and their identification using multiplex haplotype-specific PCR of mitochondrial DNA. Ichthyol Res 59: 216–222

Mérigot B, Letourneur Y, Lecomte-Finiger R (2007) Characterization of local populations of the common sole Soleasolea (Pisces, Soleidae) in the NW Mediterranean through otolith morphometrics and shape analysis. Mar Biol 151: 997–1008

McKeown NJ, Arkhipkin AI, Shaw PW (2015) Integrating genetic and otolith microchemistry data to understand population structure in the Patagonian Hoki (*Macruronus magellanicus*). Fish Res 164: 1–7

Monteiro LR, Bonato V, Dos Reis SF (2005) Evolutionary integration and morphological diversification in complex morphological structures: mandible shape divergence in spiny rats (*Rodentia, Echimyidae*) Evol develop 7: 429-439

Moreno A, Pereira J, Cunha MM. 2005. Environmental influences on age and size-at-maturity of *Loligo vulgaris*. Aqua Liv Res 18:377-384

Moreno A, Azevedo M, Pereira J, Pierce GJ (2007) Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. Mar Biol Res 3: 49–59

Morimoto A, Isoda Y, Tameishi T, Moriwaki S (2009) Seasonal variation in Tsushima warm current paths over the shelf off the San’in coast, Japan. Cont Shelf Res 29: 1437–1447

Mosegaard H, Madsen KP (1996) Discrimination of mixed Herring stocks in the North Sea using vertebral counts and otolith microstructure. Conference: International Council for the Exploration of the Sea, Pelagic Fish Committee C.M. H: 17

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 89：583-590.

Nitani H (1975) Variation of the Kuroshio south of Japan. J Ocean Soci Jap 31: 154-173

O’Dor RK, Forsythe J, Webber DM et al (1993) Activity levels of Nautilus in the wild. Nature 362:626–628

Okutani T (1984) Life history of the oval squid, *Sepioteuthis lessoniana*. Saibai Giken 13: 69–75 (In Japanese)

Onitsuka G, Yanagi T, Yoon J-H (2007) A numerical study on nutrient source in the surface layer of the Japan Sea using a coupled physical-ecosystem model. J Geophy Res 112: C05042

Pecl GT (2001) Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids.

Mar Biol 138: 93–101

Olmos-Pérez L, Pierce GD, Roura Á, González ÁF (2018) Barcoding and morphometry to identify and assess genetic population differentiation and size variability in loliginid squid paralarvae from NE Atlantic (Spain) Mar Biol 165: 136

Reichenbacher B, Kamrani E, Esmaeilli HR, Teimori A (2009) The endangered cyprinodont Aphanius ginaonis (Holly, 1929) from southern Iran is a valid species: evidence from otolith morphology. Environ Biol Fish 86: 507

Rocha-Olivares A (1998) Multiplex haplotype-specific PCR: a new approach for species identification of the early life stages of rockfishes of the species-rich genus *Sebastes* Cuvier. J Exp Mar Biol Ecol 231: 279–290

Rocha-Olivares A, Cha´vez-Gonza´lez JP (2008) Molecular identification of dolphinfish species (genus *Coryphaena*) using multiplex haplotype-specific PCR of mitochondrial DNA. Ichthyol Res 55: 389-393

Sanchez G, Tomano S, Yamashiro C, Fujita R, Wakabayashi T, Sakai M, Umino T (2016) Population genetics of the jumbo squid *Dosidicus gigas* (Cephalopoda:Ommastrephidae) in the northern Humboldt Current system based on mitochondrial and microsatellite DNA markers. Fish Res 175: 1-9

Sekiguchi H Inoue N (2005) Distribution of Scyllarid Phyllosoma Larvae (Crustacea: Decapoda: Scyllaridae) in the Kuroshio Subgyre. J Ocean 61: 389-398

Semmens JM, Pecl GT, Gillanders BM, Waluda CM, Shea EK, Jouffre D, Ichii T, Zumholz K, Katugin ON, Leporati SC, Shaw PW (2007) Approaches to resolving cephalopod movement and migration patterns. Rev Fish Biol Fish 17: 401-423

Segawa S, Hirayana S, Okutani T (1993) Is *Sepioteuthis lessoniana* in Okinawa a single species? In: Okutani T et al (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, pp 513–521

Shen K-N, Yen T-C, Chen C-H, Ye J-J, Hsiao C-D (2016) The complete mitochondrial genome of the cryptic "lineage B" big-fin reef squid, *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae) in Indo-West Pacific. Mitochondrial DNA Part A 27: 2100–2101

Stransky C, Murta AG, Schlickeiesn J, Zimmermann C (2008) Otolith shape analysis as a tool for stock separation of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean. Fish Res 89: 159-166

Swearer SE, Forrester GE, Steele MA, Brooks AJ, Lea DW (2003) Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuar Coast Shelf Sci 56: 1111–1123

Sweeney MJ, Roper CFE, Mangold KM, Clarke MR, Boletzky Sv (eds) (1992) "Larval" and juvenile cephalopods: a manual for their identification. Smithson Contr Zool 513: 1–28

Stephenson RL (1999) Stock complexity in fisheries management: a perspective of emerging issues related to population sub-unit. Fish Res 43: 247–249

Takahashi T, Nakata H, Hirano K, Matsuoka K, Iwataki M, Yamaguchu H, Kasuya T (2009) Upwelling of oxygen-depleted water (Sumishio) in Omura Bay, Japan. Oceanograph 65: 113–120

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Gene 123: 585–595

Thorpe JP, Solé-Cava AM, Watts PC (2000) Exploited marine invertebrates: Genetics and fisheries. Mar Gene, DIHY, pp 165–184

Tomano S, UetaY, Kasaoka N, Umino T (2015a) Stock identification and spawning depth of oval squid *Sepioteuthis* spp. in Tanega-shima Island inferred by DNA markers. Aquat Sci 63: 39–47 (In Japanese with English abstract)

Tomano S, Sanchez G, Ueno K, Ueta Y, Ohara K, Umino T (2015b) Microsatellite DNA variation of oval squid, *Sepioteuthis* sp. 2 reveals a single fishery stock on the coastline of mainland Japan. Fish Sci 81: 839–847

Tomano S, Sanchez G, Kawai K, Kasaoka N, Ueta Y, and Umino T (2016) Contribution of *Sepioteuthis* sp. 1 and *Sepioteuthis* sp. 2 to oval squid fishery stocks in western Japan. Fish Sci 82: 585–596

Torres G, Lombarte A, Morales-Nin B (2000) Sagittal otolith size and shape variability to identify intraspecific differences in three species of the genus Merluccius. J Mar Biol Asso UK 80: 333-342

Tracey WD, Ning X, Klingler M, Kramer SG, Gergen JP (2000). Quantitative analysis of gene function in the Drosophila embryo. Gene 154: 273--284

Tuset VM, Lozano IJ, González JA, Pertusa JF, García-Díaz MM (2003) Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). J App Ichthyol 19: 88–93

Vignon M, Morat F (2010) Environmental and genetic determinant of otolith shape revealed by a non-indigenous tropical fish. Mar Ecol Pro Ser 411: 231-241

Wang K-Y, Chanf K-Y, Liao C-H, Lee M-A, Lee K-T (2013) Growth strategies of the swordtip squid, *Uroteuthis edulis,* in response to environmental changes in the southern East China Sea — A cohort analysis. Bull Mar Sci 89: 677–698

Ward RD (2000) Genetics in fisheries management. Hydrobiologia 420: 191–201

Xavier JC, Allcock AL, Cherel Y (2015) Future challenges in cephalopod research. J Mar Biol Ass UK 95: 999–1015

Yamaguchi T, Takayama K, Hirose N, Matsuyama M (2020) Relationship between empirical water temperature and spring characteristics of swordtip squid (*Uroteuthis edulis*) caught in the eastern Tsushima Strait. Mar Biol Res 16: 93–102

Table 1. Summary information for *Sepioteuthis* spp. samples in the waters off southwestern Japan. (ML: mantle length (average, standard deviation and range); BW: body weight (average, standard deviation and range))

		Female					Male				
Location	Month-Year	*n*	ML (mm)	BW (g)	Maturity stage	Aged *n*	*n*	ML (mm)	BW (g)	Maturity stage	Aged *n*
Sasebo (SSB)	Apr-16	4	197 ± 10 (184-207)	456 ± 46 (396-516)	4	4	6	194 ± 10 (177-208)	420 ± 59 (327-508)	4	6
	Aug-16	1	185	456	4	1	4	236 ± 21 (214-264)	745 ± 16 (728-765)	4	4
Omura Bay (OMB)	Sep-16	16	120 ± 14 (95-142)	122 ± 43 (62-191)	2-3	16	17	125 ± 16 (94-148)	129 ± 41 (58-206)	2-3	17
Tameshi (TMS)	May-16	2	236 ± 47 (203-270)	774 ± 403 (489-1059)	4	2	4	271 ± 62 (213-359)	937 ± 579 (477-1783)	4	3
	Jun-16	10	198 ± 35 (156-275)	462 ± 226 (226-937)	4	8	21	252 ± 50 (177-327)	791 ± 380 (284-1324)	4	18
Eastern Nomozaki (eNMZ)	Feb-17	1	212	562	3	1	8	266 ± 54 (184-349)	1034 ± 564 (328-1977)	4	8
Western Nomozaki (wNMZ)	Oct-16	10	199 ± 26 (142-220)	510 ± 150 (258-700)	3	10	10	207 ± 48 (162-292)	571 ± 397 (260-1304)	4	10
Goto Islands (GTI)	Apr-16	2	273 ± 31 (251-295)	948 ± 394 (669-1227)	4	2	12	318 ± 53 (255-407)	1535 ± 839 (116-2977)	4	12
	Aug-16	9	168±38 (113-232)	358±179 (93-660)	2-4	7	5	249±77 (127-317)	745±16 (118-1282)	2-4	5

Table 2. Summary of mtDNA variability and diversity for *Sepioteuthis* sp.2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan.

Parameters	*Sepioteuthis* sp. 2	*Sepioteuthis* sp. 2A
Haplotype diversity (*h*)	0.6505	0.8000
Nucleotide diversity (π)	0.0076	0.0047
No. of individuals	117	16
No. of haplotypes	21	8
Tajimas' D	-2.2449**	-1.9717*
Fu's fs	-6.9818*	-2.4589*

* $P < 0.05$, ** $P < 0.01$

Table 3. Genetic distances between three taxa of *Sepioteuthis* spp. in the waters off southwestern Japan.

Taxa /Distance	*Sepioteuthis* sp. 1	*Sepioteuthis* sp. 2	*Sepioteuthis* sp. 2A
Sepioteuthis sp. 1			
Sepioteuthis sp. 2	0.1166		
Sepioteuthis sp. 2A	0.1321	0.0252	

Table 4. Results of principal components analysis for life-history traits of *Sepioteuthis* spp. in the waters off southwestern Japan. (BW: body weight; ML: mantle length; NI: number of increments; GR: average growth rate; GSI: Gonadsomatic index)

Principal components	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.010	1.106	0.769	0.112	0.004
% of value	60.20	22.11	15.38	2.23	0.088
Cumulative %	60.20	82.31	97.69	99.92	100.0
BW	0.954	-0.115	-0.062	-0.270	0.011
ML	0.995	-0.033	0.017	0.076	-0.490
NI	0.648	0.479	-0.582	0.107	0.021
GR	0.822	-0.326	0.443	0.142	0.031
GSI	0.116	0.869	0.480	-0.039	0.000

Table 5. Summary information for *Sepioteuthis* spp. samples in the waters off Japan and Taiwan. (ML: mantle length (average, standard deviation and range); BW: body weight (average, standard deviation and range))

	Female					Male				
Location	*n*	ML (mm)	BW (g)	Stage	Aged *n*	*n*	ML (mm)	BW (g)	Stage	Aged *n*
Japan	55	192 ± 41 (113-295)	183 ± 56 (58-303)	2-4	35	87	247 ± 65 (124-406)	863 ± 592 (110-2977)	2-4	63
Taiwan	105	183 ± 56 (58-303)	478 ± 354 (17-1598)	2-4	81	100	188 ± 69 (65-463)	595 ± 488 (29-2286)	2-4	84

Table 6. Monthly taxa composition of *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan.

Location	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Sum
S. sp. 1											
Japan			1				1				2
Taiwan		1	1		27	17	14	12	44	18	134
S. sp. 2											
Japan	7		13	10	13		26	30	19		118
Taiwan		7	3	1	2	17	8		2	6	46
S. sp. 2A											
Japan	1		2	1	10		3	3	1		21
Taiwan		2	3					3	1		9

Table 7. The genetic distance between three taxa of *Sepioteuthis* spp. in the waters off Japan and Taiwan.

Distance	JP_ sp. 2	JP_sp. 2A	TW_ sp. 1	TW_ sp. 2	TW_ sp. 2A
JP_sp. 2					
JP_sp. 2A	0.0127				
TW_sp. 1	0.1289	0.1365			
TW_ sp. 2	0.0032	0.0159	0.1221		
TW_sp. 2A	0.0293	0.0204	0.1397	0.0070	

Table 8. Summary of mtDNA variability and diversity for *Sepioteuthis* sp.1, *Sepioteuthis* sp.2and *Sepioteuthis* sp. 2A in the waters off Japan and Taiwan.

Parameters	JP_ sp. 2	JP_sp. 2A	TW_ sp. 1	TW_ sp. 2	TW_ sp. 2A
Haplotype diversity (*h*)	0.6505	0.8000	0.572	0.383	0.821
Nucleotide diversity (π)	0.0076	0.0047	0.00151	0.00069	0.00240
No. of individuals	117	16	97	51	8
No. of haplotypes	21	8	18	8	4
Tajimas' D	-2.2449**	-1.9717*	-2.0522*	-1.92336*	-0.22175
Fu's fs	-6.9818*	-2.4589*	-2.63948*	-2.85358*	-0.20541

Table 9. Results of principal components analysis for life-history traits of *Sepioteuthis* spp. in the waters off northwestern Pacific. (BW: body weight; ML: mantle length; NI: number of increments; GR: average growth rate; GSI: Gonadsomatic index)

Principal components	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.834	1.305	0.722	0.131	0.008
% of value	56.674	26.104	14.499	2.620	0.153
Cumulative %	56.674	82.778	97.227	99.847	100.00
BW	0.957	0.010	0.003	-0.290	0.016
ML	0.992	0.062	0.002	0.087	-.0.67
NI	0.418	0.757	-0.492	0.092	0.028
GR	0.865	-0.351	0.310	0.175	0.046
GSI	-0.108	0.777	0.620	-0.014	-0.001

Table 10. Results of principal components analysis for statolith shape of *Sepioteuthis* spp. in the northwestern Pacific. (76 Fourier coefficients)

Principal components	PC1	PC2	PC3	PC4	PC5
Eigenvalue	31.13	22.52	17.84	2.77	1.73
% of value	40.97	29.63	23.48	3.65	2.28
Cumulative %	40.97	70.6	94.01	97.72	100

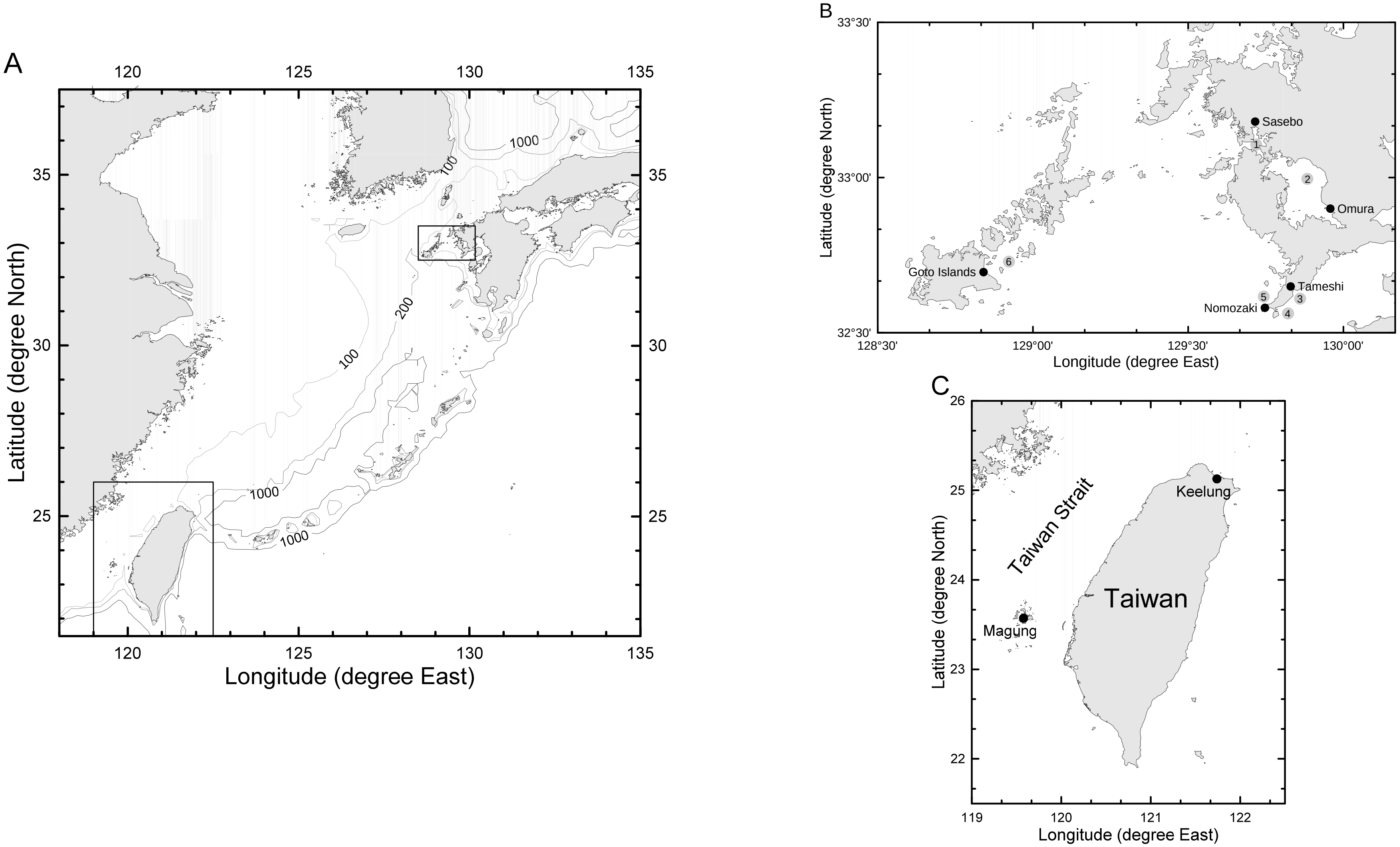

Figure 1. Map showing six locations of the *Sepioteuthis* spp. samples collected off southwestern Japan and off northern Taiwan. (A, northwestern Pacific; B, sampling locations in Japan; C. sampling locations in Taiwan)

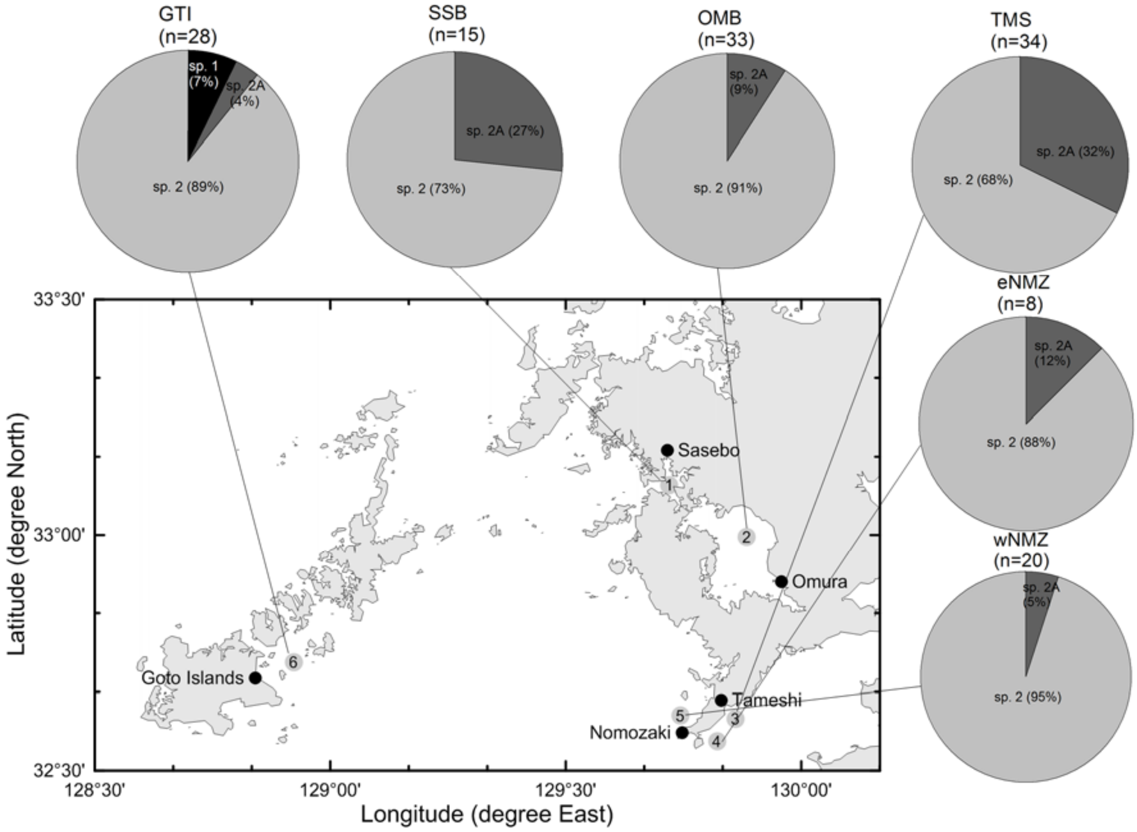

Figure 2. Taxa composition of *Sepioteuthis* spp. in six locations in southwestern Japan (SSB, Sasebo; OMB, Omura Bay; TMS, Tameshi; eNMZ, eastern Nomozaki; wNMZ, western Nomozaki; GTI, Goto Island).

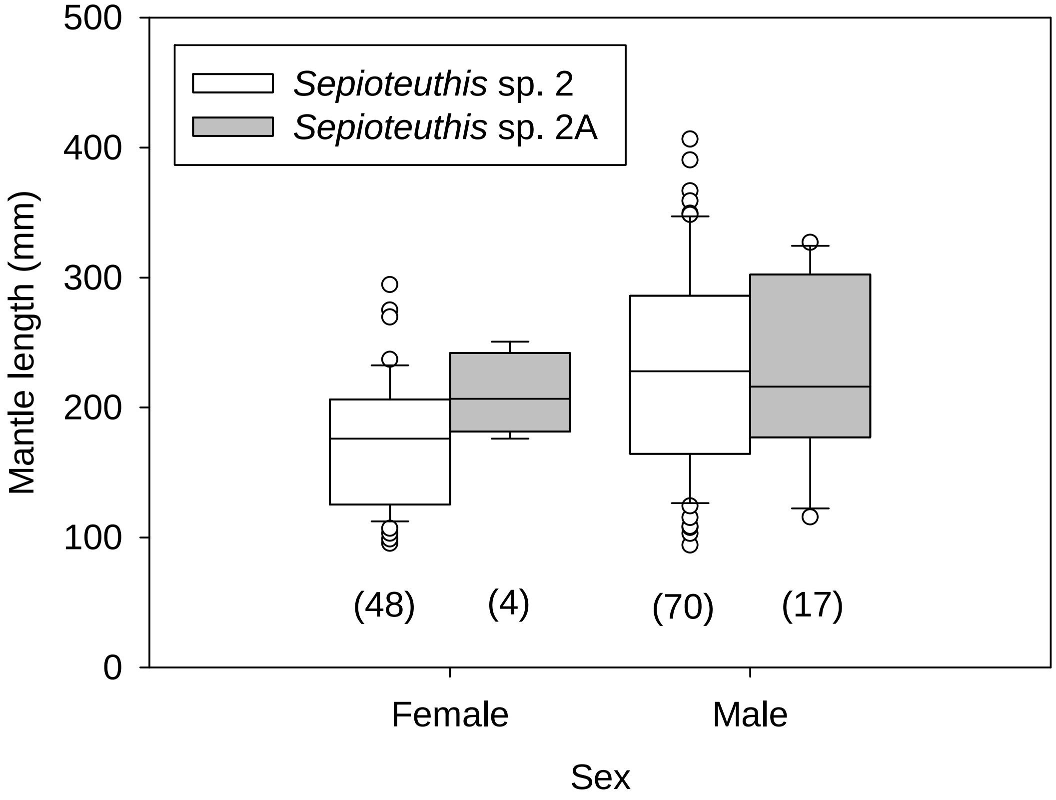

Figure 3. Box plot of mantle length for female and male *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan.

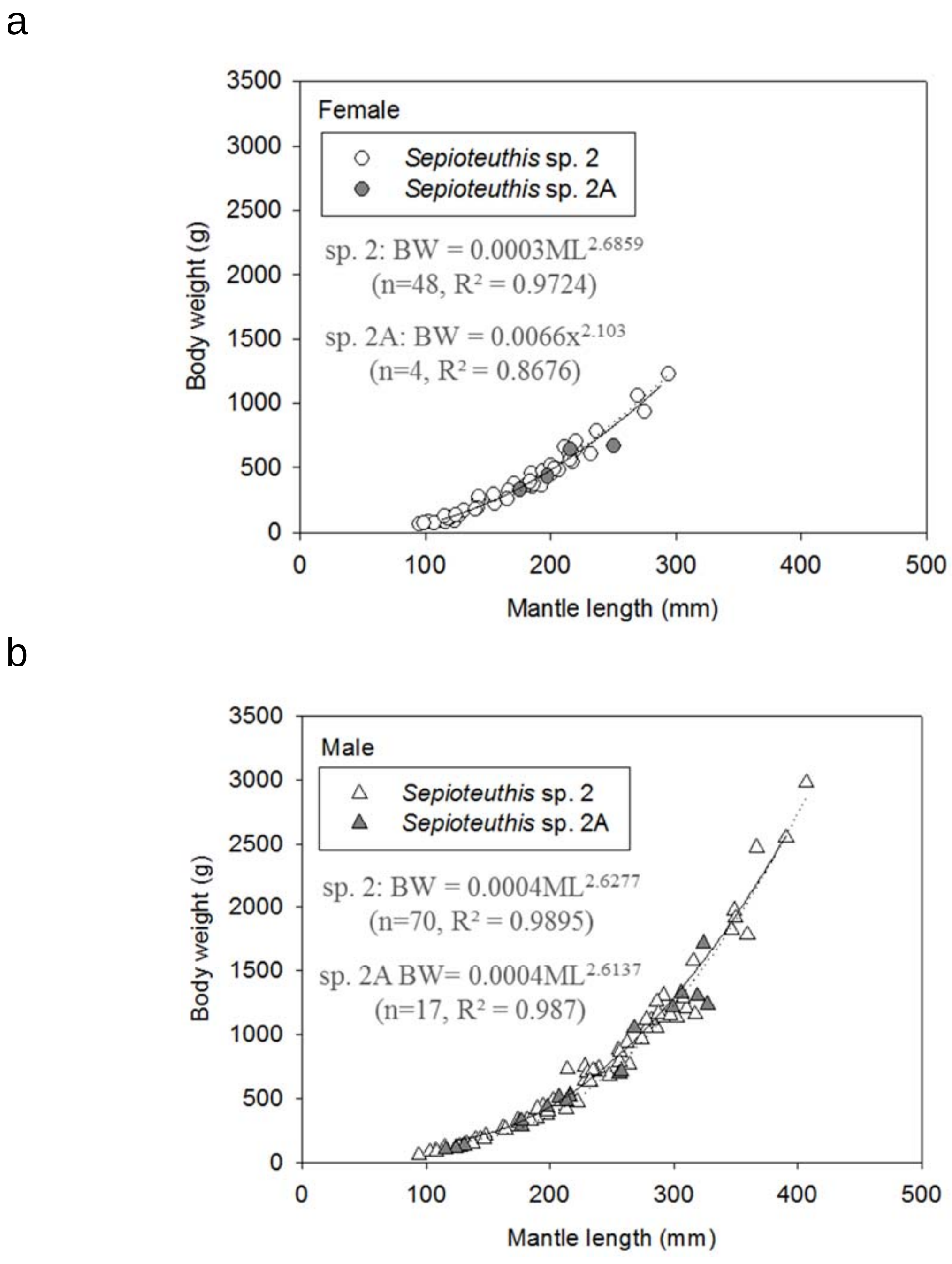

Figure 4. Relationships between mantle length and body weight for female and male *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan (a, females; b, males).

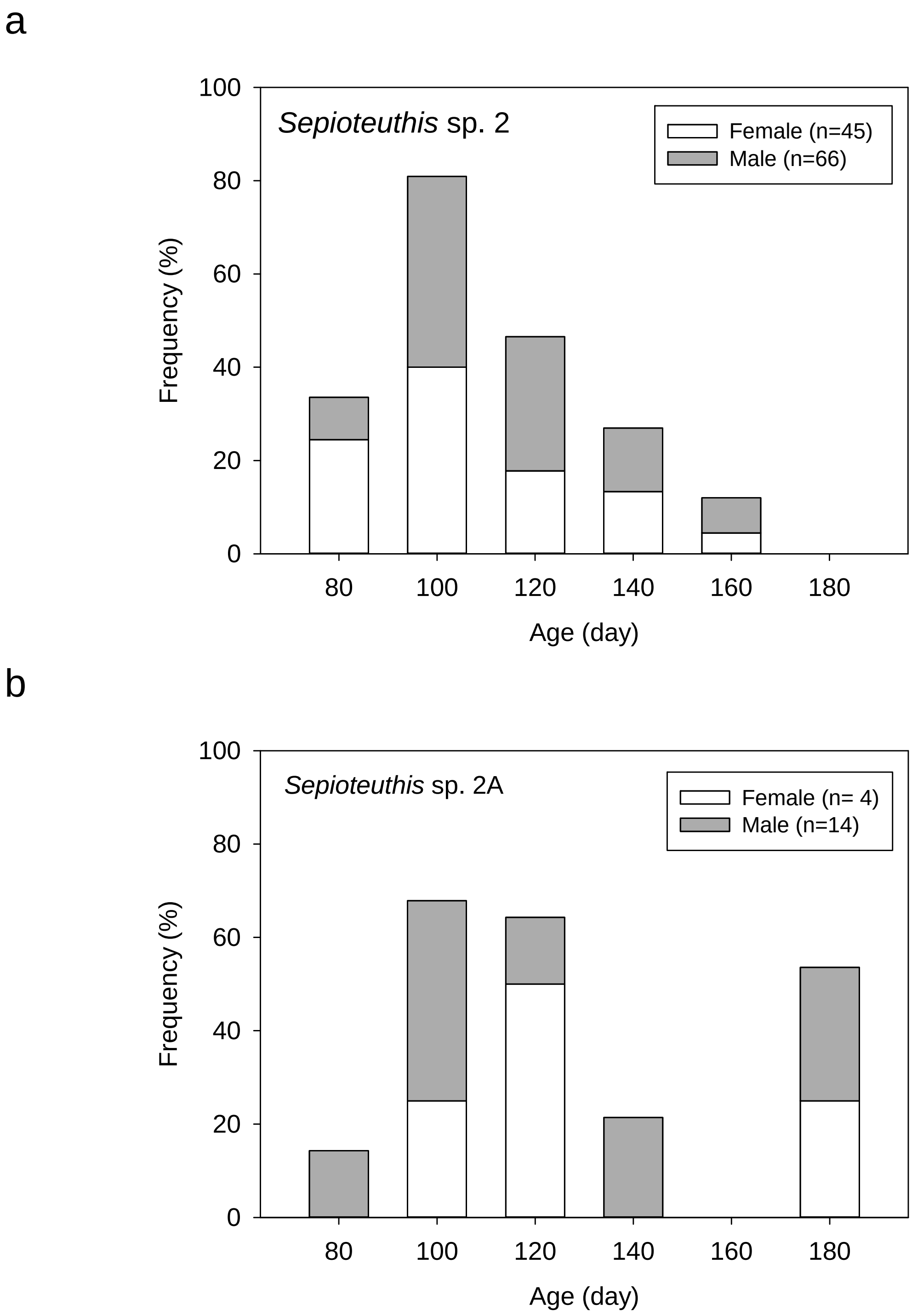

Figure 5. Frequency distribution of daily increments (age) of (a) *Sepioteuthis* sp. 2 and (b) *Sepioteuthis* sp. 2A in the waters off southwestern Japan.

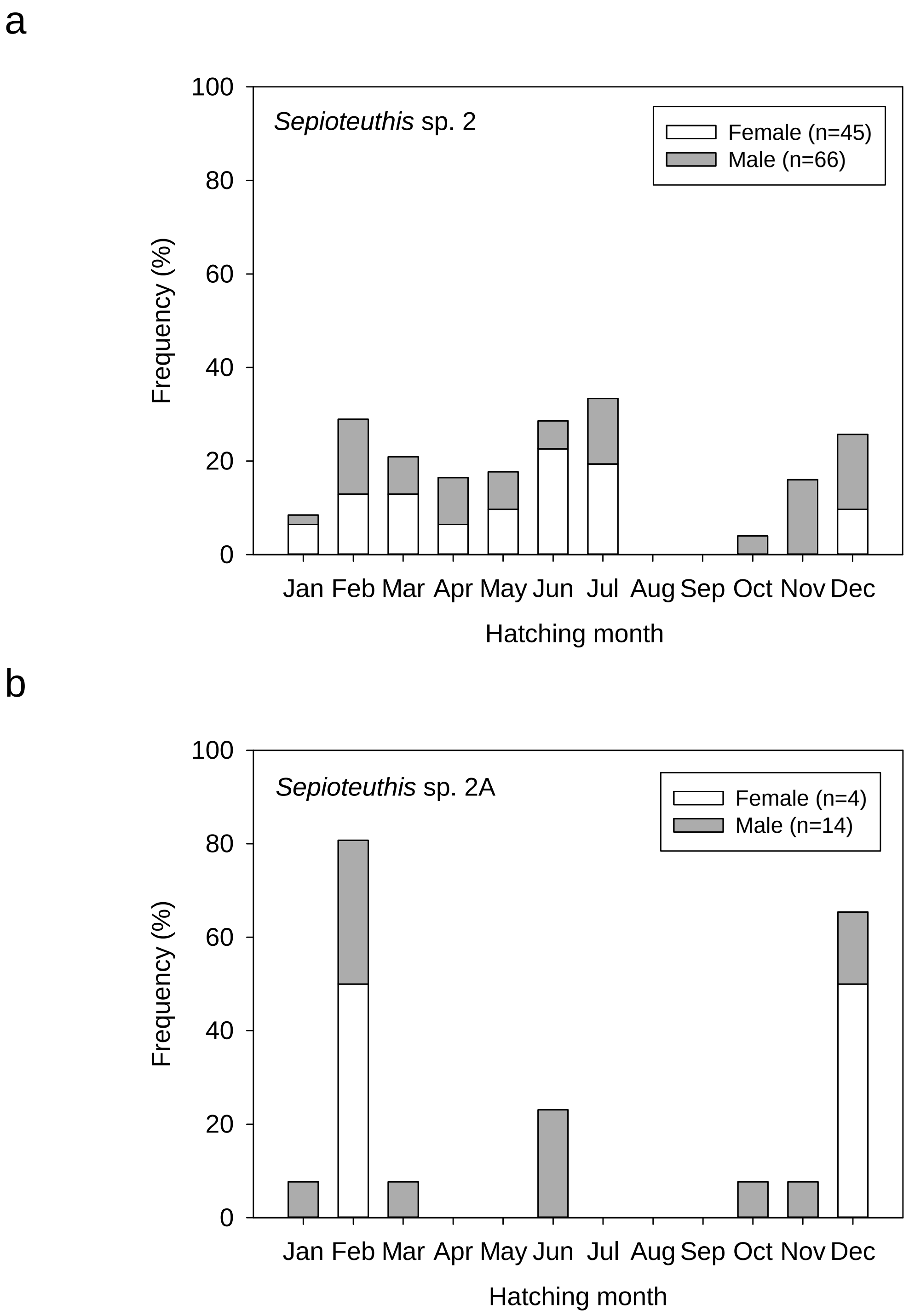

Figure 6. Frequency distribution of back-calculated hatching months of (a) *Sepioteuthis* sp. 2 and (b) *Sepioteuthis* sp. 2A in the waters off southwestern Japan.

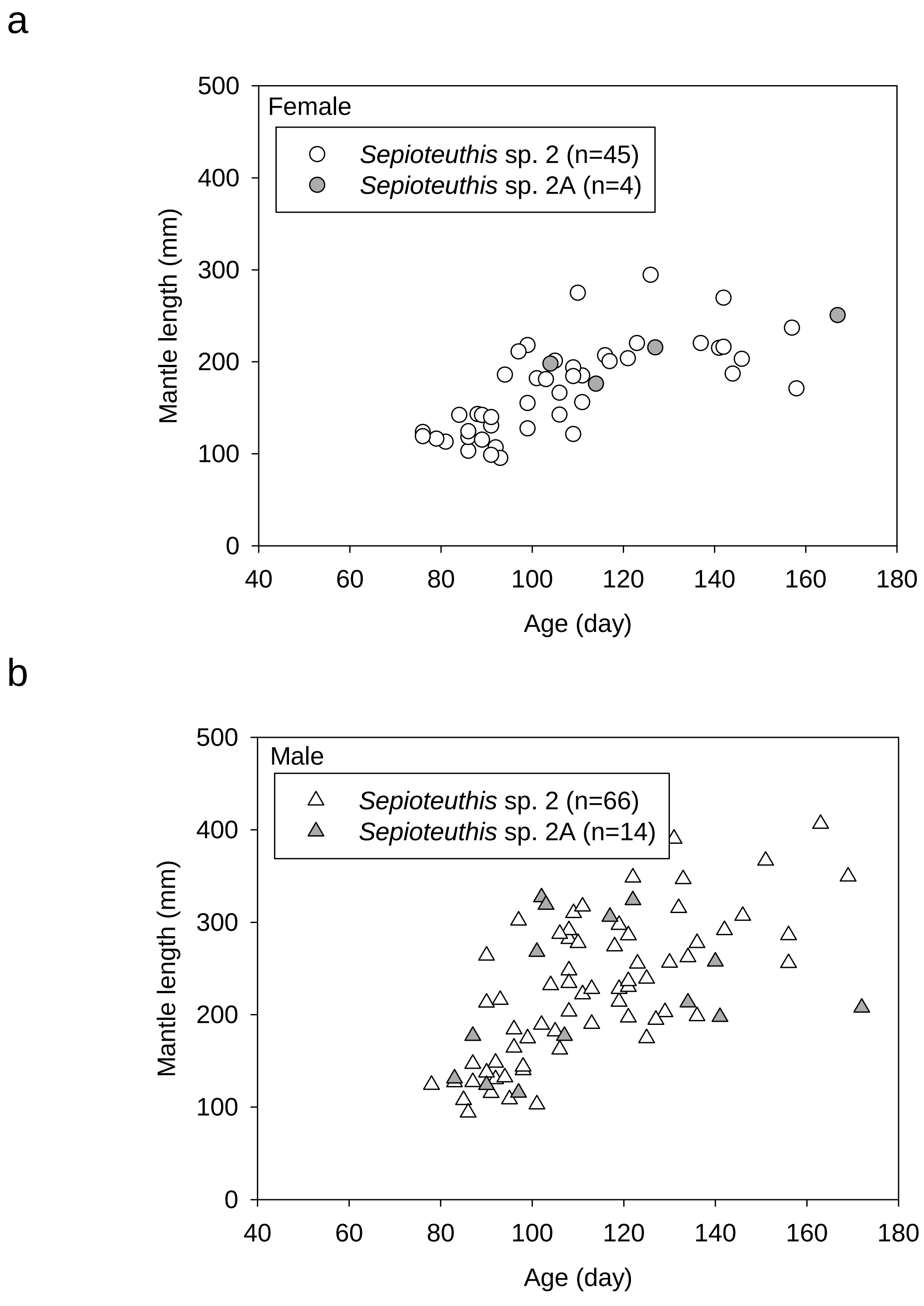

Figure 7. Relationship between mantle length and age of *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan (a, females; b, males).

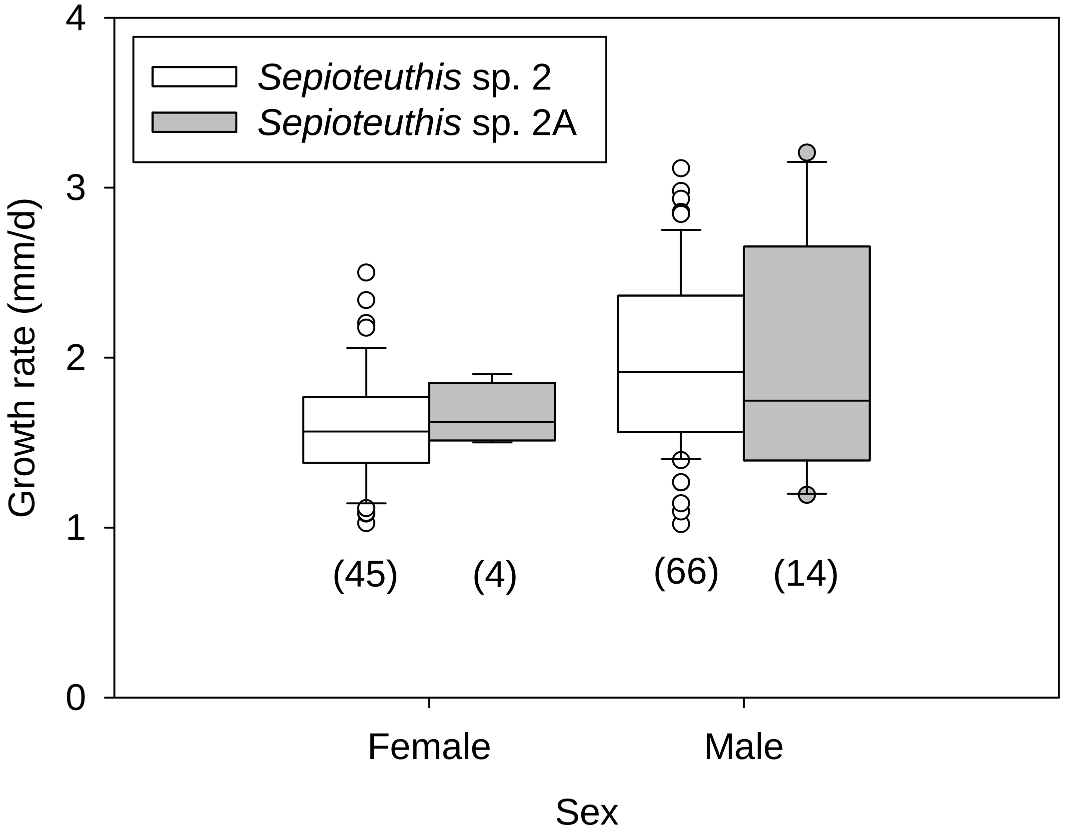

Figure 8. Growth rate box plot shown for female and male *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan.

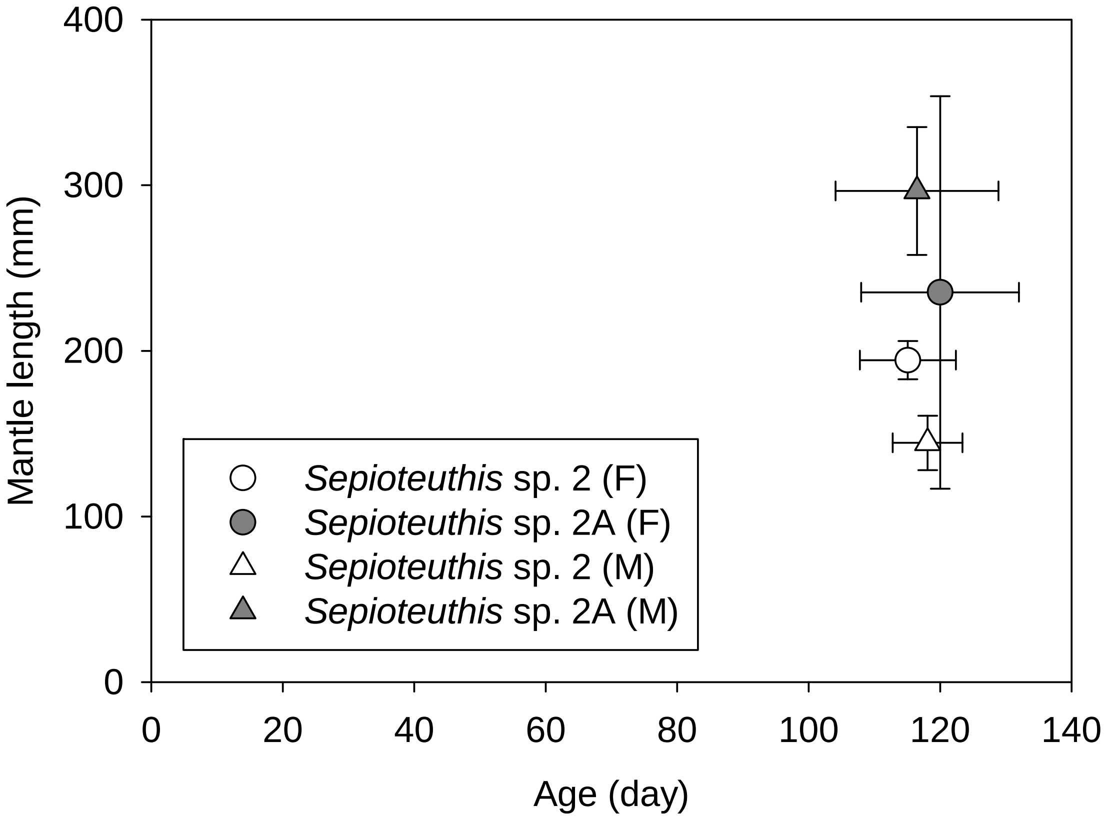

Figure 9. Mantle length and age (average and standard deviation) of mature individuals of *Sepioteuthis* sp. 2 and *Sepioteuthis* sp. 2A in the waters off southwestern Japan (circle, females; triangle, males).

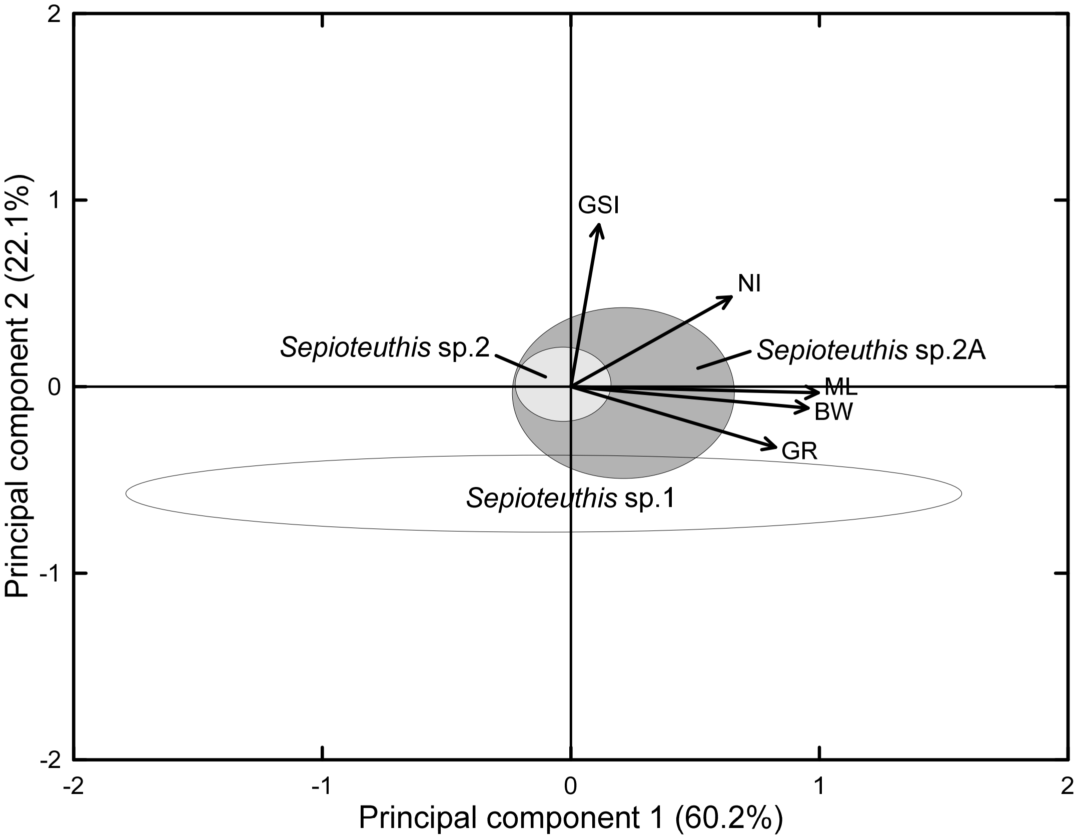

Figure 10. Scores (in average ± standard error by taxa) and loadings in two principal components from the principal component analysis for *Sepioteuthis* sp. 1 (open ellipse), *Sepioteuthis* sp. 2 (light gray ellipse), and *Sepioteuthis* sp. 2A (dark gray ellipse) in the waters off southwestern Japan based on five biological measurements (BW, body weight; ML, mantle length; NI, number of increments; GR, average growth rate; GSI, gonadosomatic index).

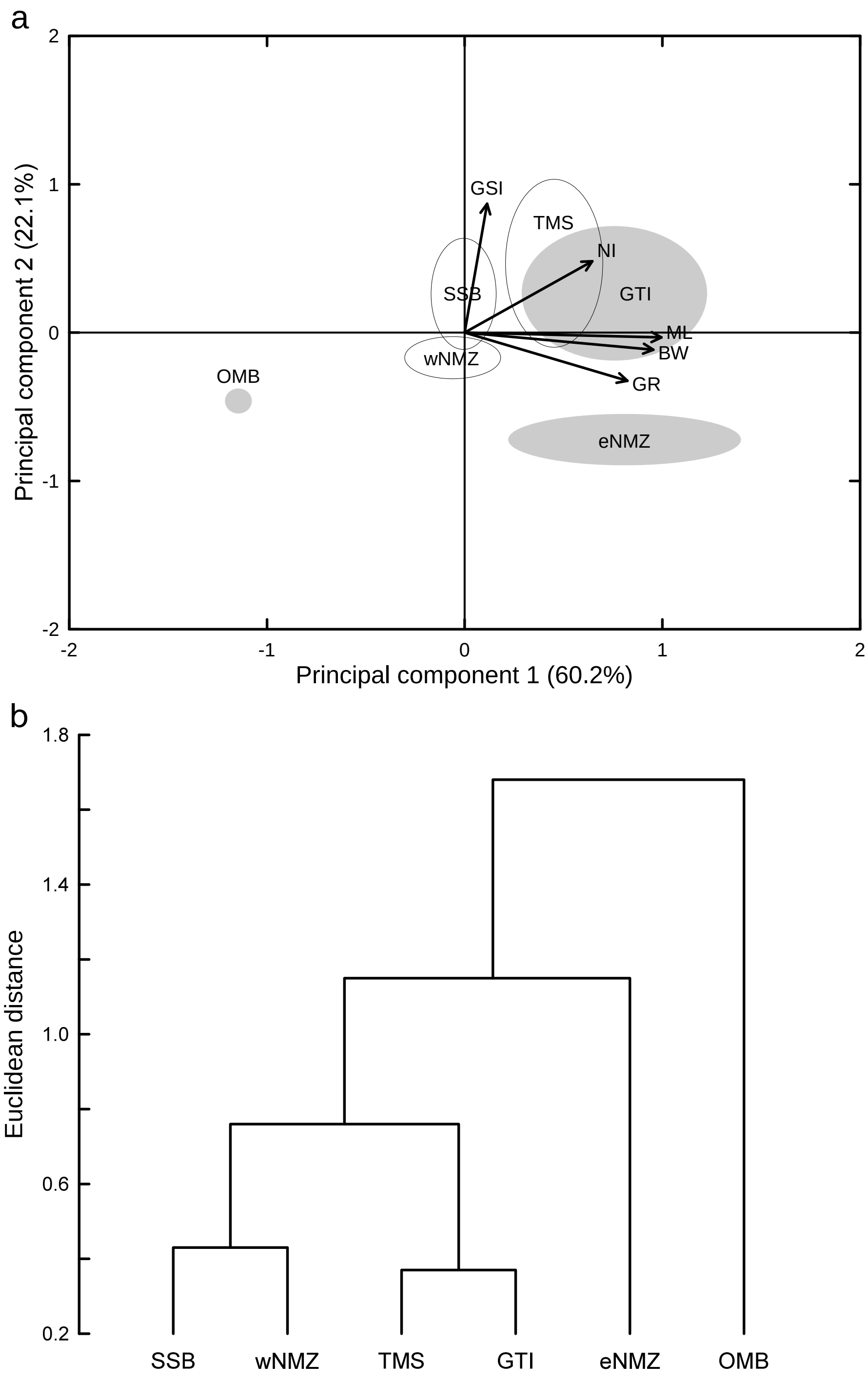

Figure 11. (a) Scores (in average ± standard error by taxa) and loadings in two principal components from the principal component analysis for *Sepioteuthis* spp. in six locations; and (b) similarity for *Sepioteuthis* spp. in six locations in the waters off southwestern Japan based on five biological measurements (BW, body weight; ML, mantle length; NI, number of increments; GR, average growth rate; GSI, gonadosomatic index).

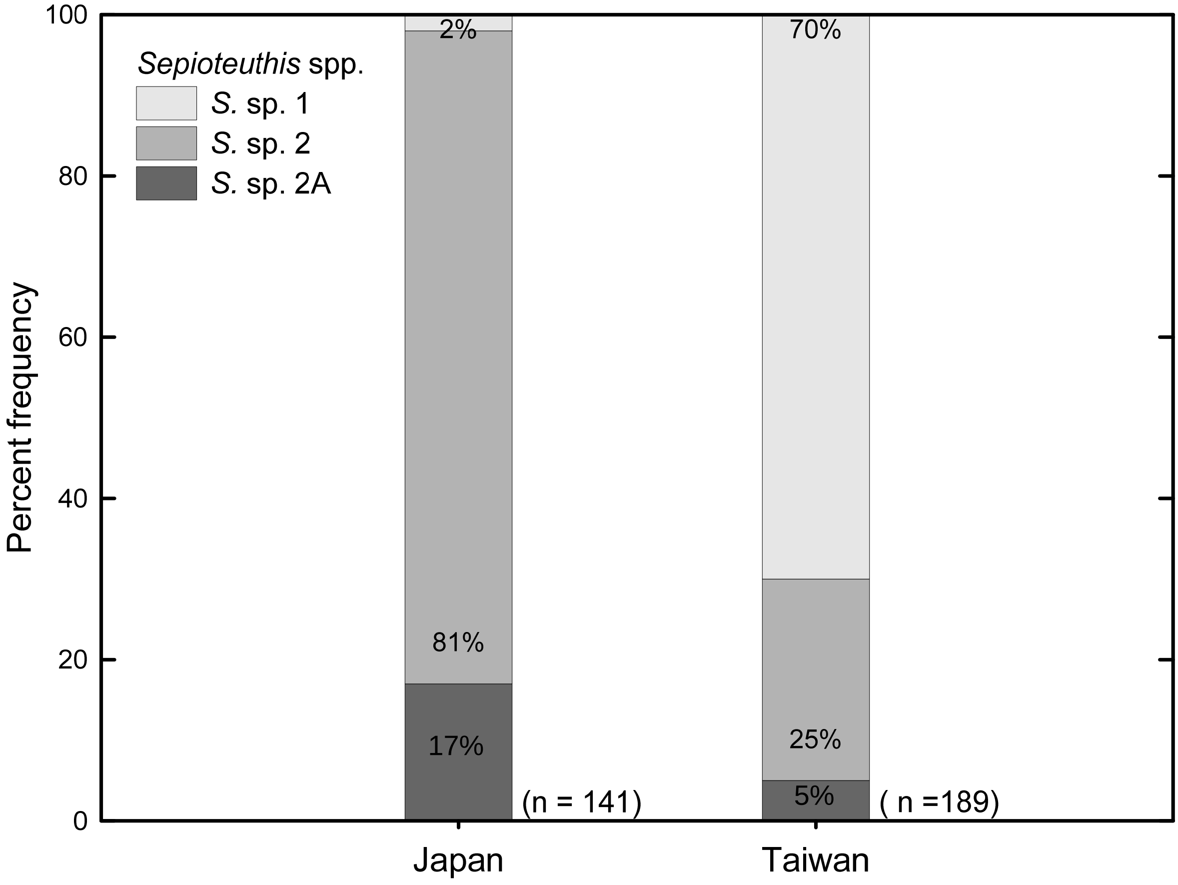

Figure 12. Taxa composition of *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

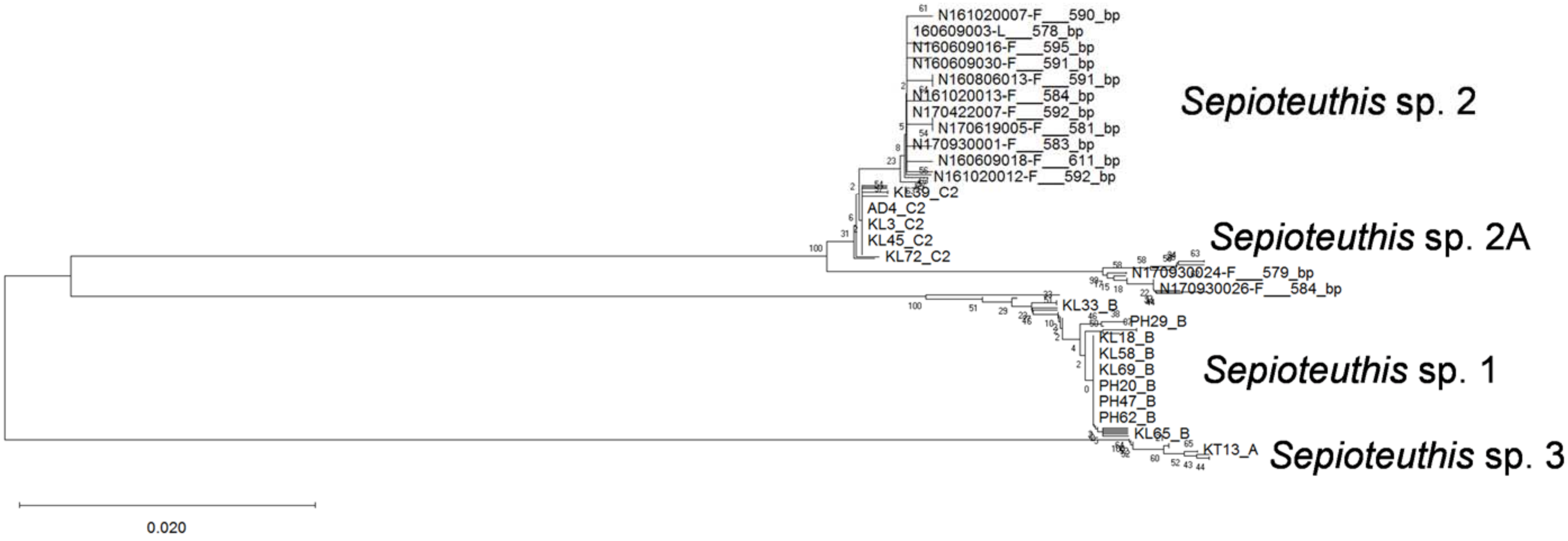

Figure 13. The neighbor joining tree of three taxa (*Sepioteuthis* sp. 1, sp. 2 and sp. 2A) in Japan and Taiwan, with the sp. 3 as the outer group.

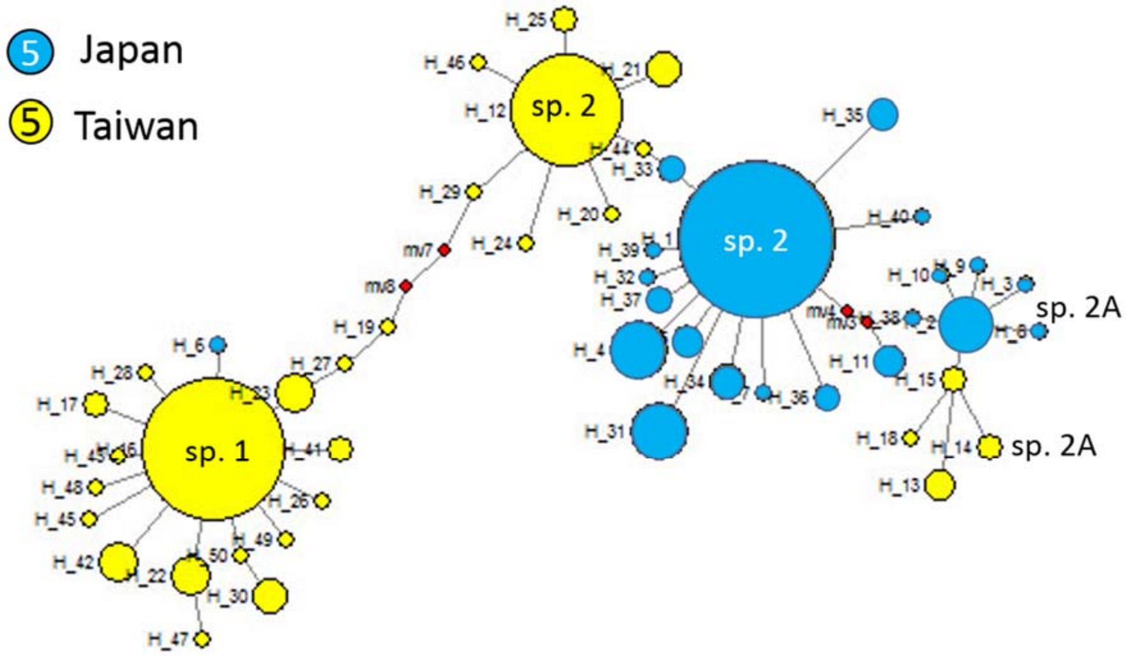

Figure 14. The haplotype network of three taxa (*Sepioteuthis* sp. 1, sp. 2 and sp. 2A) in Japan and Taiwan.

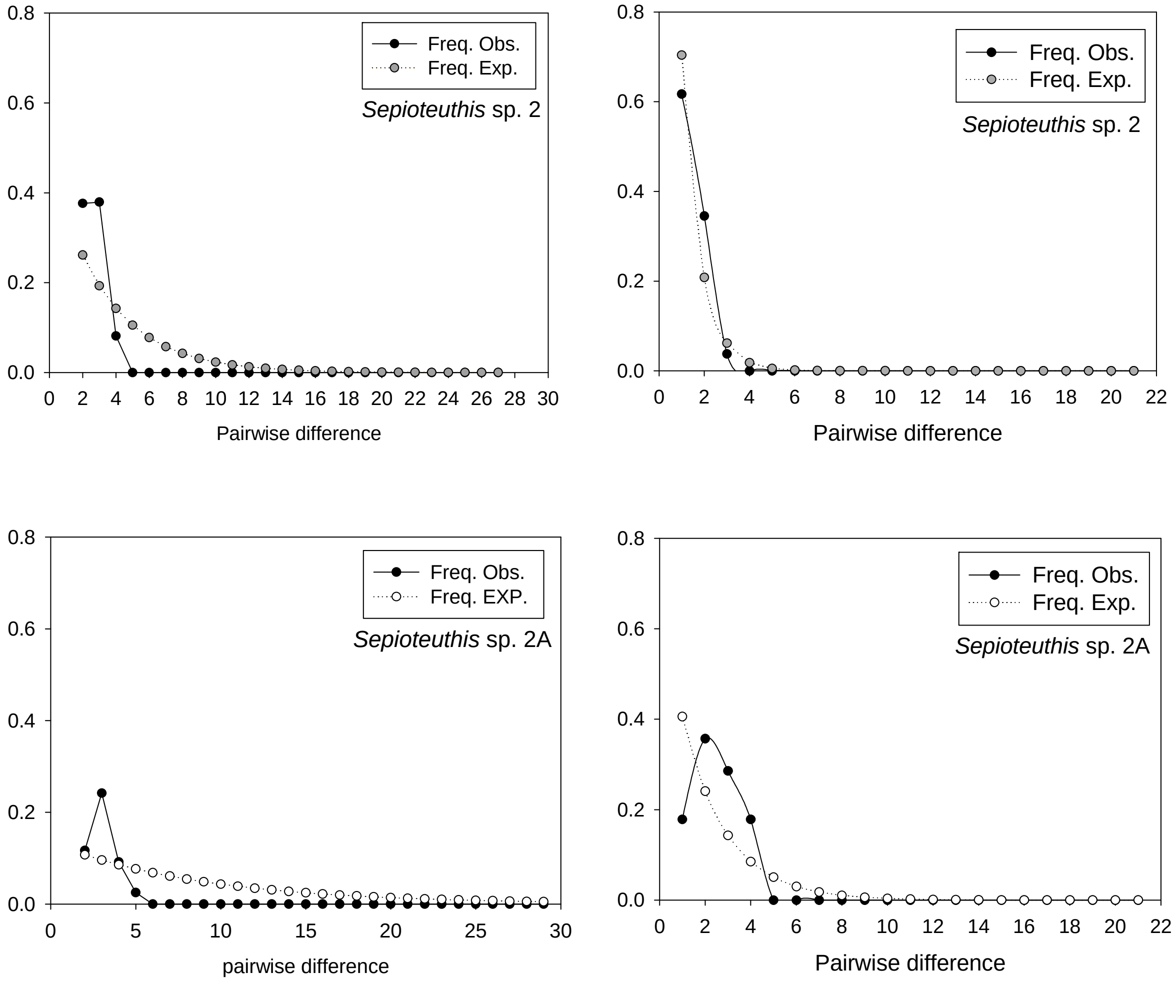

Figure 15. The mismatch distrbution of two taxa (*Sepioteuthis* sp. 2 and sp. 2A) in Japan and Taiwan.

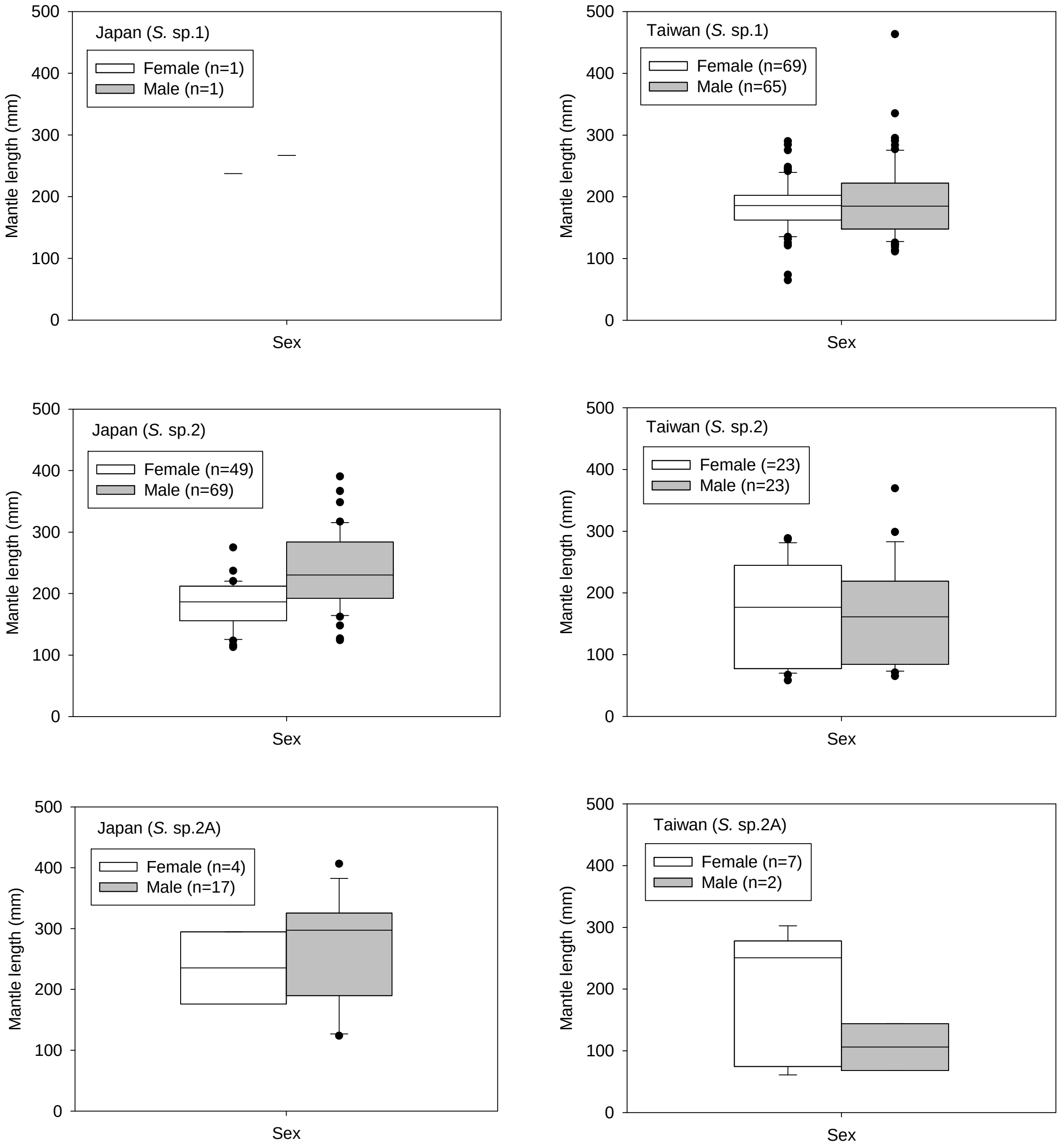

Figure 16. Box plot of mantle length for female and male *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

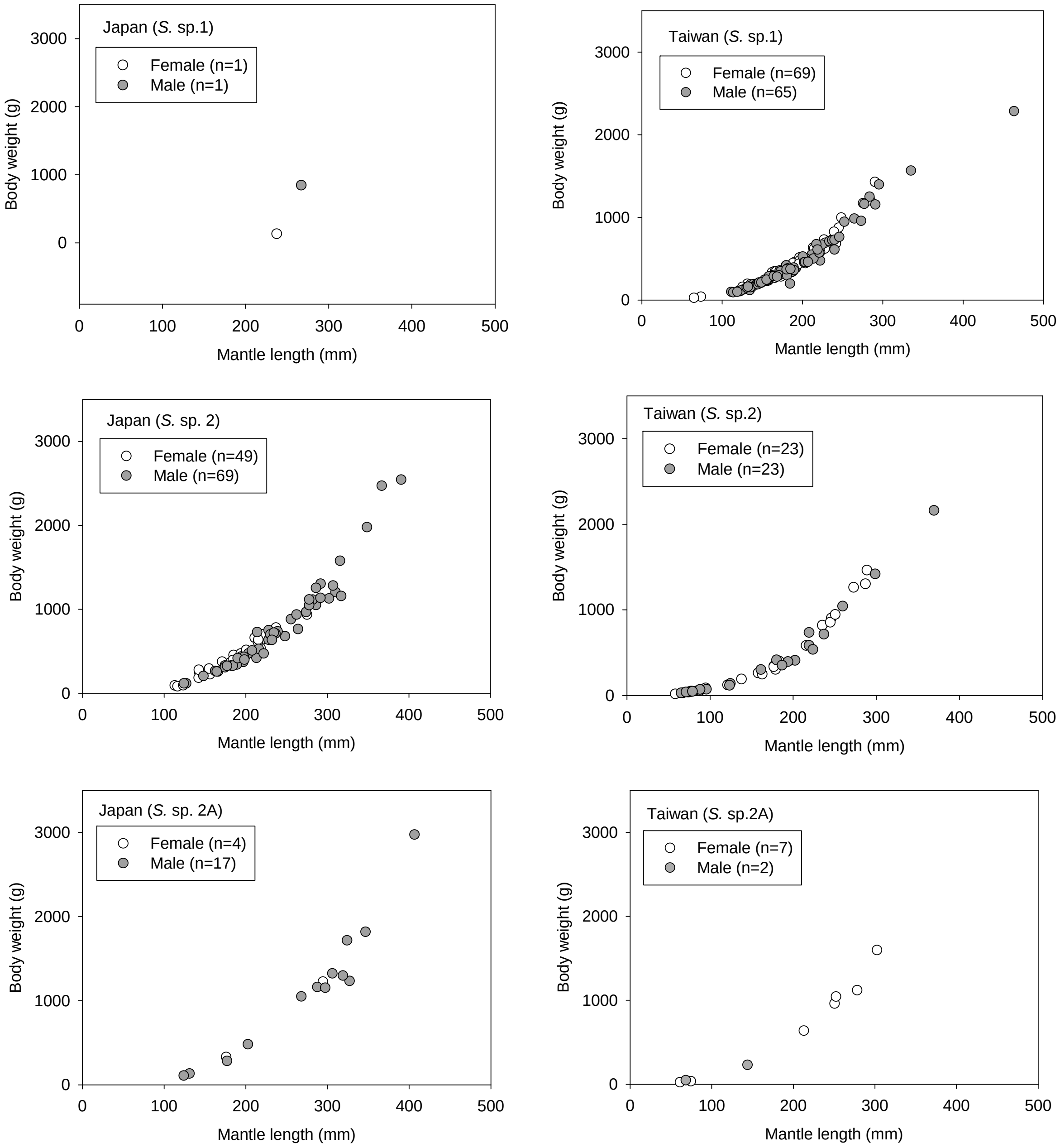

Figure 17. Relationships between mantle length and body weight for female and male *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

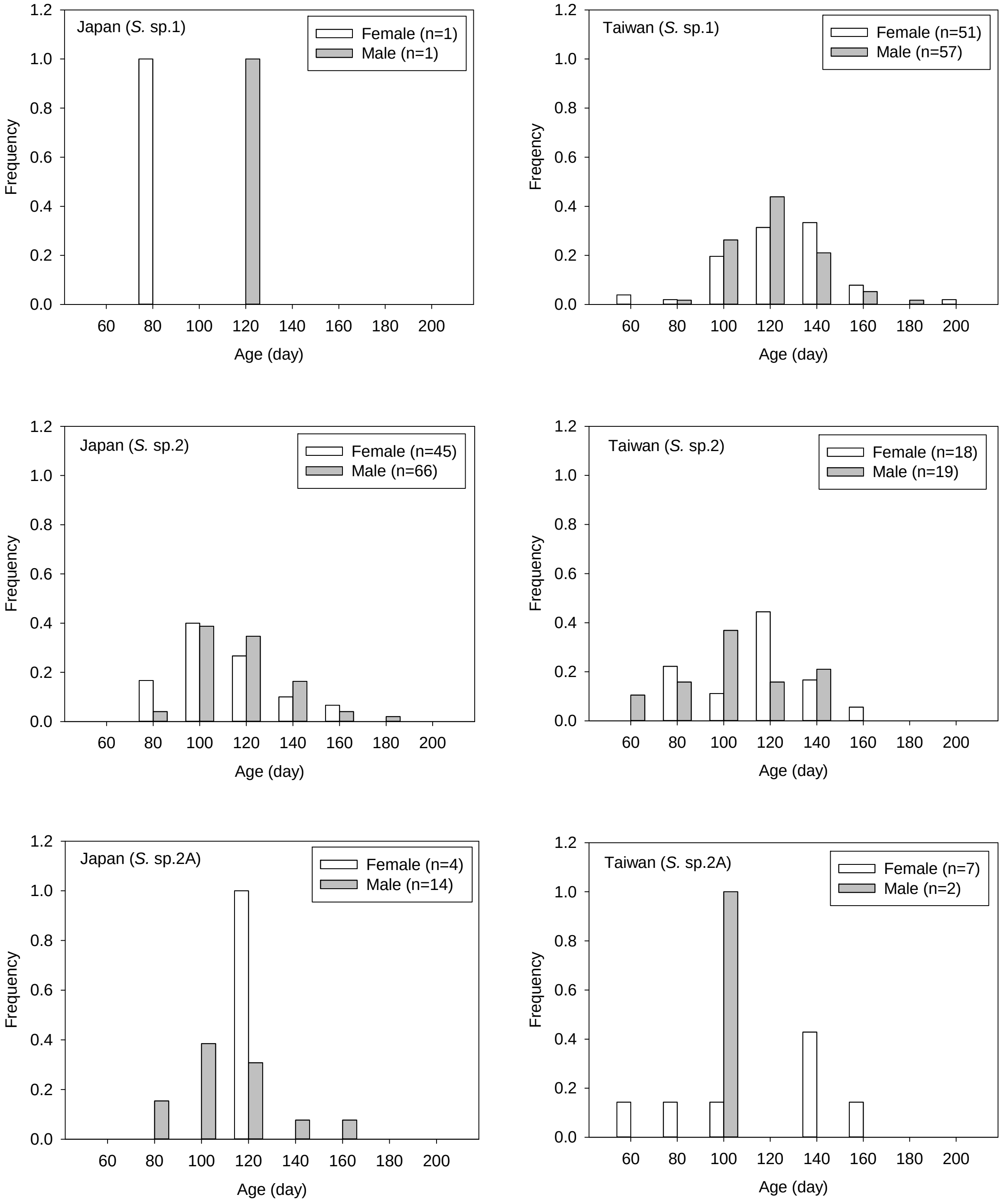

Figure 18. Frequency distribution of daily increments (age) for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

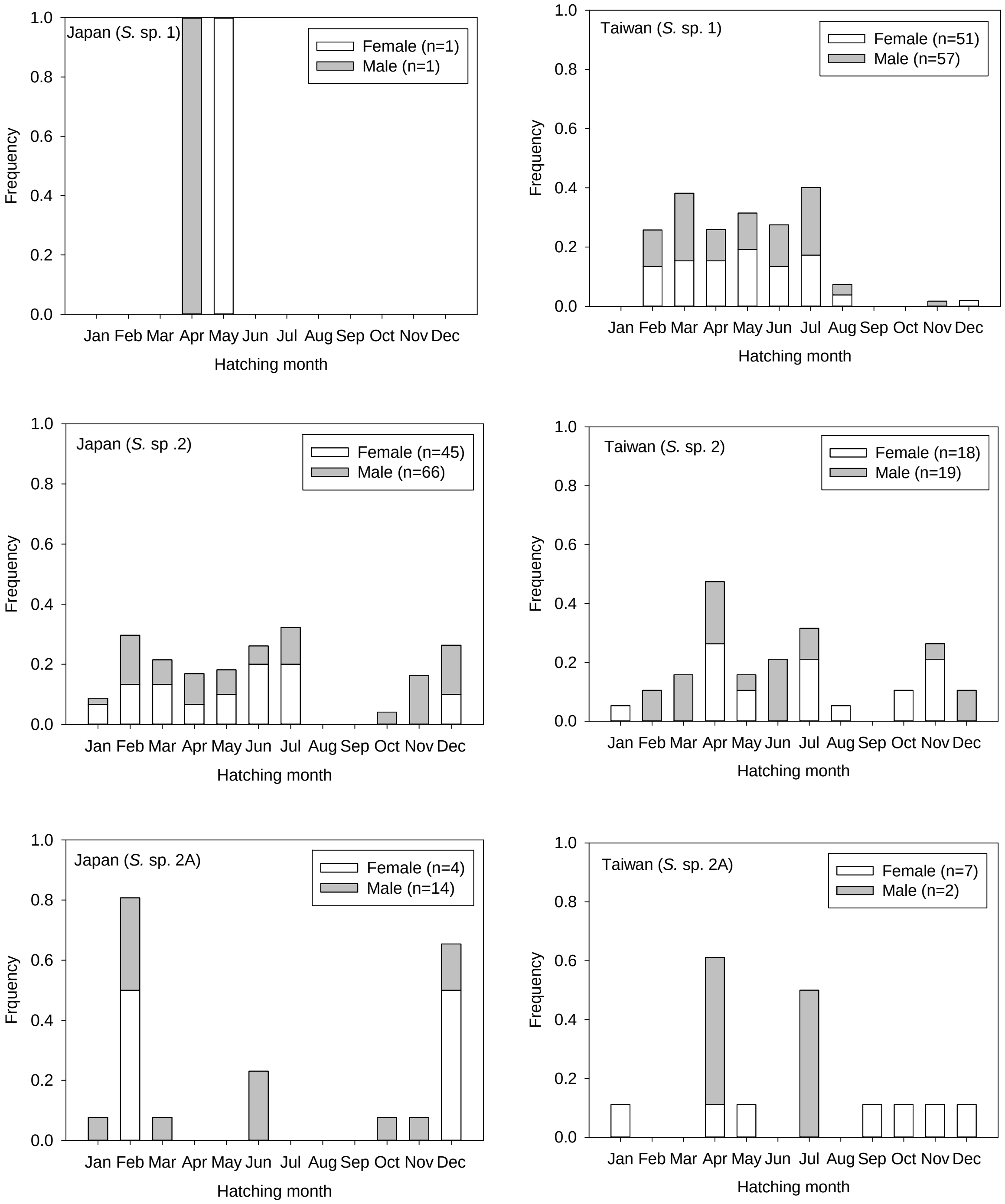

Figure 19. Frequency distribution of back-calculated hatching months for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

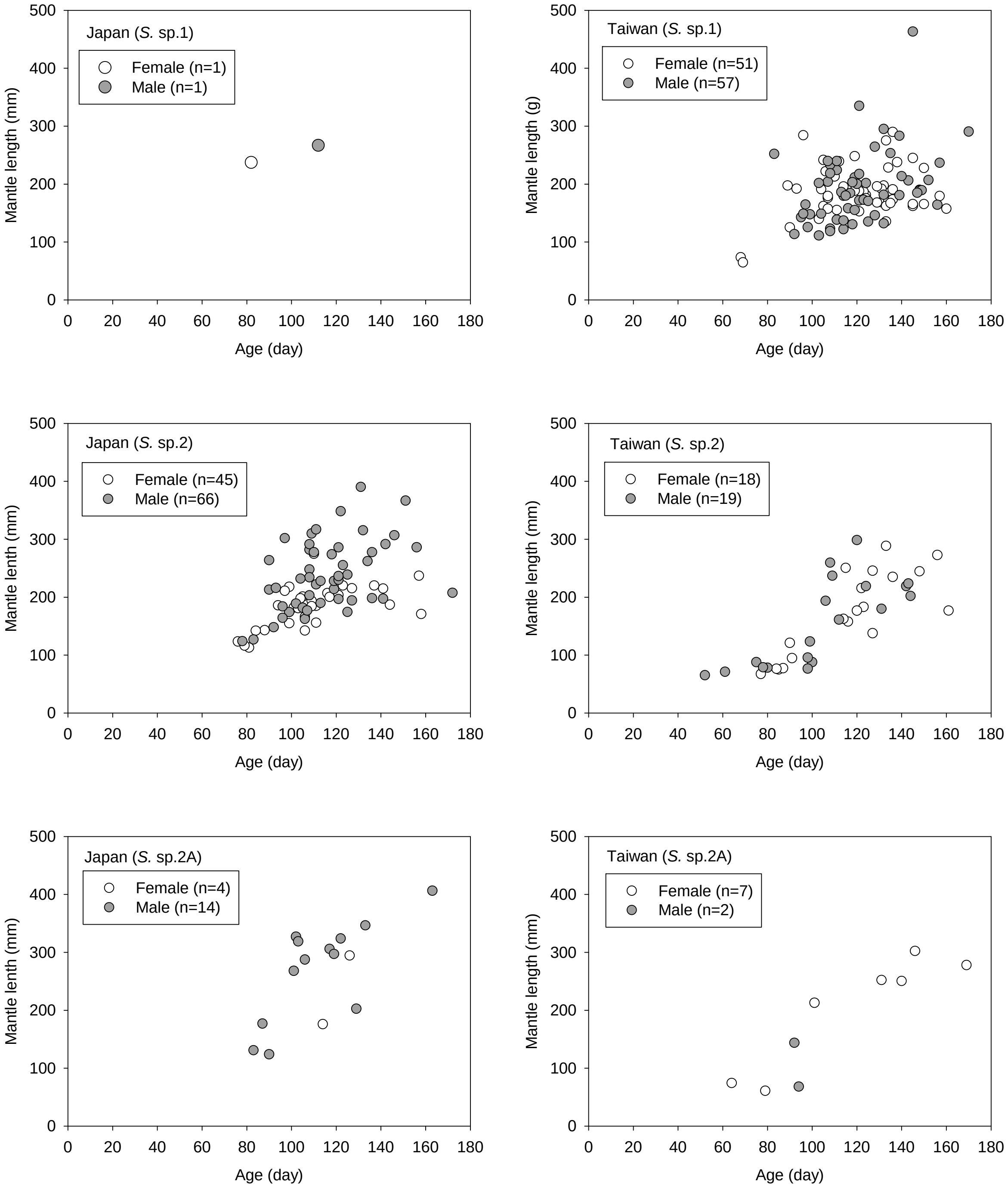

Figure 20. Relationship between mantle length and age (average and standard deviation) for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific; circle, females; triangle, males).

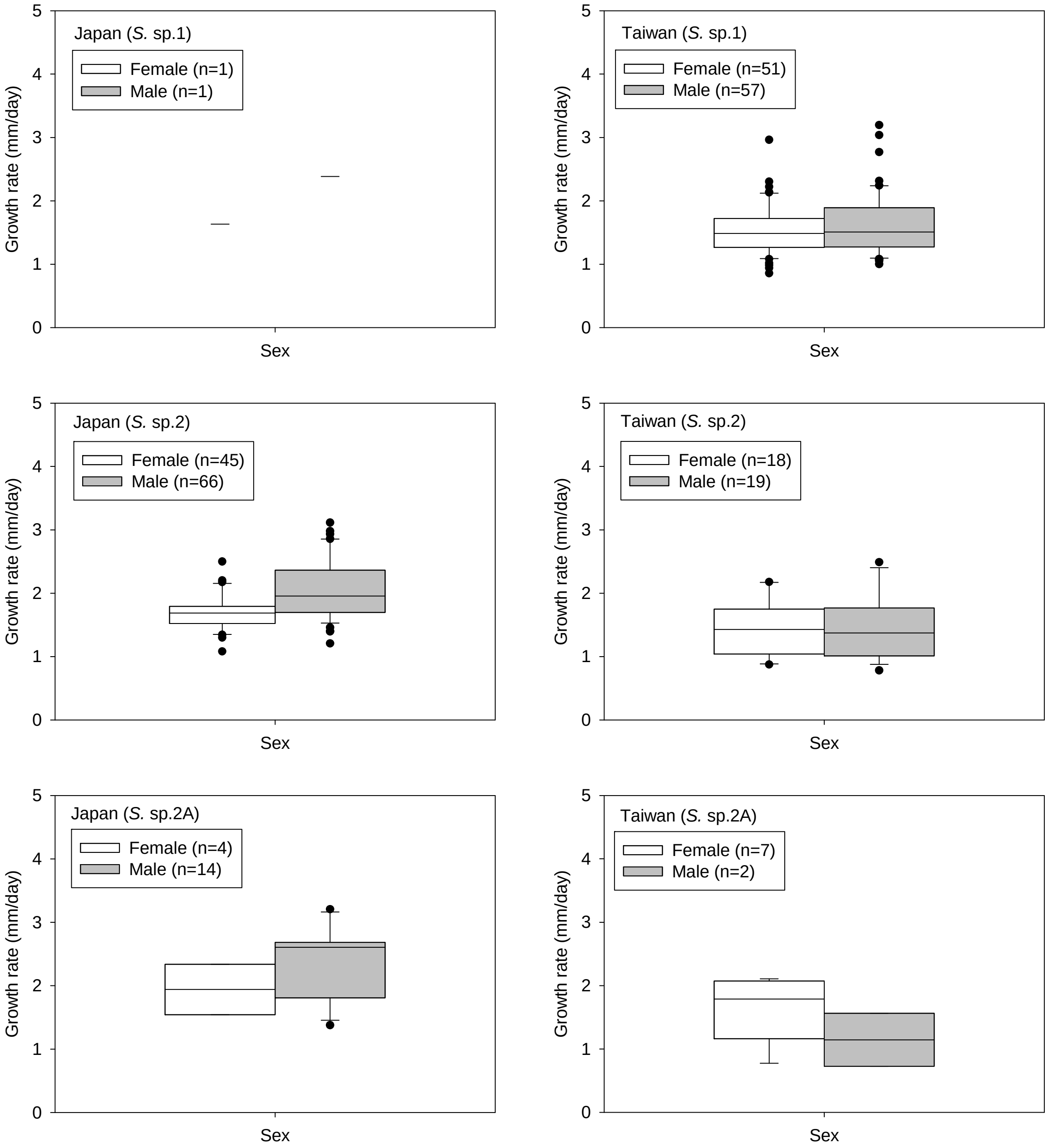

Figure 21. Box plot of growth rate for female and male for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific).

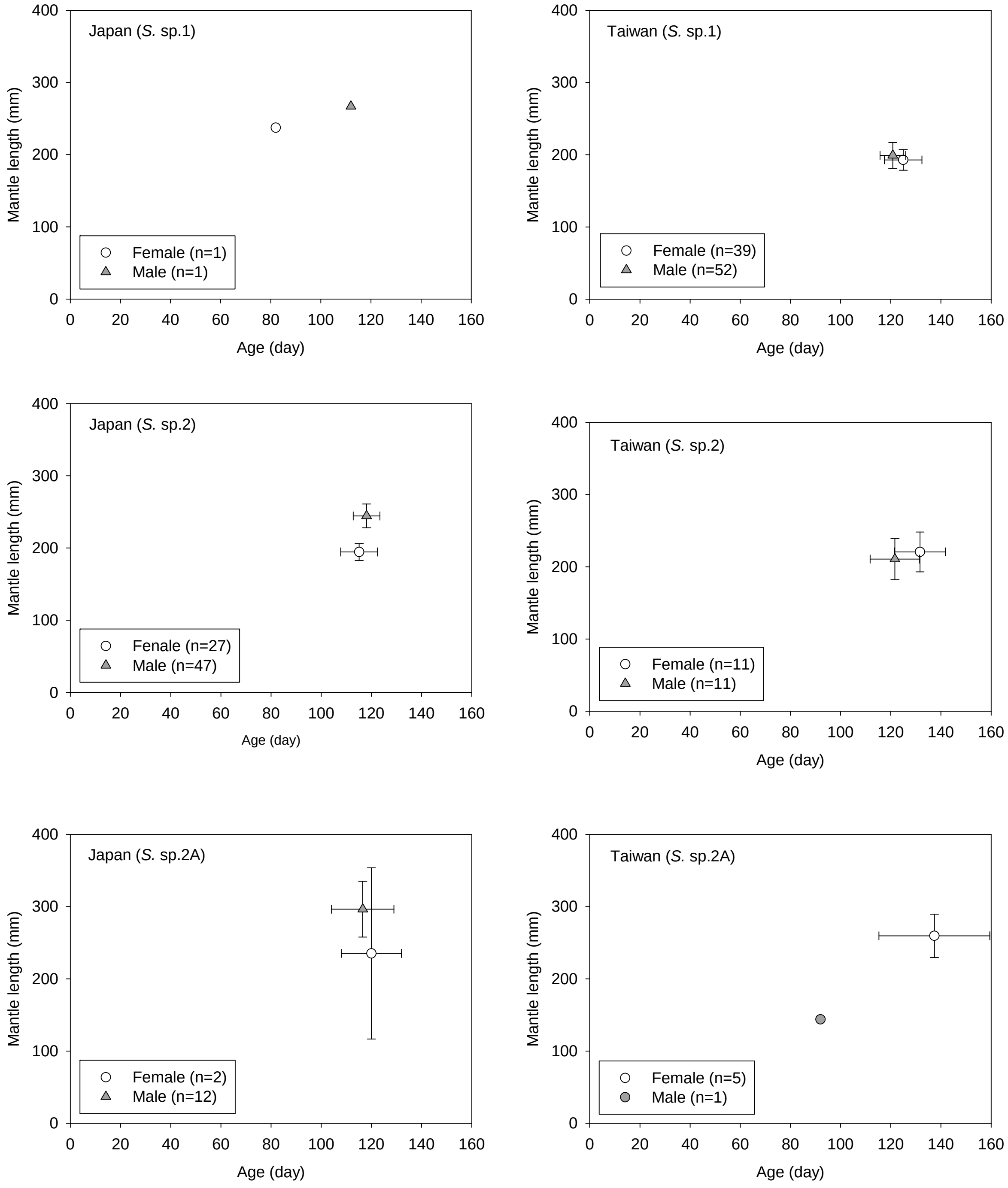

Figure 22. Mantle length and age (average and standard deviation) of mature *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan (northwestern Pacific; circle, females; triangle, males).

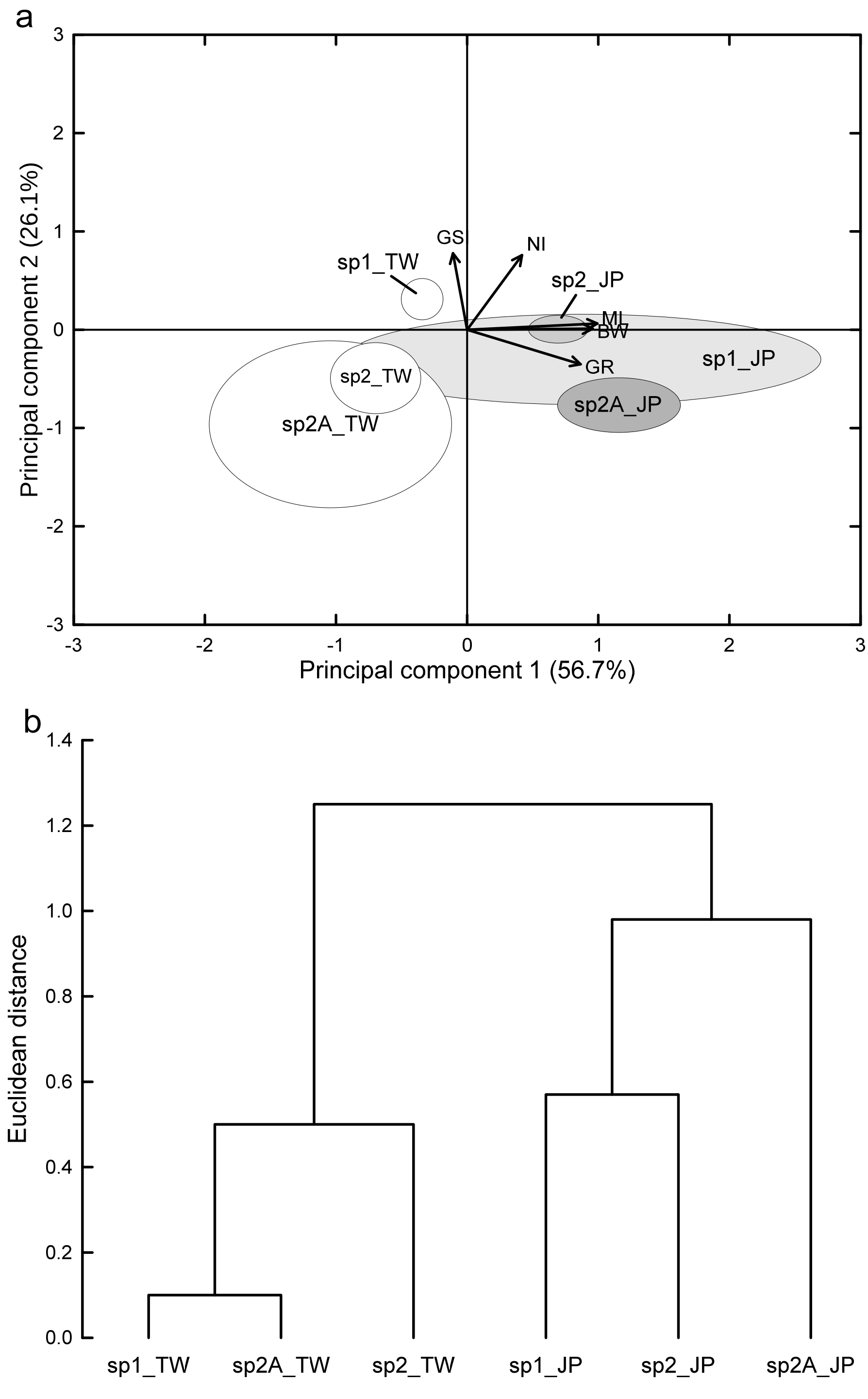

Figure 23. (a) Scores (in average ± standard error by taxa) and loadings in two principal components from the principal component analysis; and (b) similarity for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan based on five biological measurements (BW, body weight; ML, mantle length; NI, number of increments; GR, average growth rate; GSI, gonadosomatic index).

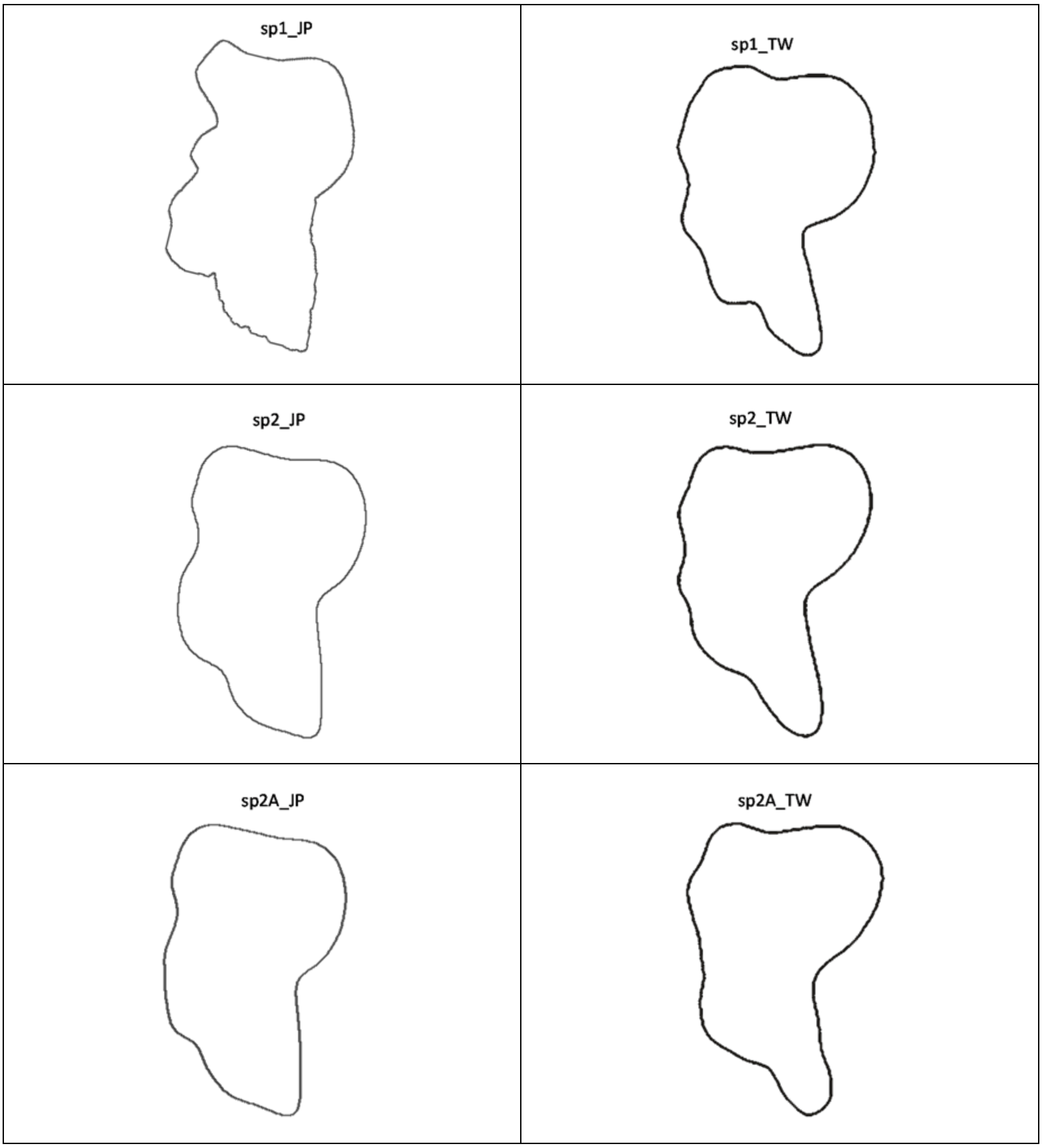

Figure 24. Morphology of statolith shape for *Sepioteuthis* spp. in the waters off southern Japan (JP) and off northern Taiwan (TW).

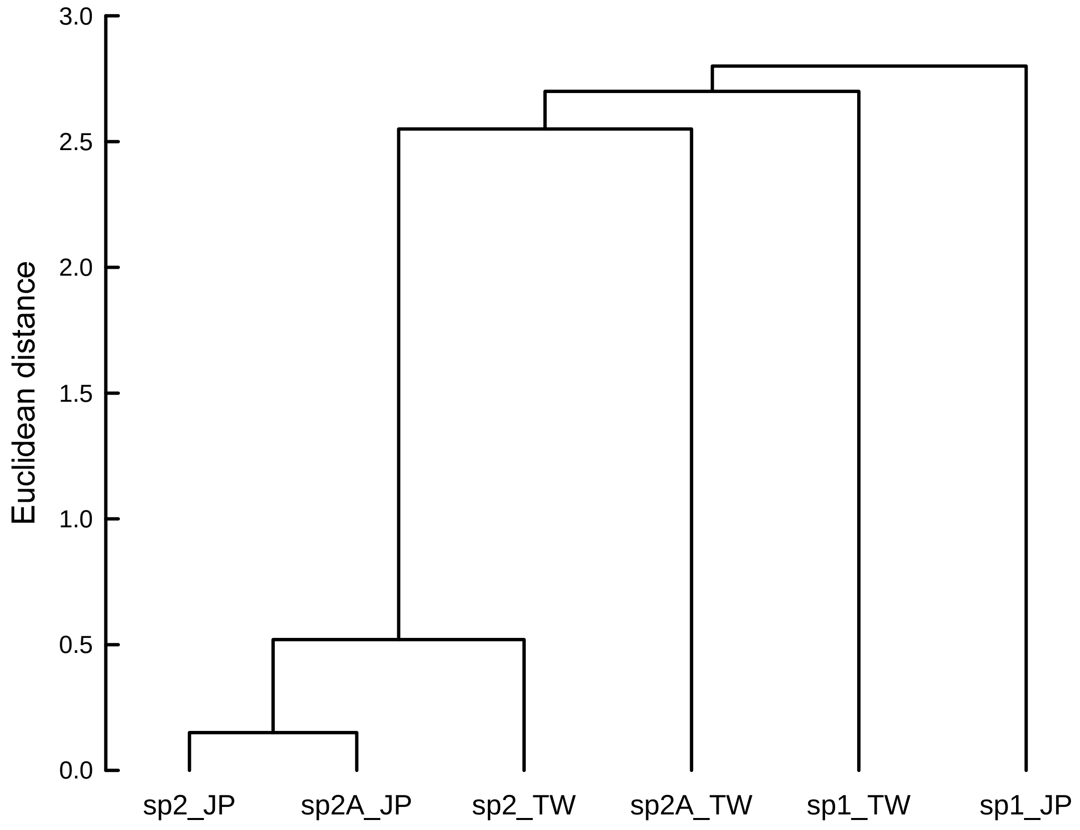

Figure 25. Similarity for *Sepioteuthis* spp. in the waters off southern Japan and off northern Taiwan based on four Fourier coefficients of 19 harmonics.

Annex

Annex1. PCR primers used to determine nucleotide sequences in this study.

Taxa	Gene	Primer	Nucleotide sequence
Sepioteuthis sp. 1 (Lineage B)	COI	BF	ATTGGGGGTTTTGGTAACTGG
	COI	BR	GATGCTAAAAGGAGTGTGAGG
Sepioteuthis sp. 2 (Lineage C1)	COI	C1F	TTAGTTGGTACCTCACTAAGG
	COI	C1R	CTCTTTCAACTGCTGAGGAC
Sepioteuthis sp. 2A (Lineage C2)	COI	C2F	TTAGTTGGTACCTCACTAAGG
	COI	C2R	GTTGATATAGAATAGGGTCTCCC
Sepioteuthis sp. 3 (Lineage A)	COI	AF	TCTCATGCTGGACCTTCAGTA
	COI	AR	TGCTCCTGCTAAAACAGGAAG

Annex 2. GenBank accession numbers for COI sequences generated in this study.

Sepioteuthis Taxa	Location	ID number	Accession number
Sepioteuthis sp. 1	GTI	SeqID160806006	MW056331
	GTI	SeqID160806019	MW056333
Sepioteuthis sp. 2	TMS	SeqID160609002	MW056322
	TMS	SeqID160609003	MW056323
	TMS	SeqID160609021	MW056326
	SSB	SeqID160806001	MW056330
	GTI	SeqID160806007	MW056332
	SSB	SeqID161020003	MW056336
	eNMZ	SeqID170227001	MW056338
	TMS	SeqIDN160609004	MW056341
	TMS	SeqIDN160609005	MW056342
	TMS	SeqIDN160609006	MW056343
	TMS	SeqIDN160609007	MW056344
	TMS	SeqIDN160609009	MW056345
	TMS	SeqIDN160609011	MW056346
	TMS	SeqIDN160609012	MW056347
	TMS	SeqIDN160609013	MW056348
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	TMS	SeqIDN160609015	MW056350
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	TMS	SeqIDN160609020	MW056355
	TMS	SeqIDN160609029	MW056360
	TMS	SeqIDN160609030	MW056361
	TMS	SeqIDN160609031	MW056362
	SSB	SeqIDN160806002	MW056363
	SSB	SeqIDN160806003	MW056364
	SSB	SeqIDN160806004	MW056365
	SSB	SeqIDN160806005	MW056366
	GTI	SeqIDN160806008	MW056367
	GTI	SeqIDN160806009	MW056368
	GTI	SeqIDN160806010	MW056369

GTI	SeqIDN160806011	MW056370
GTI	SeqIDN160806013	MW056371
GTI	SeqIDN160806014	MW056372
GTI	SeqIDN160806015	MW056373
GTI	SeqIDN160806016	MW056374
GTI	SeqIDN160806017	MW056375
OMB	SeqIDN160918003	MW056376
OMB	SeqIDN160918004	MW056377
OMB	SeqIDN160918005	MW056378
OMB	SeqIDN160918006	MW056379
wNMZ	SeqIDN161020001	MW056380
wNMZ	SeqIDN161020002	MW056381
wNMZ	SeqIDN161020004	MW056382
wNMZ	SeqIDN161020005	MW056383
wNMZ	SeqIDN161020006	MW056384
wNMZ	SeqIDN161020007	MW056385
wNMZ	SeqIDN161020008	MW056386
wNMZ	SeqIDN161020011	MW056387
wNMZ	SeqIDN161020012	MW056388
wNMZ	SeqIDN161020013	MW056389
wNMZ	SeqIDN161020014	MW056390
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wNMZ	SeqIDN161020016	MW056392
wNMZ	SeqIDN161020017	MW056393
wNMZ	SeqIDN161020019	MW056394
wNMZ	SeqIDN161020020	MW056395
wNMZ	SeqIDN161020021	MW056396
wNMZ	SeqIDN161020022	MW056397
eNMZ	SeqIDN170227003	MW056398
eNMZ	SeqIDN170227004	MW056399
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SSB	SeqIDN170422001	MW056405
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	GTI	SeqIDN170422011	MW056414
	GTI	SeqIDN170422018	MW056417
	GTI	SeqIDN170422022	MW056419
	GTI	SeqIDN170422023	MW056420
	GTI	SeqIDN170422024	MW056421
Sepioteuthis sp. 2A	TMS	SeqID160609008	MW056324
	TMS	SeqID160609010	MW056325
	TMS	SeqID160609022	MW056327
	TMS	SeqID160609023	MW056328
	TMS	SeqID160609024	MW056329
	OMB	SeqID160918001	MW056334
	OMB	SeqID160918007	MW056335
	OMB	SeqID161020018	MW056337
	SSB	SeqID170227002	MW056339
	GTI	SeqID170422012	MW056340
	TMS	SeqIDN160609025	MW056356
	TMS	SeqIDN160609026	MW056357
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	TMS	SeqIDN160609028	MW056359
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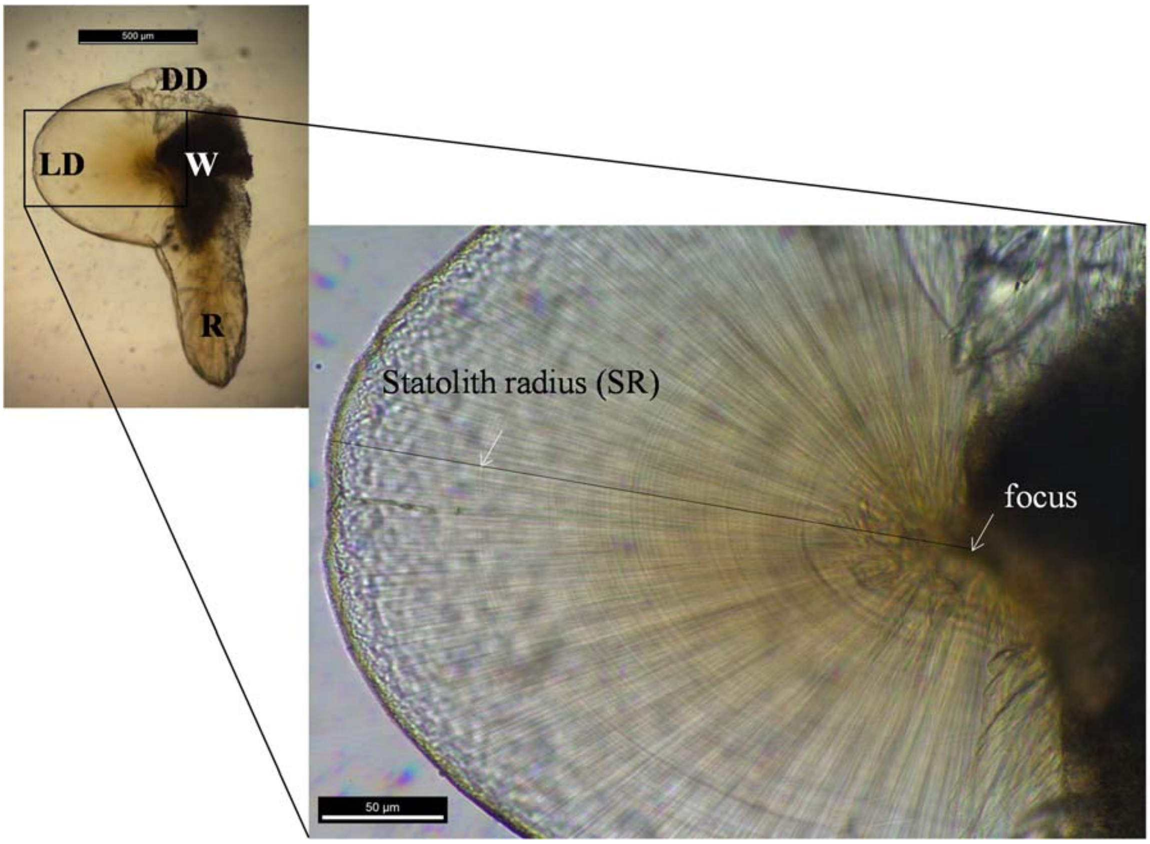
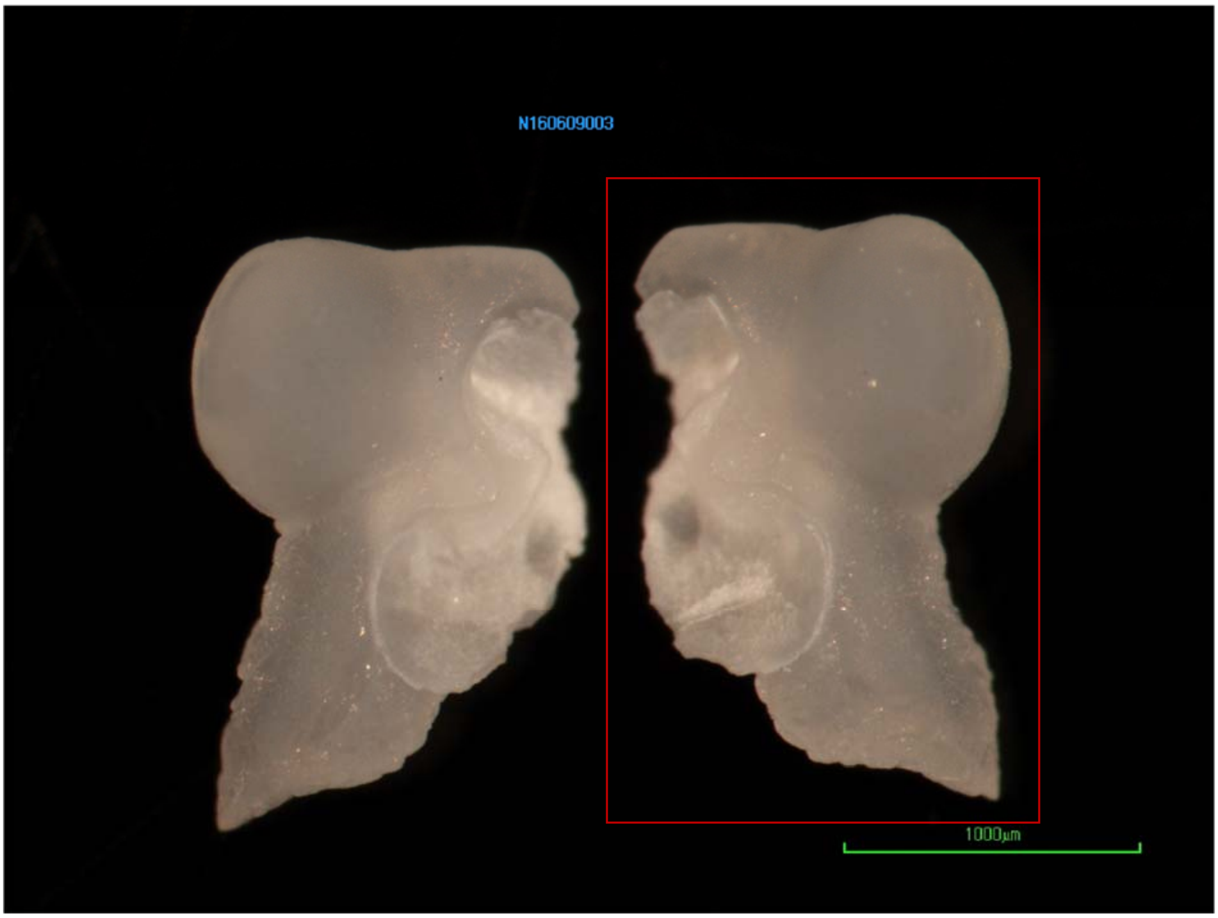
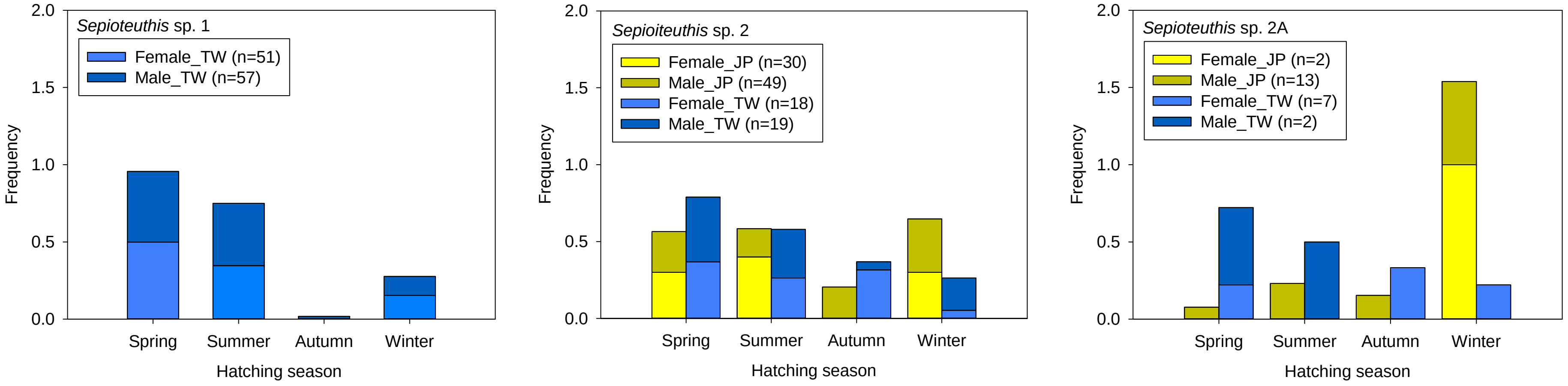


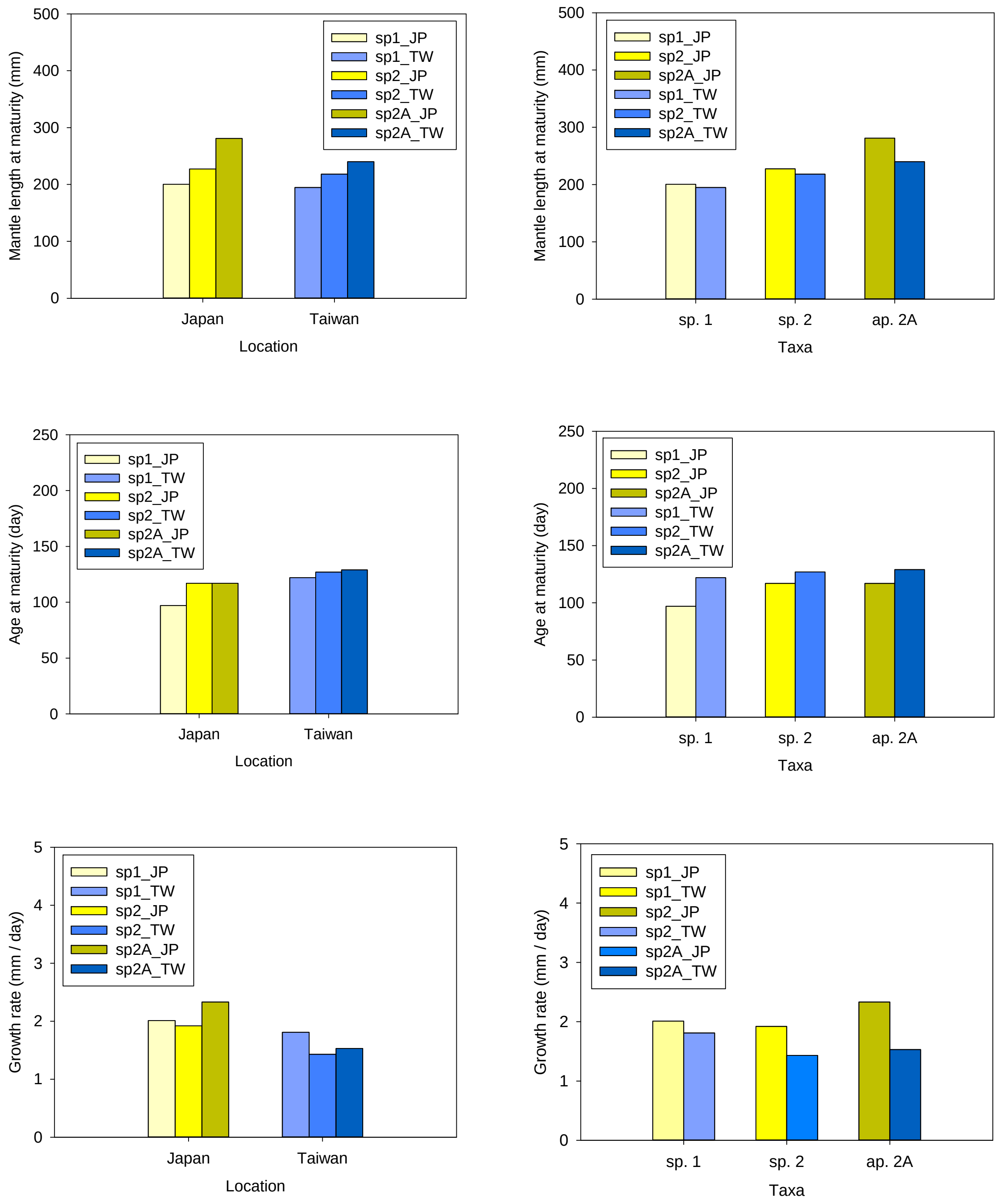
Annex 3. Statolith increments counting of the *Sepioteuthis lessoniana* and the regions (Dorsal dome (DD), Lateral dome (LD), Wing (W) and Rostrum (R)).



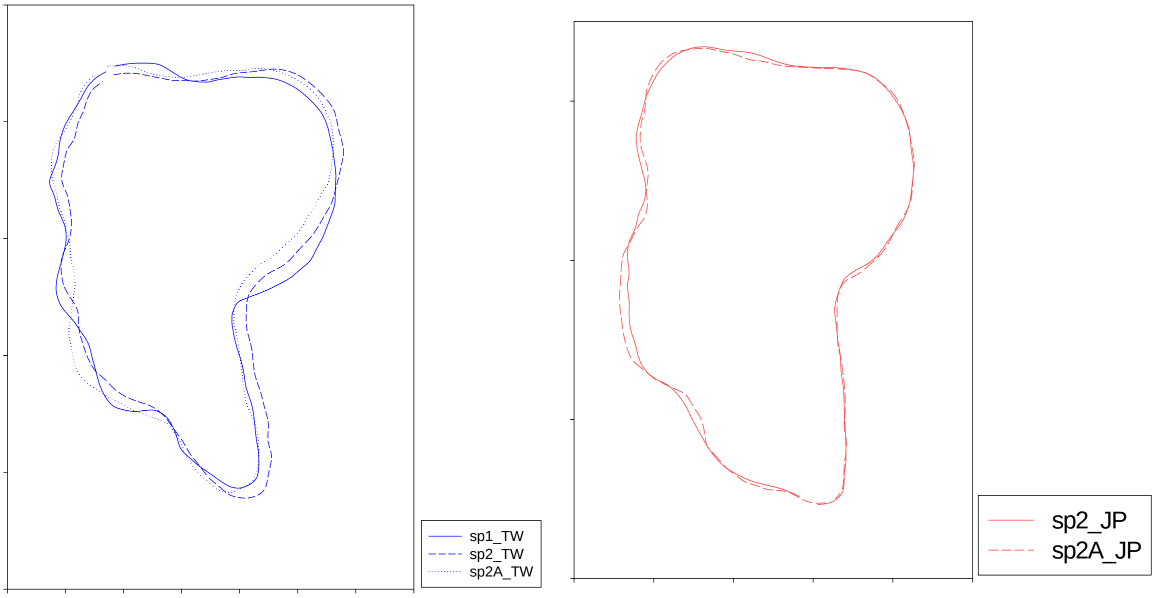
Annex 4. The photo of the statolith morphology and the area of red line is the image import to the morphology analysis.



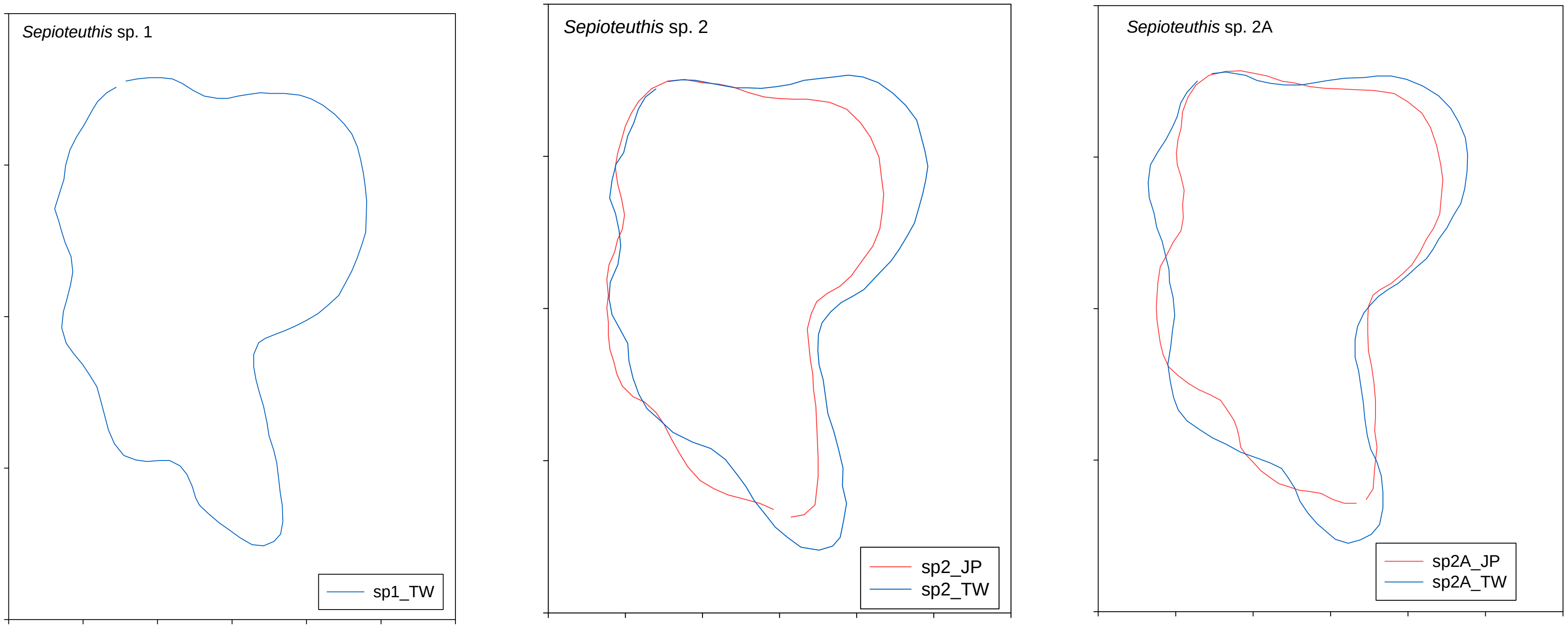
Annex 5. Frequency distribution of back-calculated hatching months of *Sepioteuthis* sp. 1, sp. 2 and sp. 2A in the waters off Japan and Taiwan.



Annex 6. Compared the ML and age at maturity, and growth rate among taxa and between locations of *Sepioteuthis* spp.



Annex 7. Statolith morphology aligned among taxa in Japan and Taiwan.



Annex 8. Statolith morphology aligned among locations in three taxa.

Publications

Peer-reviewed papers

Hsiao, C.-D., Shen, K.-N., **Ching, T.-Y.**, Wang, Y.-H., Ye, J.-J., Tsai, S.-Y., Wu, S.-C., Chen, C.-H., Wang, C.-H. (2016). The complete mitochondrial genome of the cryptic "lineage A" big-fin reef squid, *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae) in Indo-West Pacific. Mitochondrial DNA Part A, 27(4), 2433-2434 (DOI: 10.3109/19401736.2015.1030630)

Ching, T.-Y., Chen, C.-S., Wang, C.-H. (2019). Spatiotemporal variations in life-history traits and statolith trace elements of *Sepioteuthis lessoniana* populations around northern Taiwan. Journal of the Marine Biological Association of the United Kingdom 99, 203-213

Ching, T.-Y., Chen, C.-S., Yagishita, N., Yamaguchi, A., Wang, C.-H., Shen, K.-N. (2021). Variations in life-history traits and statolith shape for *Sepioteuthis* spp. in the waters off southwestern Japan. Fisheries Science DOI 10.1007/s12562-020-01485-3

Presentations

Ching, T.-Y., Chen, C.-S., Wang, C.-H. (2014). Spatial and temporal variation in statolith elemental signatures of the *Sepioteuthis lessoniana* around northern Taiwan. 5th International Otolith Symposium, Oct, Mallorca, Spain.

Ching, T.-Y., Wang, C.-H., Chen, C.-S. (2015). Applying life-history traits and statolith microchemistry to examine *Sepioteuthis lessoniana* populations around North Taiwan. 台灣水產學會103年度會員大會暨學術論文發表會，01月18日，台北，台灣。

Ching T.-Y., Wang, C.-H., Chen, C.-S. (2015). Variation in life history traits and statolith trace elements of seasonal cohorts of *Sepioteuthis lessoniana* around North Taiwan. The Cephalopod International Advisory Council Meeting (CIAC 2015 Hakodate), Nov, Hakodate, Japan.

Ching, T.-Y., Wang, C.-H., Chen, C.-S., Shen, K.-N. (2016). Population structure and spawning period analysis of *Sepioteuthis lessoniana* in the North Taiwan Seas. 台灣水產學會104年度會員大會暨學術論文發表會，01月19日，屏東，台灣。

Ching, T.-Y., Yamaguchi, A., Yagishita, N., Wang, C.-H., Chen, C.-S., Shen, K.-N. (2017). Population structure of *Sepioteuthis lessoniana* in the Northwestern Pacific: application of life-history traits and genetic markers. 日本水產學會春季大會，平成29年3月，東京，日本。

Ching, T.-Y., Yamaguchi, A., Yagishita, N., Wang, C.-H., Chen, C.-S., Shen, K.-N. (2018). Composite variations in molecular structure, life-history traits and statolith morphology of *Sepioteuthis lessoniana* populations in southwest Japan waters. 6th International Otolith Symposium, Apr, Keelung, Taiwan.

Ching, T.-Y., Yamaguchi, A., Yagishita, N., Wang, C.-H., Chen, C.-S., Shen, K.-N. (2018). Composite variations in molecular structure, life-history traits and statolith morphology of *Sepioteuthis lessoniana* populations in southwest Japan waters. 2018年海洋科學年會，5月2日，台北，臺灣。(青年論壇生物組第一名)

Ching, T.-Y., Yamaguchi, A., Yagishita, N., Wang, C.-H., Chen, C.-S., Shen, K.-N. (2019). Populations structure of *Sepioteuthis lessoniana* in Northwestern Pacific - composite variations in genetic structure, life-history traits and statolith morphology. 台灣水產學會107年度會員大會暨學術論文發表會，01月12日，台北，台灣。

Ching, T.-Y., Chen, C.-S., Yamaguchi, A., Shen, K.-N., Yagishita, N., Wang, C.-H.

(2020).Variations in life-history traits and statolith shape for *Sepioteuthis* spp. in the waters off southwestern Japan. 台灣水產學會108年度會員大會暨學術論文發表會，online。

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