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STATOLITH COMPARISON OF TWO SOUTH-WEST ATLANTIC LOLIGINID SQUID: *LOLIGO SANPAULENSIS* AND *LOLIGO GAHI*

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The statoliths of two South-West Atlantic loliginid squid, *Loligo gahi* and *Loligo sanpaulensis,* were studied to determine if they could be a useful tool for species differentiation. Allometric equations were employed to examine differences in statolith shape and growth. Statolith dimensions were standardized by total length and dome length and principal components analysis was employed to compare the shapes. The first component was used as a discriminant function to classify species. There is clear species differentiation in both size and shape of the statoliths. The statoliths of *Loligo gahi* are significantly larger than those of *Loligo sanpaulensis. Loligo gahi* statoliths are characterized by the presence of a prominent dorsal dome and a relatively long, thin rostrum, whereas those of *Loligo sanpaulensis* have a rounded dome and short rostrum. The discriminant function correctly separated 92.9% of *Loligo gahi* and 88.5% of *Loligo sanpaulensis* statoliths in additional samples. Relationships between mantle length and statolith length and between total mass and statolith length were determined for both species.

Loligo gahi d'Orbigny, 1835 is found in the eastern Pacific Ocean from southern Peru to southern Chile and in the South-Western Atlantic from Tierra del Fuego to 36°S on the continental slope off Argentina. It is a cold-temperate (5.5–8.5°C) neritic species occurring from shallow waters to 400 m water depth (Castellanos and Menni 1968, 1969, Castellanos and Cazzaniga 1977, 1979, Roper *et al*. 1984, Pineda *et al*. 1998). *Loligo sanpaulensis* Brakoniecki, 1984 is found in the South-West Atlantic from 20°S off Brazil (Roper *et al*. 1984) to 46°S off the San Jorge Gulf (Castellanos 1967). It is a neritic species of coastal temperate waters (12–18°C), living in water 3 –120 m deep (Costa *et al.* 1990, Andriguetto and Haimovici 1991).

The two species overlap between 42 and 46°S, over the intermediate shelf off Argentina (Pineda *et al*. 1998). Their general morphology is similar, especially when they are juveniles, making identification quite difficult. The relative growth patterns of some parts of the beak were used recently to identify them (Pineda *et al*. 1996).

Statoliths have been shown to be useful in species identification (Clarke *et al*. 1980, Lipiński *et al*. 1993) and age determination (Hatfield 1991, Natsukari and Komine 1992, Arkhipkin and Nekludova 1993, Arkhipkin 1995, Bettencourt *et al*. 1995) in several species of squid. The objective of this study was therefore to investigate the possibility of distinguishing *Loligo gahi* from *Loligo sanpaulensis* by means of differences in the statoliths. In addition, the relationships between mantle length and statolith length and between total mass and statolith length are presented.

MATERIAL AND METHODS

Samples of *Loligo sanpaulensis* (n = 280) and *Loligo gahi* (n = 388) were collected during research cruises of the R.V. *Dr. Holmberg* and *Captain Oca Balda* and from commercial landings at Mar del Plata during the years 1992 and 1993 (Fig. 1). An almost complete size range (30-250+340 mm dorsal mantle

Table I: Comparisons of statolith mean morphometric characters of *Loligo gahi* and *L. sanpaulensis*

Variable	Species	Mean	Standard deviation	F
Rostral length	*Loligo gahi*	0.576	0.0705	1 234.58
	L. sanpaulensis	0.367	0.0758	HS*
Dome width	*Loligo gahi*	0.796	0.0986	704.74
	L. sanpaulensis	0.588	0.0997	HS*
Statolith length	*Loligo gahi*	1.577	0.1891	473.60
	L. sanpaulensis	1.254	0.1891	HS*
Dome length	*Loligo gahi*	1.000	0.1310	120.28
	L. sanpaulensis	0.886	0.1323	HS*

*Highly significant difference HS: $p < 0.01$

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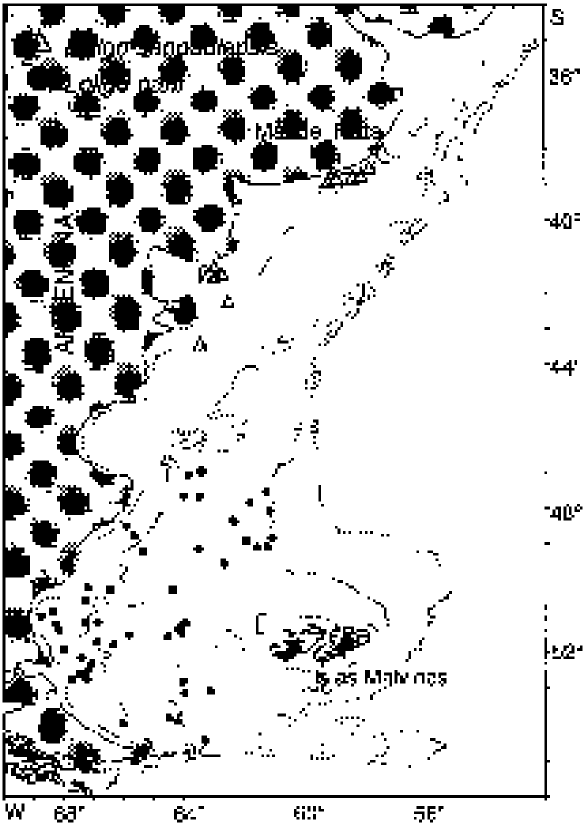

Fig. 1: The distribution of *Loligo gahi* and *Loligo sanpaulensis* sampling stations

length *ML* for *Loligo gahi*, 20-170 mm *ML* for *Loligo sanpaulensis*) and all maturity stages of each species were represented in the samples.

Dorsal mantle length, total mass (*TM*), sex and maturity stage (Lipiński 1979, Vigliano 1985) were also recorded. Statoliths were dissected from fresh squid and stored in 96% ethanol for later analysis. Terminology and statolith measurements were according to those of Clarke (1978). Four measurements were made on the posterior side of the right statolith: total length (*TL*), dome length (*DL*), rostrum length (*RL*) and dome width (*DW*) – Fig. 2. All were made under a binocular microscope, using an eyepiece micrometer with a precision of 0.01 units and transformed to mm.

In order to study statolith morphology of the two species, three approaches were followed:

(i) The mean values of *TL, RL, DL* and *DW* were compared (one-way ANOVA) to determine differences in statolith size.

(ii) After log-transforming the values, an allometric equation was employed to examine differences in statolith growth using four pairs of variables, *DL/TL, RL/TL, DW/TL* and *DW/DL.* The regression lines were tested between species by a maximum likelihood ratio test that compares the slope (b) and the intercept (ln a) coefficients together (Fomby *et al*. 1984). The slopes were then tested for isometry.

(iii) The standardized statolith dimensions (*RL/TL, DW/TL* and *DW/DL*) were compared to determine differences in statolith shape (Mosimann 1970). Principal components analysis was employed to identify the relative contribution of each index for discrimination between species. From that analysis, the indices were ranked by the proportion of the variation explained by the first component.

The same measurements were taken from additional samples of squid to examine the significance of the results obtained (n = 26 for *Loligo sanpaulensis* and n = 28 for *L. gahi*). The theoretical probability of mistaking one species for the other (misclassification) was calculated.

Finally, relationships between mantle length and statolith length and between total mass and statolith length were determined for each species.

RESULTS

Size differences

Differences between mean morphometric characters for each species are shown in Table I. The means for all characters of *Loligo gahi* were significantly greater (p < 0,01) than those for *Loligo sanpaulensis.* Mean characters per size-class were also greater in *L. gahi* (Fig. 3).

Growth differences

Relationships between statolith characters were approximated by a power function for each species. The differences between the slopes and the intercepts of each regression line between the two species were statistically significant (p < 0.001), demonstrating the

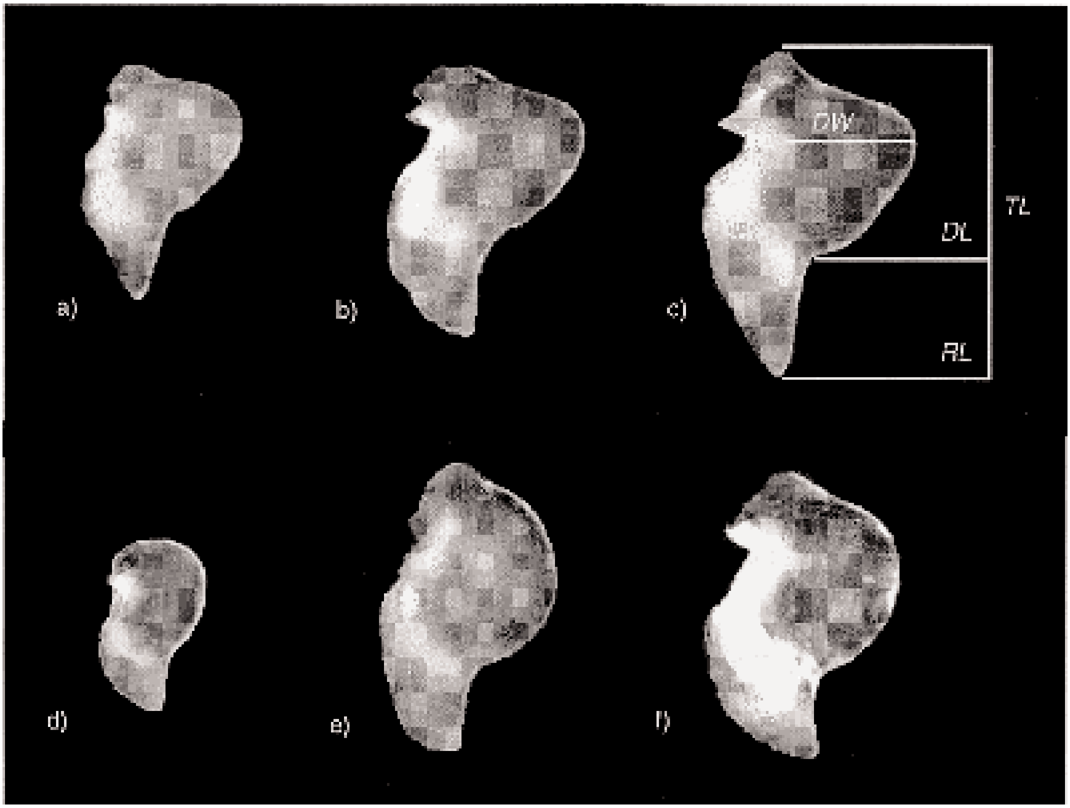

Fig. 2: Posterior views of statoliths of (a–c) *Loligo gahi* and (d–f) *L. sanpaulensis.* Mantle lengths and statolith lengths (mm) were (a) 55 and 1.21; (b) 129 and 1.53; (c) 213 and 1.76; (d) 49 and 1.08; (e) 113 and 1.39 and (f) 181 and 1.50. *DW* = dome width, *DL* = dome length, *RL* = rostral length, *TL* = total length

difference between statolith growth of each species (Table II, Fig. 4). Growth was positively allometric ($p < 0.001$) in both species for all relationships, the only exception being that of *DW/TL* for *Loligo sanpaulensis*, for which isometry was obtained. The results suggest that shape changes during the process of statolith development in both species.

Shape differences

All statolith dimensions were standardized by statolith length and dome length and principal components analysis was employed to investigate statolith shape.

The principal components analysis revealed positive values on the first component (C1) for *Loligo gahi* and negative values for *Loligo sanpaulensis,* the two species being clearly separated (Fig. 5). The first two principal axes explained 88.4 and 11.5% of the total variation respectively. Table III lists the weightings on each index for the two principal axes. The correlations between the indices and the first component revealed high, positive values.

Most of the positive C1 values (*RL/TL*, *DW/TL* and *DW/DL*) corresponded to *Loligo gahi* and the majority of the negative ones to *Loligo sanpaulensis* (Figs 4, 5). In other words, *Loligo gahi* had larger standardized dimensions than *Loligo sanpaulensis.* These results also indicate a more prominent rostrum and dome in the *Loligo gahi* statolith than in that of *Loligo sanpaulensis.*

The first component was also used as a discriminant function, classifying positive and negative values as *Loligo gahi* and *Loligo sanpaulensis* respectively.

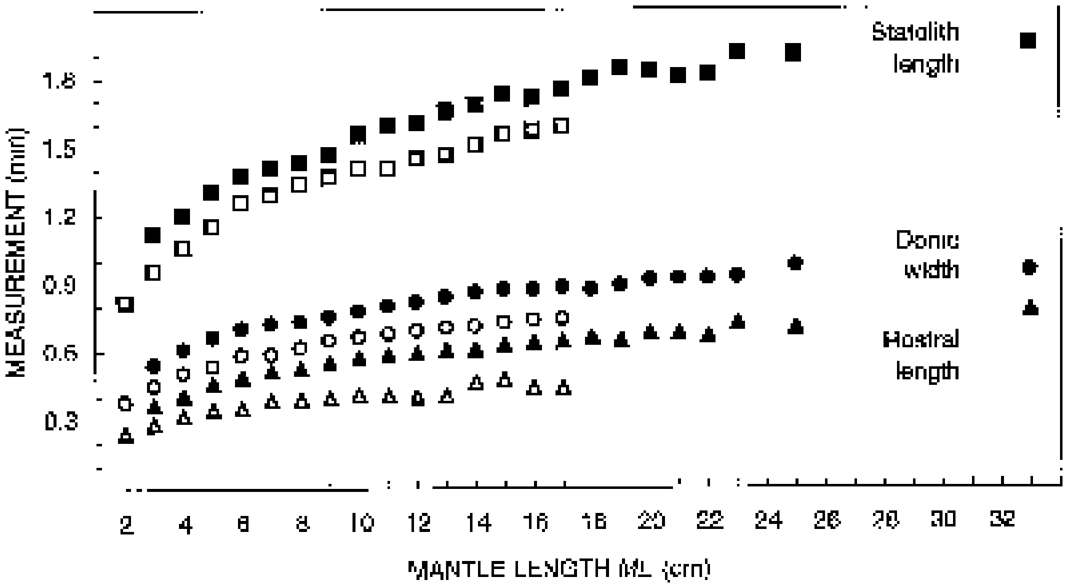

Fig. 3: Mean statolith length, dome width and rostral length calculated per size-class for each species (solid symbols *Loligo gahi;* open symbols *L. sanpaulensis*

The best discrimination between the species was based on the function

$$C1 = Z1*(RL/TL - MRT) + Z2*(DW/TL - MDT) + Z3*(DW/DL - MDD),$$

where Z1 = 0.3726, Z2 = 0.3096 and Z3 = 0.8748 are the loadings of the first component, and *MRT*= 0.033528, *MDT* = 0.49019 and *MDD* = 0.74186 are the mean values of the *RL/TL*, *DW/TL* and *DW/DL* indices respectively.

Table II: Isometry test and comparison between statolith relative growth curves for *Loligo gahi* and *Loligo sanpaulensis*

Species	*n*	r^2	*SEE*	ln a	b	Isometry	Likelihood ratio test
				ln *DL* = ln a + b* ln *TL*			
Loligo gahi	388	0.8991	1.0000	–0.4304	0.9457; *sb* = 0.0161	*t* = –3.37; HS	*F* = 300.35
Loligo sanpaulensis	280	0.9317	1.0000	–0.3616	1.0564; *sb* = 0.0171	*t* = 3.30; HS	*df* = 2 and 664; HS
				ln *RL* = ln a + b* ln *TL*			
Loligo gahi	388	0.7966	1.0000	–1.0549	1.1008; *sb* = 0.0283	*t* = 3.56; HS	*F* = 286.06
Loligo sanpaulensis	280	0.6085	1.0000	–1.1990	0.8548; *sb* = 0.0411	*t* = –3.53; HS	*df* = 2 and 664; HS
				ln *DW* = ln a + b* ln *TL*			
Loligo gahi	388	0.7533	0.0658	–0.6541	0.9295; *sb* = 0.0271	*t* = –2.60; HS	*F* = 72.96
Loligo sanpaulensis	280	0.8269	0.0719	–0.7565	0.9849; *sb* = 0.0270	*t* = –0.56; NS	*df* = 2 and 664; HS
				ln *DW* = ln a + b* ln *DL*			
Loligo gahi	388	0.6968	0.0729	–0.2316	0.8963; *sb* = 0.0301	*t* = –3.44; HS	*F* = 467.75
Loligo sanpaulensis	280	0.8112	0.0752	–0.4249	0.8913; *sb* = 0.0258	*t* = –4.21; HS	*df* = 2 and 664; HS

r^2 = coefficient of determination, *SEE* = standard error estimate, *sb* = standard deviation of the slope, *TL* = statolith total length, *DL* = dome length, *RL* = rostal length, *DW* = dome width
No significant difference (NS): $p > 0.05$
Significant difference (S): $0.01 < p < 0.05$
Highly significant difference (HS): $p < 0.01$

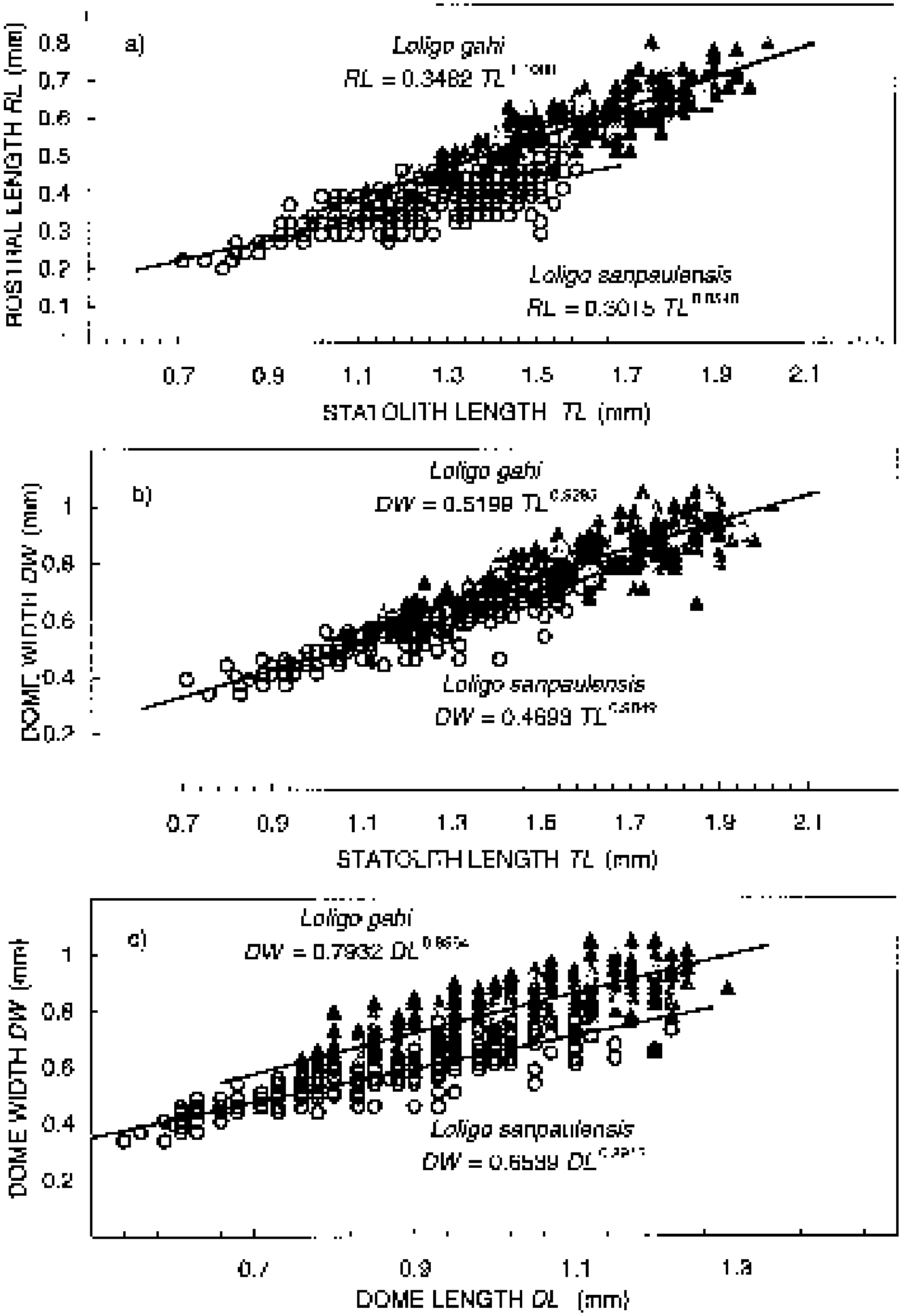

Fig. 4: Relationships between (a) rostral length and statolith length, (b) dome width and statolith length, and (c) dome width and dome length for *Loligo gahi* and *L. sanpaulensis*

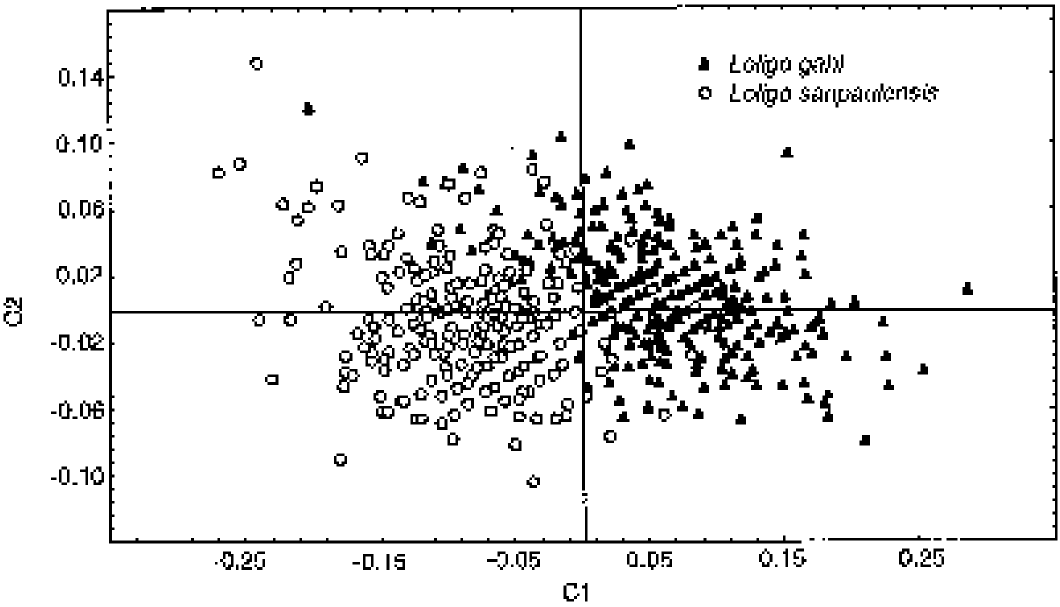

Fig. 5: First two principal axes plot for statolith dimensions of *Loligo gahi* and *L. sanpaulensis,* standardized by statolith and dome length

This discriminant function was validated with additional samples of both species, and 92.86% of *Loligo gahi* and 88.46% of *Loligo sanpaulensis* were correctly classified.

Relationships between mantle length and statolith length and between total mass and statolith length

Allometric relationships between mantle length and statolith length and between total mass and statolith length are shown in Tables IV and V. The power function revealed the best fit for both relationships. Statistically highly significant differences were found between the relationships for each sex in both species, and between species when comparing the same sex, with the exception of *TM*/*TL* in *Loligo sanpaulensis*.

Growth in mantle length and body mass also were positively allometric with growth in statolith length in both species and sexes (Tables IV, V).

Table III: Weightings on each index for the two principal axes

Indices	C1	C2
RL/TL	0.7995	0.6000
DW/TL	0.8087	−0.5862
DW/DL	0.9989	−0.0453

DISCUSSION

The long-finned squid *Loligo gahi* and *L. sanpaulensis* are possibly sister species allopatrically distributed and potentially important for the coastal fishery of Argentina. Both species are common on the intermediate shelf off Argentina between 42 and 46°S (Pineda *et al*. 1998), and there is an overlap in almost all of the diagnostic characters (Castellanos and Cazzaniga 1979, Brakoniecki 1984), making identification difficult, especially between the juveniles.

Features and dimensions of statoliths and principal components analysis have been defined as of great value for evaluating differences between closely related species of squid (Clarke *et al*. 1980, Clarke and Maddock 1988). Comparison of statolith characters between species was made with the purpose of determining their practical value as a tool to distinguish between the species in the field. They proved to be a reliable means of distinguishing between the two species of *Loligo* in Argentine waters, permitting accurate identification over the whole size range found.

Table IV: Comparison between statolith length *TL* – mantle length *ML* relative growth curves for *Loligo gahi* and *Loligo sanpaulensis* ($\ln ML = \ln a + b * \ln TL$)

Parameter	*n*	r^2	*SEE*	ln a	b	Likelihood ratio test
Loligo gahi	388	0.8533	1.0000	3.2700	3.2307; *sb* = 0.0682	*F* = 110.84
Loligo sanpaulensis	280	0.8361	1.0000	3.7084	2.6442; *sb* = 0.0702	*df* = 2 and 664; HS
Loligo gahi						
Males	230	0.8497	0.1631	3.3223	3.1726; *sb* = 0.0883	*F* = 7.41
Females	158	0.8655	0.1627	3.2124	3.2763; *sb* = 0.1034	*df* = 2 and 384; HS
Loligo sanpaulensis						
Males	135	0.8578	0.1958	3.7029	2.8010; *sb* = 0.0989	*F* = 6.43
Females	145	0.8191	0.1711	3.7240	2.4460; *sb* = 0.0961	*df* = 2 and 276; HS
Males						
Loligo gahi	230	0.8497	1.0000	3.3222	3.1726; *sb* = 0.0883	*F* = 56.81
Loligo sanpaulensis	135	0.8578	1.0000	3.7029	2.8010; *sb* = 0.0989	*df* = 2 and 361; HS
Females						
Loligo gahi	158	0.8655	0.1627	3.2124	3.2763; *sb* = 0.1034	*F* = 59.55
Loligo sanpaulensis	145	0.8191	0.1711	3.7240	2.4460; *sb* = 0.0961	*df* = 2 and 299; HS

r^2 = coefficient of determination, *SEE* = standard error estimate, *sb* = standard deviation of the slope
No significant difference (NS): $p > 0.05$
Significant difference (S): $0.01 < p < 0.05$
Highly significant difference (HS): $p < 0.01$

Statistically significant differences were evident in all morphometric characters and indices selected (Figs 2, 3). The principal components analysis, as a discriminant function, successfully classified *Loligo gahi* and *L. sanpaulensis* as positive and negative values respectively, revealing changes in shape. The species

Table V: Comparison between statolith length *TL* – total mass *TM* relative growth curves for *Loligo gahi* and *L. sanpaulensis* ($\ln TM = \ln a + b * \ln TL$)

Parameter	*n*	r^2	*SEE*	ln a	b	Likelihood ratio test
Loligo gahi	388	0.8700	1.0000	–0.1209	7.8962; *sb* = 0.1553	*F* = 240.88
Loligo sanpaulensis	280	0.8413	1.0000	1.1932	6.6539; *sb* = 0.1733	*df* = 2 and 664; HS
Loligo gahi						
Males	230	0.8720	0.3640	0.0039	7.7722; *sb* = 0.1972	*F* = 9.98
Females	158	0.8773	0.3766	–0.2555	7.9755; *sb* = 0.2388	*df* = 2 and 384; HS
Loligo sanpaulensis						
Males	135	0.8511	0.4893	1.1742	6.7981; *sb* = 0.2465	*F* = 0.52
Females	145	0.8295	0.4375	1.2204	6.4735; *sb* = 0.2454	*df* = 2 and 276; NS
Males						
Loligo gahi	230	0.8720	1.0000	0.0039	7.7722; *sb* = 0.1972	*F* = 107.17
Loligo sanpaulensis	135	0.8511	1.0000	1.1742	6.7981; *sb* = 0.2465	*df* = 2 and 361; HS
Females						
Loligo gahi	158	0.8773	0.3766	-0.2555	7.9755; *sb* = 0.2388	*F* = 135.71
Loligo sanpaulensis	145	0.8295	0.4375	1.2204	6.4735; *sb* = 0.2454	*Df* = 2 and 299; HS

r^2 = coefficient of determination, *SEE* = standard error estimate, *sb* = standard deviation of the slope
No significant difference (NS): $p > 0.05$
Significant difference (S): $0.01 < p < 0.05$
Highly significant difference (HS): $p < 0.01$

were distinguished by differences in dome width and length, in rostral length and in statolith length. Dome width showed the best discriminatory value when standardized by dome length. The statoliths of *Loligo gahi* have a prominent lateral dome and a relatively long, thin rostrum, whereas the notable characteristics of *Loligo sanpaulensis* statoliths are a rounded dome and a short rostrum.

Sexual dimorphism in statolith size was observed in both species, as has been reported for other loliginids (Hatfield 1991, Arkhipkin and Nekludova 1993, Lipiński and Durholtz 1994, Arkhipkin 1995, Bettencourt *et al*. 1995).

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