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Morphology and morphometry of *Doryteuthis plei* (Cephalopoda: Loliginidae) statoliths from the northern shelf off São Paulo, southeastern Brazil

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This study aims to describe and evaluate statoliths of *Doryteuthis plei* from the northern shelf off São Paulo (23° S), to provide details useful for taxonomic identification, sexual differentiation and estimation of individual size from statolith dimensions. A total of 390 statolith pairs were analysed and their morphology and morphometry were described according to growth and maturity stage. There was a tendency for the extremity of the rostrum and the domes to narrow with growth. A morphological comparison with the statoliths of the congeneric *Doryteuthis sanpaulensis* was also undertaken. No obvious gross morphological difference among statoliths between sexes was found. A logarithmic model provided the best fit of the relationship between statolith length and mantle length, which can be useful to estimate the squid's length. Power function equations indicating the allometric relationships are also shown, allowing comparison with other squid populations elsewhere.

Keywords: *Doryteuthis plei*; *Doryteuthis sanpaulensis*; statoliths; morphometry

Introduction

Statoliths are calcareous structures, composed primarily of calcium carbonate in the aragonite crystal form and, in lesser proportions, organic material (mainly proteins). They are attached to the anterior surface of the statocyst, which, in turn, is located on the ventral side of the squid skull (Radtke 1983). The crystals occur in a protein matrix, radiating from the core to the edge in statolith-shaped increments. The statolith is composed of four parts: dorsal dome, lateral dome, rostrum and wing. The wing has opaque colouring compared with other statolith parts, which are partially translucent. Statoliths vary for different species (Clarke 1978; Jackson 1994) and serve mainly to detect accelerations (linear, angular and gravitational) of the body during the squid's movement in water (Stephens and Young 1978; Arkhipkin and Bizikov 2000).

Because statoliths are formed early during the embryogenesis and persist until the end of the post-hatching life, they can also function as "black boxes" to record accurate information about the life of the squid (Arkhipkin 2005). Age and growth estimates can be obtained by identifying and interpreting growth increments deposited in statoliths (Villanueva et al. 2003; Boyle and Rodhouse 2005; Perez et al. 2006).

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In addition, statoliths are one of the few remains of cephalopods in the fossil record, and their remains can serve as a resource to infer hypotheses about the lifestyle of extinct species (Arkhipkin 2005).

Positive correlations between statoliths' morphometric measurements, with mantle length and body weight of several species of squid, have been empirically established. These correlations, combined with the taxonomic value of the morphology of the statolith, are useful in estimating the size and weight of squid preyed upon by several predatory species (Brunetti and Ivanovic 1991). In studies of trophic ecology, statoliths are found in the stomach content of predatory species of fish and cephalopods. They are used for identification purposes (Clarke and Fitch 1975; Clarke 1978; Vega et al. 2001). The morphological description of statoliths allows the identification of the prey species. In addition, statolith morphometrics can be used to identify and compare subpopulations (Vega et al. 2001; Schwarz and Perez 2007). This highlights the value of the statoliths as taxonomic tools (Clarke 1978; Brunetti and Ivanovic 1991; Flores and Garland 2002).

In Brazil, statoliths have been used for studies of age and growth of some species, such as *Doryteuthis plei* caught off the Santa Catarina coast (25–29° S) through the reading of growth increments on polished statoliths (Aguiar 2002; Perez et al. 2006). Hence, the statolith morphometrics were preliminarily used to establish relationships between statolith length and mantle length (Aguiar 2002). Considering the concepts above, this study aimed to describe and evaluate statoliths of *D. plei* caught off the São Paulo coast to provide detailed data for taxonomic identification, sexual differentiation and estimation of individual squid size from statolith dimensions.

Material and methods

A total of 390 *D. plei* was caught during the austral summer months (from September 2011 to February 2012). Samples were obtained from the north coast of São Paulo, at the following sites: São Sebastião Island (23°46′ S; 45°21′ W) and Ubatuba (23°31′ S; 45°06′ W). The specimens were collected using hand-jigging ("zangarilhos"), dip-nets (under light attraction), and artisanal bottom trawls and pound nets (Japanese style floating trap nets). The identification of the squid caught followed Roper et al. (1984) and Jereb and Roper (2010).

In the laboratory, squid were weighed to the nearest gram and had their dorsal mantle length (ML) measured to the nearest millimetre. After dissection of the mantle, the sex and stage of maturity were obtained according to the macroscopic scale proposed by Juanicó-Rivero (1979), modified by Perez et al. (2002): I, immature or juvenile; II, maturing; III, mature; IV, spent.

The statoliths were extracted through a ventral incision in the back of the head cartilage. The statolith pairs of each individual were removed with the aid of forceps, put on tissue paper to remove the moisture and stored in 70% ethanol in 2-ml Eppendorf flasks for later analysis. The statoliths were analysed under a stereoscopic microscope attached to a computer system at 3.2–6.3× magnifications. Statoliths were measured for their length (SL, mm) and dome length (DL, mm) following Clarke (1978). Other measurements included the: (1) angle of the rostrum (ÂR, degrees); (2) angle of the dorsal dome (ÂD, degrees); and (3) statolith area (AS, mm^2) (Figure 1). Measurements were performed on digitalised images using the AxioVision$^©$ software (version 4.8).
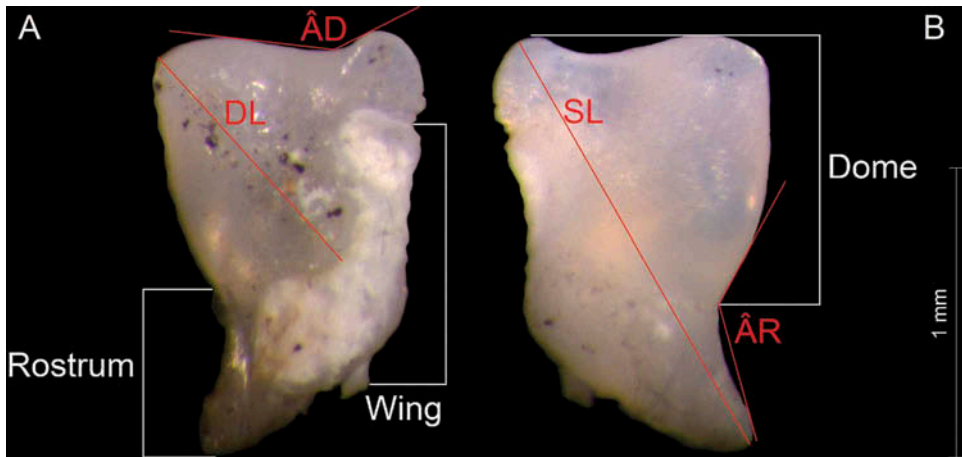
Figure 1. *Doryteuthis plei* statolith measurements. (A) Anterior face: angle of dome (ÂD), dome length (DL); (B) rear face: estimated statolith length (SL), angle of rostrum (ÂR).

As a component of the description of *D. plei* statoliths, we compared the *D. plei* statolith morphology with the congeneric *Doryteuthis sanpaulensis* statolith (which was described only morphologically).

The distribution of the measurements of statoliths and ML were tested using the Shapiro–Wilk test, indicating non-parametric data ($p < 0.05$). This condition having been satisfied, a Kruskal–Wallis test was used to check both whether differences existed between the right and left statoliths, and for data standardisation. As there was no significant difference between right and left statoliths (Kruskal–Wallis $p = 1$), further analyses were performed using the measurements on the right statolith.

To detect any possible difference between sexes, the Kruskal–Wallis test was also applied to the statolith measurements of males and females. The relationships between SL and ML for males and females and with respect to the maturity stages were tested using analysis of covariance (ANCOVA; test of homogeneity of slopes), where ML was the dependent variable, sex (or maturity) the categorical factor, and SL the continuous predictor (Zar 1996). Data were log-transformed before analysis, and calculations were performed using the Statistica$^{©}$ software (version 10).

The relationships between SL and ML were tested with the fitting models: linear, exponential, logarithmic and power function. Following a criterion of choice, the best fit was selected according to the highest values of R^2 and the Akaike Information Criterion (AIC) (Akaike 1973). This analysis was performed using the R$^{©}$ software (R Development Core Team 2012).

Results

Sample characterisation

From the 390 squid collected, 223 were female, 140 were male and 27 were juvenile (with undefined sex). Mantle length averaged 112.06 mm for the whole sample (sexes pooled), ranging from 25 to 291 mm ML. The average MLs were 101.75 mm and 126.71 mm for males and females respectively. Juveniles showed a mean size of 44.48 mm ML.

Morphology and morphometry of D. plei statoliths

Statoliths were whitish in colour. The wing was more whitish in relation to the rest of the structure, with a thin rugged surface, comprising, on average, nearly 28.51% of the statolith area. The dome and rostrum were also whitish, but translucent in comparison to the wings. The bulk of the statolith is made up of the dorsal and lateral portions of the dome plus the rostrum, occupying as much as 71.49% of the statolith area (Figure 1). The rostrum is narrow medially, and extends to the end of the wing, having a relatively sharp tip (Figure 1). The rostrum tip tends to become sharper as the squid grows (Figure 2).

The SL averaged 1.35 mm in length, ranging from 0.8 to 1.83 mm (Table 1; Figure 4). The average angle of the dorsal dome (ÂD) was 161.87°, ranging from 123.14 to 179.81°. However, the ÂD in statoliths of juvenile specimens was close to 180°. The average angle of the rostrum (ÂR) was 140.51° (range: 114.63–166.64°). Table 1 and Figure 4 highlight other results obtained about statolith structure measurements.

Differentiation between male and female statoliths

There were no obvious visual gross morphological or morphometric differences between male and female statoliths (Kruskal–Wallis test $p = 1$; ANCOVA $p = 0.83$).

Descriptions of statoliths by size and maturity stage

A tendency for the extremity of the rostrum and the domes to narrow with size was observed in *D. plei*. Figure 2 shows major changes in 0.85–1.83 mm SL statoliths in respect to squid size (25–263 mm ML). Individuals between 25–100 mm ML showed statoliths with average 1.18 mm SL, rounded domes and angle dome of about 180°, the rostrum angle ranging from 121° to 166° (Figure 2A–G). Squid between 101–200 mm ML presented expansion of areas of the dome, rostrum and wing, with dome angles ranging from 123° to 179°, and SL ranging between 1.34–1.80 mm (Figure 2H–M and O). In individuals larger than 200 mm ML, the average SL was 1.45 mm and, in general, the statoliths showed a prominent dorsal and lateral dome (Figure 2N and P).

The statoliths of immature *D. plei* (Figure 3A) are characterised by an average size of 1.02 mm (range: 0.88–1.13 mm) (Table 1). Some statoliths of immature individuals showed a small dark blue stain on the dome (Figure 3A). The rostrum has a tapered shape and the dome is rounded. The angle of the rostrum is more accentuated in juveniles (139.03°), forming an edge (Table 1; Figure 4).

Maturing squid (Figure 3B) have statoliths averaging 1.16 mm in SL (range: 0.8–1.63 mm) (Table 1; Figure 4). The rostrum was characterised by a rounded tip. The region of union between the rostrum and the lateral dome tended to be round, as did the junction between the dorsal and lateral domes (mean angle of 143.97° and 163.58° for the rostrum and dorsal dome, respectively), which forms a sharp tip at its end.

Mature squid (Figure 3C) showed larger statoliths, averaging 1.56 mm in SL (range: 1.06–1.83 mm) (Table 1; Figure 4). The dorsal dome was characterised by a conspicuous angle, and the DL ranged from 0.6 to 1.12 mm (Table 1). There were no visual gross differences between the statoliths of mature individuals and spent squid (Figure 3D).

There was a significant difference in SL with respect to ML only between mature and immature stage squid (ANCOVA $p < 0.0000001$).
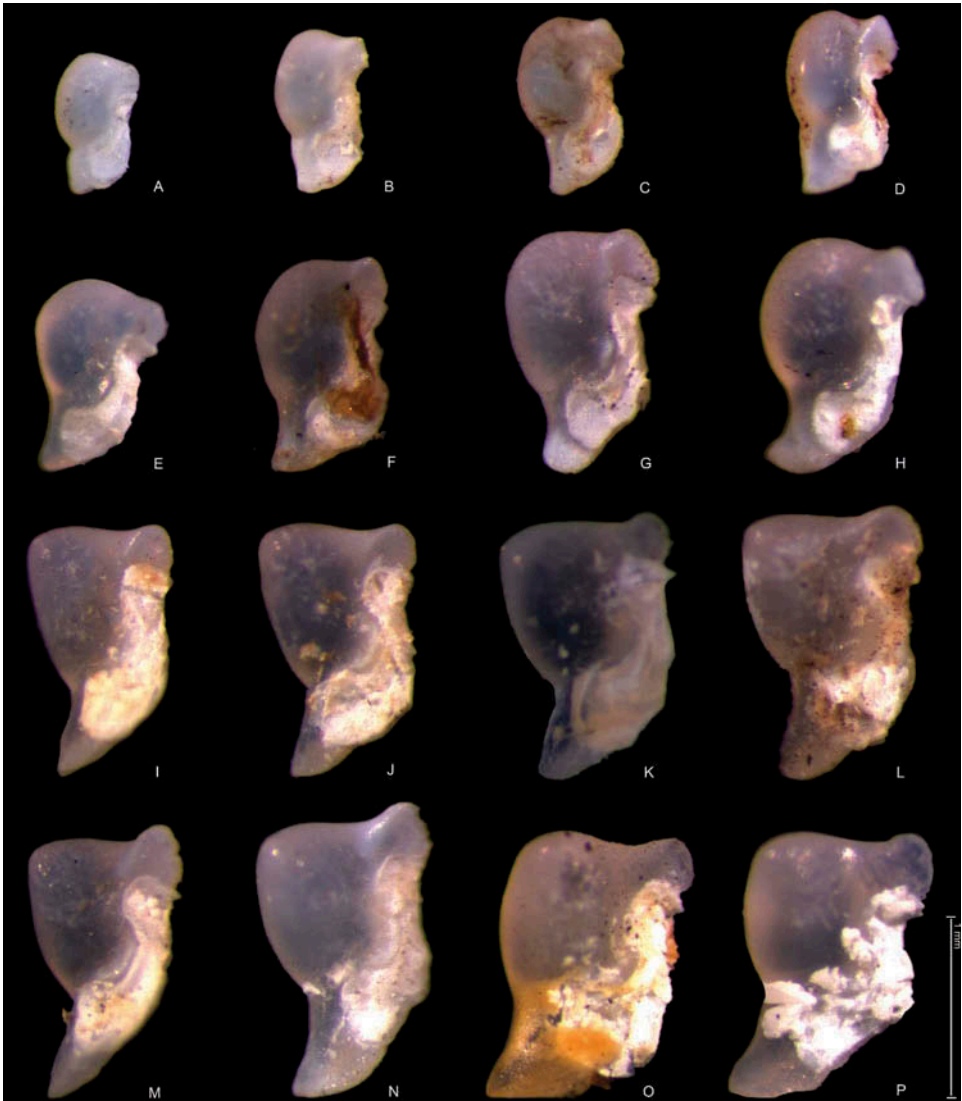
Figure 2. Statoliths of *Doryteuthis plei* at different sizes, represented from (A) to (P). The first number between brackets indicates the SL (in mm) and the second the respective individual ML (in mm), as follows: A (0.85; 25), B (0.96; 35), C (1.08; 45), D (1.16; 50), E (1.25; 57), F (1.32; 82), G (1.46; 85), H (1.49; 109), I (1.51; 111), J (1.56; 123), K (1.64; 139), L (1.66; 166), M (1.69; 157), N (1.78; 221), O (1.80; 194) and P (1.83; 263).

Relationship between SL and ML

A logarithmic function provided the best fit for most relationships (for the total sample; males, females and mature), with the best R^2 and AIC. Table 2 shows the fitting values for the total sample, by sex and maturity stages for all models proposed. For individuals in the immature stage, the power and exponential models provided the best fit, although it was very close to the logarithmic model. The exponential fit was indicated

Table 1. Morphometric comparison between *Doryteuthis plei* statolith length, dome length, angle of the rostrum and dome for the total sample, by sex and maturity stages.

|	Statolith length (mm)		Dome length (mm)		Angle of the rostrum (°)		Angle of the dome (°)	
	Average	Range	Average	Range	Average	Range	Average	Range
Total	1.35 ± 0.2	0.8–1.83	0.77 ± 0.1	0.45–1.12	140.51 ± 8.7	114.63–166.64	161.87 ± 9.3	123.14–179.81
Male	1.27 ± 0.3	0.8–1.83	0.73 ± 0.1	0.45–1.12	141.42 ± 8.8	117.06–163.96	162.75 ± 9.7	129.98–179.77
Female	1.44 ± 0.2	0.95–1.8	0.82 ± 0.1	0.52–1.08	140.12 ± 8.8	114.63–166.64	160.09 ± 8.4	123.14–179.81
Immature	1.02 ± 0.1	0.88–1.13	0.58 ± 0.04	0.52–0.67	139.03 ± 6.8	121.57–157.18	171.98 ± 7.1	155.33–179.47
Maturing	1.16 ± 0.2	0.95–1.63	0.67 ± 0.1	0.57–1.01	143.97 ± 9.0	123.33–166.64	163.58 ± 9.1	137.99–177.40
Mature	1.56 ± 0.1	1.06–1.83	0.89 ± 0.1	0.6–1.12	137.56 ± 7.5	114.63–160.65	158.85 ± 8.4	123.14–178.90
Spent	1.58 ± 0.02	1.56–1.6	0.91 ± 0.1	0.83–0.96	142.72 ± 6.4	137.79–149.90	163.24 ± 5	158.08–168.06
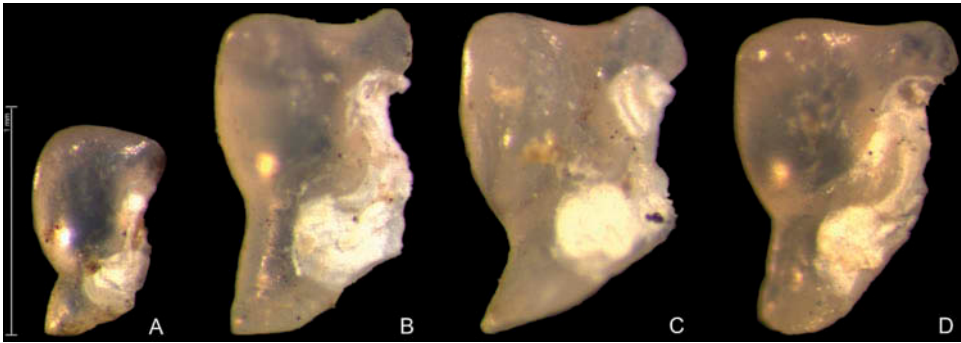
Figure 3. Statoliths of *Doryteuthis plei* at different stages of maturity. (A) Immature, 42 mm ML and 1.04 mm SL; (B) maturing female, 144 mm ML and 1.6 mm SL; (C) mature male, 201 mm ML and 1.59 mm SL; (D) spent female, 159 mm ML and 1.58 mm SL.
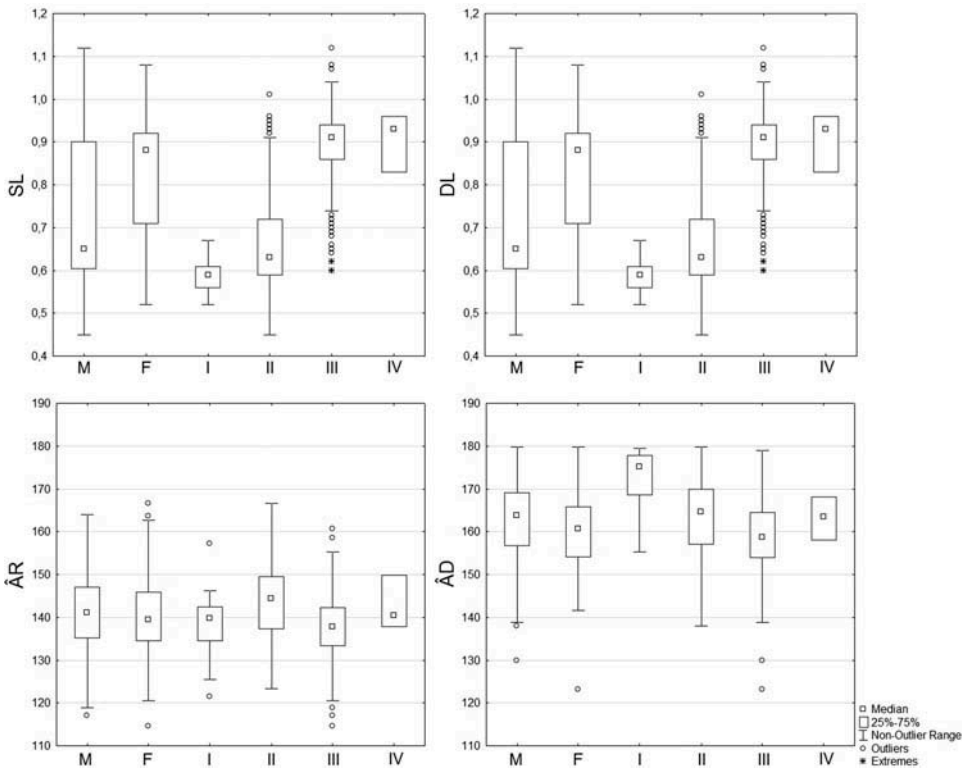

Figure 4. Morphometric values of the *Doryteuthis plei* statoliths into categories (M) males, (F) females, (I) immature, (II) maturing, (III) mature and (IV) spent by statolith measures (SL) statolith length, (DL) dome length, (ÂR) angle of the rostrum and (ÂD) angle of the dome.

Table 2. *Doryteuthis plei* relationships between statolith length (SL) and mantle length (ML) for the total sample, males, females, immatures, maturing and mature stages, with values of R^2 and Akaike Information Criterion for linear, logarithmic, power function and exponential models.

Models	Total		Male		Female		Immature		Maturing		Mature	
	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2	AIC
Linear	0.90	−834.78	0.91	−291.44	0.91	−567.18	0.72	−110.06	0.93	−488.83	0.66	−462.01
Logarithmic	0.96	−1142.62	0.97	−422.47	0.93	−644.20	0.73	−111.45	0.94	−534.14	0.76	−524.62
Power function	0.95	−1080.82	0.96	−396.92	0.93	−632.61	0.73	−111.16	0.94	−534.66	0.74	−514.36
Exponential	0.95	−1080.82	0.96	−396.92	0.93	−632.61	0.73	−111.56	0.94	−534.66	0.74	−514.36

as being the best for immature squid. For the mature stage, all models showed the same R^2 values but the lower AIC values were found in the power function model. Figure 5 displays the best fits for the relationship between SL and ML for the total sample, by sex and maturity stages with their respective equation models.

However, it can be noted that the power function showed fitting criteria quite close to the best fitted models (Table 2). In this case, considering that the power function shows allometric coefficients that can be compared to other squid, the equations of the power models are here available for the total sample ($y = 0.3323\ x^{0.3054}$); males ($y = 0.3407\ x^{0.2963}$); females ($y = 0.3499\ x^{0.2971}$); immature ($y = 0.2506\ x^{0.3712}$) and mature individuals ($y = 0.4851\ x^{0.2312}$).

Comparison between D. plei and D. sanpaulensis statoliths

The most striking difference between statoliths of *D. plei* and *D. sanpaulensis* was the shape of the tip of the rostrum, the rostrum size and angle, and the roundness of the dome (Figure 6). The *D. plei* rostrum was larger than that of *D. sanpaulensis*, and tends to taper. The angle of the rostrum was also sharper in *D. plei* (140.51°) than in *D. sanpaulensis* (nearly 180°). The lateral dome of the *D. plei* statolith had an oval shape, differing from *D. sanpaulensis*, which showed a more rounded dome.

Discussion

The statoliths of *D. plei* described here seemed to be very similar to those described by Perez et al. (2006) for squid caught off the Santa Catarina coast. In the strata of both populations, the statolith area was characterised by having a prominent dorsal and lateral dome, but in juvenile squid, this area was more rounded and smaller. However, the size of the specimens analysed in our study (range: 25–291 mm; average: 112.06 mm) was smaller than those sampled by Perez et al. (2006); range: 35–342 mm.

As noted by Aguiar (2002), morphological differences were also observed between statoliths of juveniles and adults. The statoliths of juvenile squid tend to be smaller in size (average 1.02 mm), with a rounded dorsal dome and a short rostrum. The statolith tends to change in shape as the squid matures, and the morphology and the morphometry become quite different in adult squid in relation to the juveniles. According to Lombarte et al. (1997) this is due to the squid's short life and rapid growth. Aguiar (2006) also observed changes in the morphology of the statoliths of *D. sanpaulensis* between juveniles and adults. Adult squid had statoliths that were more rounded and robust when compared with juvenile squid.

In terms of the relationship between ML and SL, the logarithmic model provided the best fit for *D. plei* and can be used to estimate the squid's length knowing only the statolith length. Previous SL–ML relationships for the species have also been described by the logarithmic function, but with poorer fits (Aguiar 2002; Perez et al. 2006). In the southwestern Atlantic Ocean, for the *Illex argentinus* squid, the power function showed the best fit for the relationship between SL and ML (Brunetti and Ivanovic 1991).

The power function is suitable for describing differences in the growth of body structures, showing allometric coefficients (Gould 1966) most useful for comparison of the growth of a given structure with other proportions of the organism. Allometric coefficients are also considered useful for comparison of the SL × ML relationships between different species of Loliginidae, such as *D. sanpaulensis*, *Doryteuthis gahi*,
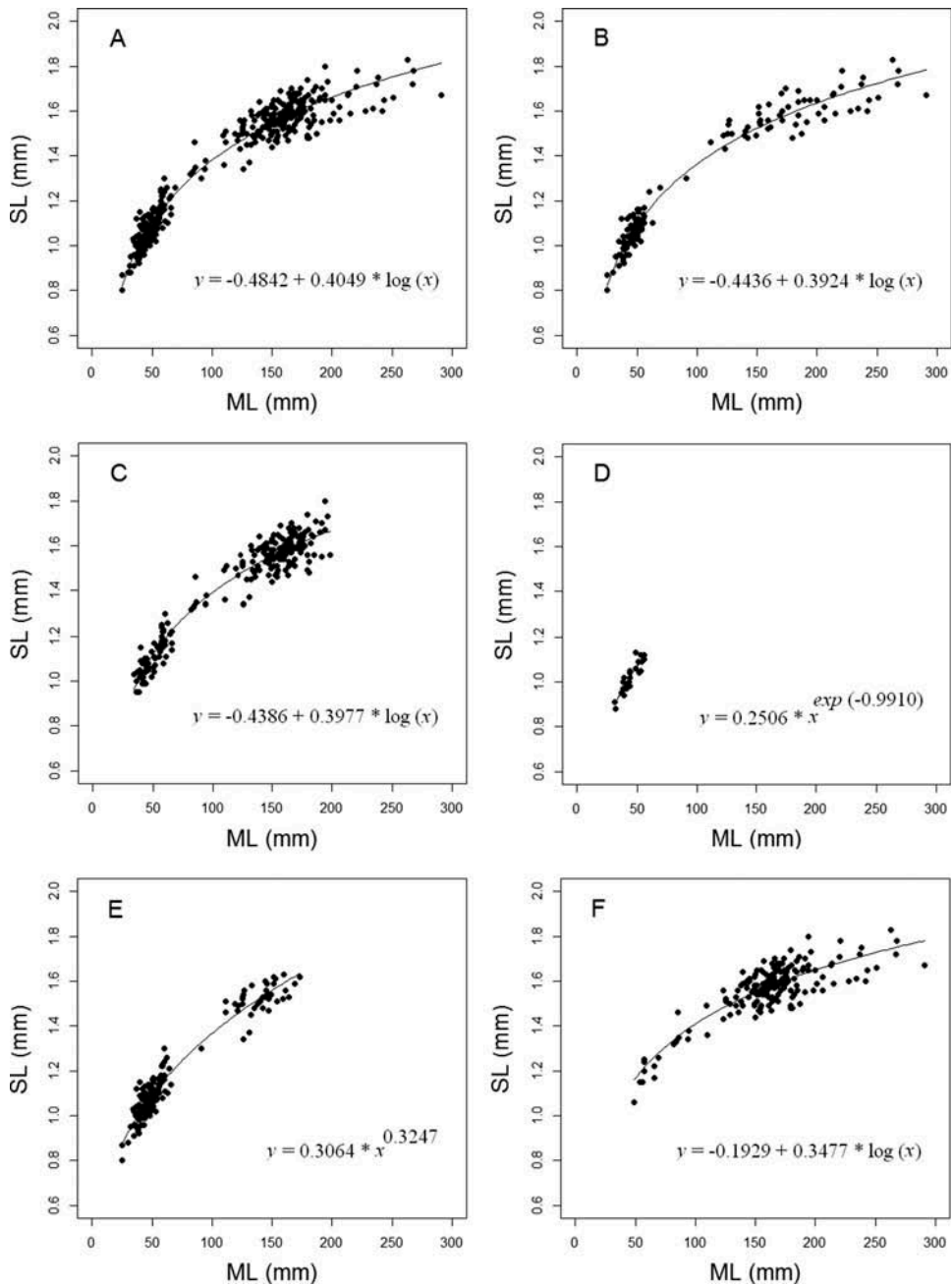
Figure 5. Relationship between *Doryteuthis plei* SL (statolith length, in mm) and ML (mantle length, in mm) for (A) total sample, (B) males, (C) females, (D) immature, (E) maturing and (F) mature. Values of R^2 and Akaike Information Citerion for each relationship are available in Table 2.
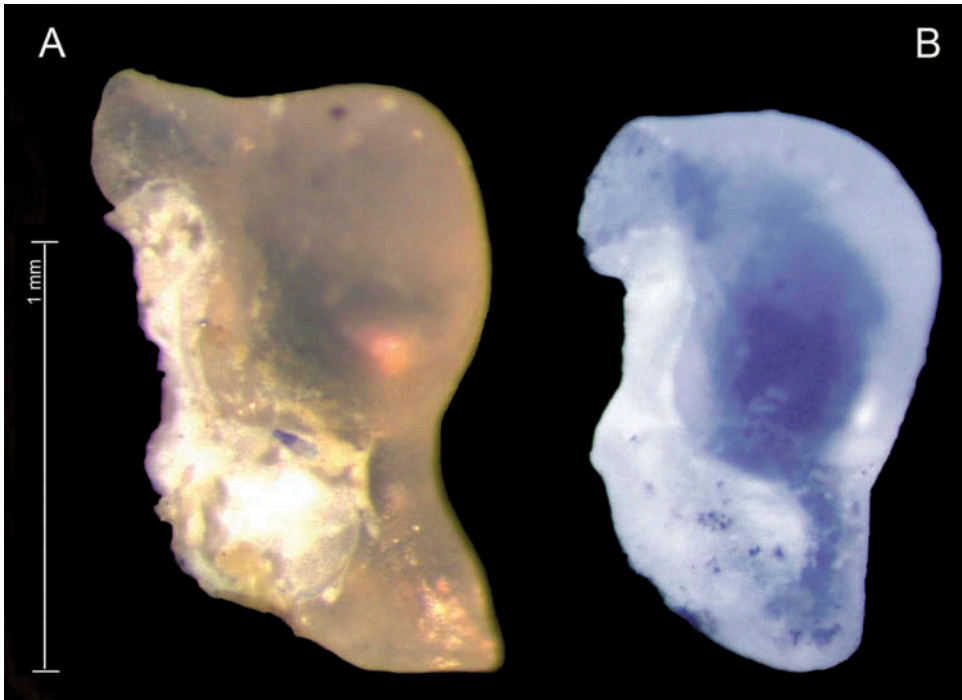
Figure 6. Comparison between squid statoliths at the same stage of maturity. (A) *Doryteuthis plei*; (B) *Doryteuthis sanpaulensis*. The bluish appearance of the *D. sanpaulensis* statolith is probably due to refractive properties, because it is more transparent than the *D. plei* statolith.

Sepioteuthis lessoniana (Pineda et al. 1998; Thomas and Moltschaniwskyj 1999; Flores and Garland 2002).

Conclusion

The morphology of statoliths of *D. plei* seems to be similar to that of other loliginids. However, they have a specific morphology, including a tendency to narrow the extremity of the rostrum and the domes with growth. No obvious gross morphological difference among *D. plei* statoliths of males and females was found, but statoliths of adult squid were significantly different in morphology and morphometrics in relation to that of juvenile squid. The logarithmic model provided the best fit for the relationship between mantle length and statolith length and can be used to estimate the squid's length using only values of statolith length.

Acknowledgements

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