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RESEARCH ARTICLE

Biology, distribution and geographic variation of loliginid squids (Mollusca: Cephalopoda) off southwestern Atlantic

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ABSTRACT. The biology and ecology of southwestern Atlantic loliginid squids have been intensively researched in the last few decades, mostly off the Brazilian southern coast. However, information gathered by scientific research cruisers, either past or recent, is limited. Three species of loliginid squids – the warm-tempered *Doryteuthis sanpaulensis* (Brakoniecki, 1984), plus the tropical *D. pleii* (Blainville, 1823) and *Lolliguncula brevis* (Blainville, 1823) – were sampled along 16 degrees of latitude on the southwestern Atlantic (22–38°S). The samples were obtained mostly from oceanographic surveys, but also included squids caught by commercial fisheries, and a few specimens from museum collections. Squid response to abiotic variables, morphological variation and circadian behaviour were surveyed in detail. *Doryteuthis sanpaulensis* can be divided into at least six distinguishable geographical groups, which do not form a cline. In contrast, *D. pleii* can be divided into only two morphological groups that are very similar. *Doryteuthis* spp. were heterogeneously distributed on the shelf, whereas *L. brevis* was confined nearshore. Our data extended the southernmost distribution range of *D. pleii* by at least nine degrees of latitude, owing to specimens obtained at ~38°S (Mar del Plata, Argentina). Small, immature *D. sanpaulensis* were sampled inside the Patos Lagoon estuary (~32°S). The morphologically similar *Doryteuthis* spp. apparently avoid direct competition by concentrating at different depths, displaying different thermal preferences, and inverse circadian levels of activity. The information reported herein may be regarded as a "snapshot" of the ecology of sympatric squids in a marine environment that has not been deeply affected by climate change.

KEY WORDS. Competition, morphology, Myopsida, population biology, reproduction.

INTRODUCTION

Loliginid squids are typical inhabitants of coastal and shelf waters (Jereb et al. 2010). Many species are widely distributed geographically, and for this reason they experience significant shifts in environmental conditions such as water temperature and food availability (Pelc and Jackson 2008). In addition to the geographic location, depth and oceanographic regimes also affect the environmental conditions of these mollusks. Loliginid squids display noticeable variations in body proportions, some of which result in cryptic speciation, or distinct population groups that are clearly discernible based on their morphology, (Jackson and Moltschaniwskyj 2001, Herke and Foltz 2002, Sales et al. 2013, 2014, 2017, van der Vyver et al. 2016). Those groups may differ not only in body proportions, but also in other biological aspects, such as body condition, adult size, fecundity, reproductive cycle and lifespan (Pelc and Jackson 2008).

The veined squid, *Loligo forbesii* Steenstrup, 1856 from the Azores Islands form a distinct population, with individuals that are much larger (and robust) than their con-specifics from the European and north-western African Atlantic continental shelves (Pierce et al. 1994). Despite these differences, which include DNA microsatellite divergences, veined squid from the Azores are conspecific with *L. forbesii* distributed elsewhere (Shaw et al. 1999). Similar (and sometimes almost identical) phenotypes of the Atlantic brief squid *Lolliguncula brevis* (Blainville, 1823) are found scattered along the Brazilian coast (Perez and Zaleski 2013). Interestingly, distinct groups inhabiting nearby areas are often separate by indistinguishable groups of this squid located far apart (Zaleski et al. 2012). In addition, a recent genetic analysis of *L. brevis* has confirmed cryptic speciation within the species (Sales et al. 2014), as previously suggested from the morphometrics that distinguish populations from the North and South Atlantic (Zaleski et al. 2012).

Three loliginid squid species are sympatric and are commonly found in shelf waters off southern Brazil: the "tropical arrow squid" *Doryteuthis pleii* (Blainville, 1823), the "São Paulo squid" *Doryteuthis sanpaulensis* (Brakoniecki, 1984) and the "Atlantic brief squid" *Lolliguncula brevis* (Blainville, 1823) (Haimovici and Perez 1991a). *Doryteuthis pleii* is currently the best-studied species, mostly due to its importance for industrial and artisanal fisheries (e.g. Martins and Perez 2007, Arkhipkin et al. 2015). *Doryteuthis sanpaulensis* was intensively studied during the 1980s owing to its high abundance in the upwelling zone of Cabo Frio (22°S, Rio de Janeiro State) and along the coast of the state of Rio Grande do Sul (29–32.5°S) (Vidal et al. 2013). *Lolliguncula brevis* is the least-studied species thus far, perhaps because the species has little or no commercial value (Coelho et al. 2010, Perez and Zaleski 2013, Vaske et al. 2014).

Of interest, these three western Atlantic loliginids inhabit the so-called Paulista Province (22–33°S), a marine transitional biogeographical zone that shares biota with the Caribbean and Northern Patagonian Provinces, and has a relatively high level of endemism (Palacio 1977). The regional physiography and environmental conditions of the Paulista Province vary widely due to heterogeneous coastal features (including sedimentation patterns, oceanic sandy beaches, rocky shores, sheltered bays and bights, estuaries, mangroves, islands and reefs, and shelf width), oceanographic variability (circulation and water masses), climatic seasonality, and levels of biological productivity. This province is limited to the north and to the south by the 23.0 °C isotherm, and the area between 33–38°S acts as a barrier for the tropical fauna, mostly due temperature fluctuations (Palacio 1982).

The Paulista Province is regarded as the southernmost distributional limit for tropical species such as *D. pleii* and *L. brevis*, whereas the warm-tempered *D. sanpaulensis* extends its southern distributional range towards the Northern Patagonian Province and beyond (Haimovici and Perez 1991a, Vidal et al. 2013). Therefore, these squids are exposed to a suite of environmental conditions (and their fluctuations) in this transitional zone that certainly may affect their biology and ecology (Pierce et al. 1994, Pelc and Jackson 2008).

In Brazil, before the1970s, most studies on cephalopods involved their taxonomy and biogeography. From the 1950s on, the growing importance of squids as fishing targets in the coastal waters of southern Brazil created a demand for studies on their biology and ecology (Yesaki et al. 1976, Juanicó 1980, Gasalla et al. 2005). Even though our knowledge on the various important aspects of the biology of these cephalopods has increased ever since, some basic information on the population structure, reproduction, distribution, and trophic relationships of squids have remained unclear. In addition, there are taxonomic issues pertaining common species that are still unresolved (e.g. Brackoniecki 1984, Vecchione et al. 2005, Sales et al. 2013, 2014, 2017). Despite those gaps mentioned above, Brazilian research on the biology, ecology and fisheries of loliginid squids has developed considerably in the last four decades, mostly on populations off the southeast and south portions of the Brazilian coast (see Perez and Zaleski 2013, Vidal et al. 2013 and Arkhipkin et al. 2015 for comprehensive reviews).

Basic morphometric and sexual maturity studies on the tropical arrow squid, the São Paulo squid and the Atlantic brief squid were published in the late 1970s and early 1980s (Juanicó 1978, 1983). This study comes to complement these efforts by giving the first appraisal on loliginid squid biology and ecology and a first description of loliginid squid population structure, distribution in relation to environmental variables and geographic morphological variation off the southwestern Atlantic, between Rio de Janeiro and Mar del Plata (22–38°S).

MATERIAL AND METHODS

The study area ranges from 22°S (Cabo Frio, Brazil) to 38°S (Mar del Plata, Argentina), covering the entire Paulista Province plus part of the Northern Patagonic Province, encompassing the continental shelf from coastline to shelf break (200 m isobath) (Fig. 1). The seabed within this range is comprised mostly of soft sediments (Mahiques et al. 2004, Acha et al. 2008). Two distinct oceanographic systems are included: the South Brazil Bight (SBB, 22–29°S), and the shelf between Rio Grande do Sul and the northernmost part of the Argentinean shelf (29–38°S).

The SBB is characterized by seasonal interplay between onshore bottom intrusions of the South Atlantic Central Water (SACW) in spring-summer and surface equatorward penetration of the La Plata River Plume in autumn-winter (Pimenta et al. 2005, Castro et al. 2006). South to SBB, the oceanographic regime is influenced by the seasonal oscillation of the Subtropical Convergence (a mesoscale oceanographic feature originated from interactions between Brazil and Falkland currents), and the steady injections of fresh water due to continental drainage of the La Plata River and Patos Lagoon (Garcia 7997). Thus, biotic (biological productivity) and abiotic (temperature, salinity, pH, dissolved oxygen, etc.) variables vary widely throughout the study area.

The bulk of the loliginid samples used in this study were obtained during two oceanographic surveys conducted with the R/V Prof. W. Besnard between 23 and 30°S in September and November of 1975 (FAUNEC Cruisers III and IV) (Figs 1, 2). Squids were caught using a bottom otter trawl (16.7 m headrope fitted with floaters and stretched meshes of 60 mm in the net wings/body and 30 mm in the cod end). Hauls lasted 1.5 h at a velocity of 3.5 knots and were carried out between 10 and 132 m. Thirty-seven hauls were accomplished in the first leg (19–27 September 1975) and 41 in the second leg (07–27 November 1975), totalling 78 oceanographic stations (Fig. 2). Surface and bottom temperatures were obtained *in situ* with inversed thermometers fitted to Nansen bottles. Salinities of surface and bottom water samples were measured onboard using a Kahlsico

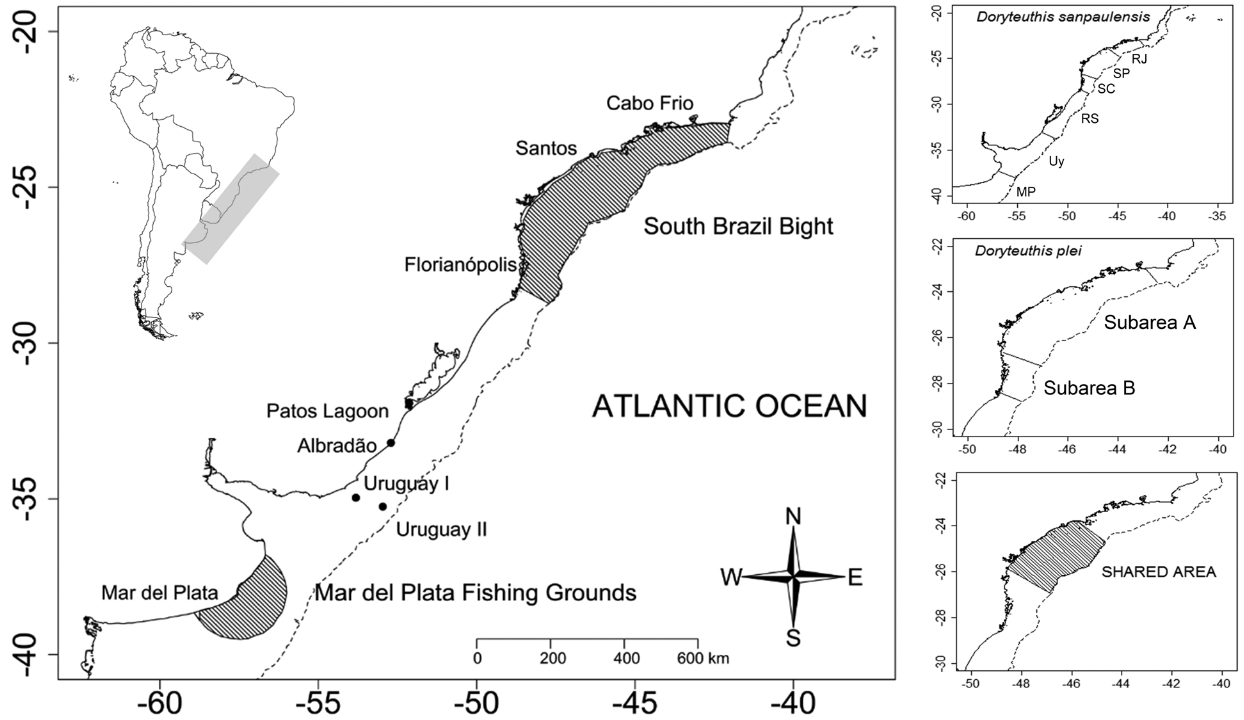

Figure 1. Study area, showing the areas and sites from where squid samples were obtained, also including the subareas used for the geographic differentiation in *Doryteuthis sanpaulensis* and *D. pleii* plus the shared area of co-occurrence of both species. The relative position of the study area on the South American Atlantic coast is boxed. RJ: Rio de Janeiro, SP: São Paulo, SC: Santa Catarina, RS: Rio Grande do Sul, Uy: Uruguay, MP: Mar del Plata. Subarea A: Cabo Frio (23°S) – Paranaguá (26°S), Subarea B: Paranaguá (26°S) – Santa Catarina Island (28°S). Latitudes and longitudes are decimal transformed. The 200 m isobath is showed (dashed line).

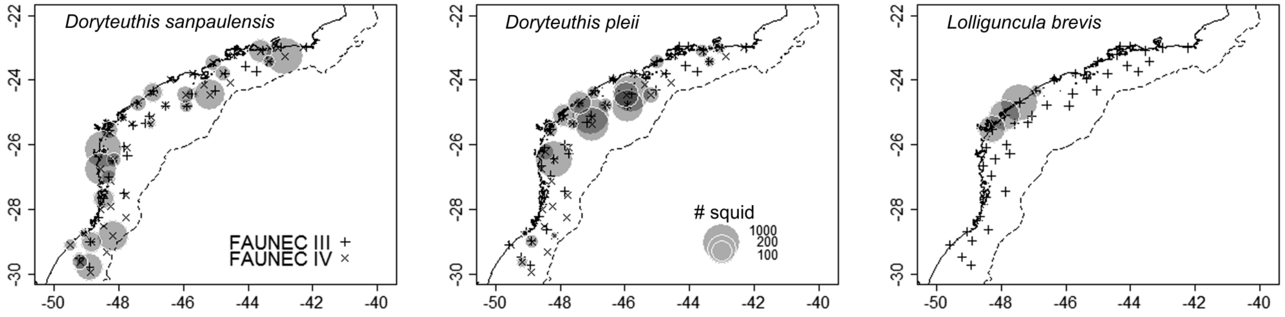

Figure 2. Maps of FAUNEC surveys from where squid where obtained. Abundance data were log-transformed for better visualization. Latitudes and longitudes are decimal transformed. The 200 m isobath is showed (dashed line).

Electrodeless Induction Salinometer (model S/N 21705). Squids were sorted from the remaining catch and were frozen following each haul. After landing, the samples were fixed and preserved in 10% buffered formalin solution in the laboratory.

The sampling effort was further supplemented with squids from Rio Grande do Sul, Uruguay and Mar del Plata (Argentina) coasts (29–38°S) (obtained from scientific surveys, commercial fisheries, and fish markets) to expand the study area domain (Fig. 1). Some few specimens were obtained from the malacological collection of the National Museum located in Rio de Janeiro. Details of the analysed material are given in the electronic Suppl. material 1.

In the laboratory, eight morphometric characters were selected following Cohen (1976) and Clarke (1962): mantle length (ML), fin length (FL), fin width (FW), gill length (GL, right only), raquis width (RW), gladius width (GW) and lower beak rostral length (LBRL). Measurements were taken from formalin-preserved specimens to the nearest millimetre (FL, FW, GL, RW, GW) and to the nearest 0.05 mm (LBRL), with the latter done under a stereoscopic microscope. All measurements (except ML) were taken with a Vernier calliper. The RW and GW were obtained by gently pressing the structure against a flat surface to standardize the lateral angle.

After dissection of the mantle, the sex and maturity stage of specimens were determined according to the five-step maturity scale proposed by Juanicó (1983): juvenile, (A) immature, (B) in maturation, (C) mature and (D) spent (Table 1). The presence of implanted spermatophores in the buccal receptacle (in *Doryteuthis* spp.) or in the inner wall of the mantle cavity, near the tip of gills (in *L. brevis*) was recorded as evidence of mating (Juanicó 1983).

We believe that tissue shrinkage in formalin-preserved squids that had been previously frozen is negligible and did not affect the measurements (Andriguetto and Haimovici 1988). To achieve standardized results , all morphometric measurements, sex identification and maturity scoring were carried out by one person, the second author (Pierce et al. 1994).

Morphometric analyses were conducted for samples collected during FAUNEC surveys. Samples from the Patos Lagoon obtained from the southern coast of Rio Grande do Sul were not used because those samples were collected with different gear and levels of the effort. However, squid samples from Uruguay and Mar del Plata were retained in the analysis to include the southernmost part of the study area (Fig. 1). Small samples ($n \leq 10$) were rejected regardless of their origin.

To address geographic diversification, Juanicó (1978) modified the discriminant method proposed by Royce (1957), originally developed to discriminate fish stocks. The Royce method compares the superposition of normal curves obtained from linear regressions between morphometric characters to discriminate distinct phenotypes, and thus different populations (Royce 1957). However, this method has three inherent problems: (1) it is valid only when the regression slopes are equal, (2) it cannot deal with different variances and (3) it cannot handle large sample sizes. Juanicó (1978) adapted the Royce method (hereafter referred to as the Modified Royce Method – MRM) to resolve these issues. Details on the MRM method are given in Suppl. material 2.

Since the MRM is a statistically based decision method that neither relies on significance levels nor on confidence intervals, it can be applied for paired comparisons among several samples. For the sake of simplicity, a grand mean of superposition ($\bar{\Omega}$) was calculated by averaging all average morphological characters ($\bar{S}$). This value, when multiplied by 100, yields the mean superposition in terms of percentage. Squid were considered morphologically distinct when $\bar{\Omega} < 70$.

For comparative purposes, the study area was divided into subareas according to the species investigated and the squid groups bounded within each subarea were analysed accordingly. Those divisions were based on latitudinal discontinuities in the catches of both species, and were named after geographic references on the coast (six subareas for *D. sanpaulensis*) and latitudinal intervals (two subareas for *D. pleii*) (Fig. 2).

The size structure of males, females and juveniles of the three loligind species was ascertained by visual analysis of ML histograms depicting the percentage of squids of each sex and juveniles in pooled 10 mm intervals. Histograms were made for each of the geographic divisions used in the morphometric analysis of *Doryteuthis* spp. (see Fig. 2). All *L. brevis* individuals obtained were analysed regardless their source due the small number of individuals sampled (see Suppl. material 1).

A hypothesis regarding the parity of sexes (i.e., 1 female: 1 male) was tested by means of a two-tailed chi-square test with Yates correction for continuity (Zar 2009). The sex ratio was further examined by subarea (*D. sanpaulensis* only) and depth. The latter analyses were carried out at specific depth strata

Table 1. Juanicó's (1983) loliginid squid maturity scale.

Stages	Females	Males
Juvenile	Reproductive system unrecognizable	Reproductive system unrecognizable
A – Immature	Nidamental glands distinguishable under dissection microscope. The ovary is small and filiform, and lacks a granulose structure	Spermatoforic organ and sac very small and distinguishable only under dissection microscope. The spermatoforic sac lacks spermatophores and testis resemble a thin membrane or a small lobule
B – In maturation	Nidamental glands small to large in size. Early stages differs from stage **A** by a bulged ovary with a clearly granulose structure containing small oocytes or ovules. Latter stages differ from stage **C** by an opaque whitish ovary with a reticular system on the walls of oocytes and ovules (particularly evident in *Doryteuthis sanpaulensis* and *Lolliguncula brevis*)	There are few and small spermatophores in the spermatoforic sac, the testis is clearly distinguishable to the naked eye
C – Mature	Ovary and oviduct full of oocytes, occupying half of the posterior mantle cavity. Nidamental glands swollen and firm. All or most ovules amber in color, semi-transparent, and lacking a reticular system. Mature ovules filling the oviduct, but in some females they may also fill the ovary	Spermatoforic sac full of completely developed spermatophores. Testis at its largest size. When males mature, they produce spermatophores continuously, making it difficult to tell them apart from spent individuals
D – Spent	Large females with flaccid or reduced nidamental glands. Ovary and oviduct flabby with few or no mature ovules, but with some immature ovules and tissue remain	–

for both *Doryteuthis* species, namely <31 m and >55 m for *D. sanpaulensis* and <30 m and >45 m for *D. pleii*. This approach was taken because there were few or no squid of each species caught at the intermediate depths between these bathymetric intervals. All statistical tests were considered significant at a significance level of 0.05.

Size-at-maturity (ML_{50}) was estimated for each species by fitting logistic regression models to the proportion of mature squid (Martins and Perez 2007). Regressions were fitted with generalized linear models (GLMs) using binomial family and logit link functions (Crawley 2007). The ML_{50} was estimated for five out the six subareas established for *D. sanpaulensis* (squid from Uruguay were not included due to decay problems in females), and for the whole SBB for the remaining two species.

The distribution of *D. sanpaulensis* and *D. pleii* along the SBB (FAUNEC surveys) was exanimated according to environmental variables recorded at the oceanographic stations, namely surface and bottom temperature, surface and bottom salinity, time of day and depth strata. Time of day was divided into four intervals of 6 h each (3:00 am – 9:00 am, 9:00 am – 3:00 pm, 3:00 pm – 9:00 pm and 9:00 pm – 3:00 am). Depth was also stratified into four bathymetric intervals: 10–40, 40–70, 70–100 and 100–130 m (10–30, 30–50, 50–70 and 70–90 m in the "shared area", see Fig. 2).

Spatial overlap among the three loliginid species over all samples were using the Fager affinity index (*I*) (Fager and McGowan 1963):

$$I = \left(\frac{J}{\sqrt{N_A \cdot N_B}}\right) - \left(\frac{1}{2\sqrt{N_B}}\right)$$

where *J* is the number of joint occurrences of both species, and N_A and N_B are the numbers of occurrences of species *A* and *B*, where N_B is larger or equal to N_A. Since this index quantifies the overlap of only two species at a time, calculations were done separately for each pair of each species. A minimum value of 0.5 indicates a positive relationship between the pair of species.

Food and feeding were examined only for *D. pleii* from FAUNEC samples. Stomachs were dissected, and their degrees of fullness were scored according the following criteria: (1) empty, (2) 50% full and (3) 100% full or distended. Since stomach contents were considerably digested and mostly unrecognizable (unpublished results), a refined description of the diet was not attempted. However, stomach fullness data were pooled by sex and ontogeny and analyzed in relation to the time of the day to describe the feeding schedule of the species.

RESULTS

Characterization of geographic groups in *Doryteuthis sanpaulensis*

Figures 3 and 4 show the standardized dimensions of each morphological measurement as a result of linear regressions against the ML. Differences can clearly distinguish female and male squid according to the geographic subarea. Despite those differences, however, these groups do not form a cline along the latitudinal gradient.

Among the female squid, individuals from the coast of Santa Catarina were the most distinct, with the largest FL, FW, RW, GW, LBRL and the smallest GL amongst all areas. Squid from Mar del Plata were also very distinct, since all measurements (except FL and LBRL) were the smallest among subareas. Squid from Rio de Janeiro, São Paulo and Rio Grande do Sul were similar, but individuals from São Paulo had the largest GL and those from Rio de Janeiro had the smallest LBRL (Fig. 3).

Morphological differences were also evident in males. Except for the FW, differences were noticeable for the remaining measurements. Squid from Rio de Janeiro had the smallest FL and LBRL, whereas those from São Paulo had the largest GL and a relatively large RW. Individuals from Santa Catarina had the smallest GL and RW, whereas Rio Grande do Sul squid had the smallest FL and largest RW, GW and LBRL. Uruguayan squid were fairly alike to Rio de Janeiro individuals, but differed in RW, GW and LBRL. Squid form Mar del Plata had the largest FL and FW (Fig. 4).

Geographic variation in *Doryteuthis* spp.

The two species of *Doryteuthis* presented distinguishable morphological differences along the latitudinal gradient. In *Doryteuthis pleii*, males and females from the two subareas overlapped >70%, implying that the latitudinal differences were subtle. In contrast, *D. sanpaulensis* can be separated into at least six distinguishable geographical groups (five in the case of female squid) (Fig. 5).

Doryteuthis sanpaulensis females inhabiting Santa Catarina and Mar del Plata diverged morphologically from their immediate counterparts to the north and south (São Paulo and Rio Grande do Sul, $\bar{\Omega}$ = 60.5–67) and to the north (Rio Grande do Sul coast, $\bar{\Omega}$ = 55.8) respectively. Squid from Mar del Plata also overlapped very little in their morphologies within all remaining subareas. Squid from São Paulo and Rio Grande do Sul were very similar ($\bar{\Omega}$ = 75.2). The smallest overlap between groups were between squids from Santa Catarina and Rio de Janeiro ($\bar{\Omega}$ = 49.3) (Fig. 5).

Doryteuthis sanpaulensis males from Santa Catarina also overlapped very little with their closest neighbours to the north and to the south ($\bar{\Omega}$ = 64.1–68.6) and, again, squid from the São Paulo and Rio Grande do Sul coasts were very similar ($\bar{\Omega}$ = 75.9). The Uruguayan group diverged strongly from their immediate northern neighbour (Rio Grande do Sul, $\bar{\Omega}$ = 53) and was very similar to squid from the northernmost geographical area (Rio de Janeiro, $\bar{\Omega}$ = 72.2) (Fig. 5).

Population size structure, maturity, sex ratio and size at maturity (ML_{50})

The size structure histograms constructed for *D. sanpaulensis* were plotted in three figures for better visualization (Figs 6–8). The size of individuals ranged from 14 to 114 mm ML. The

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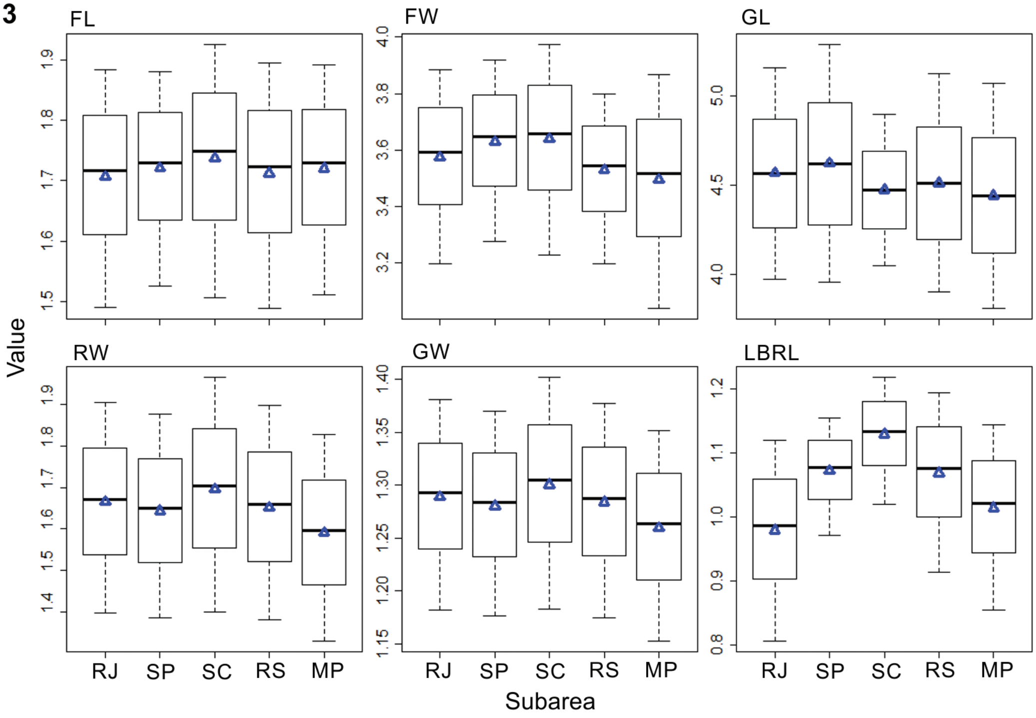

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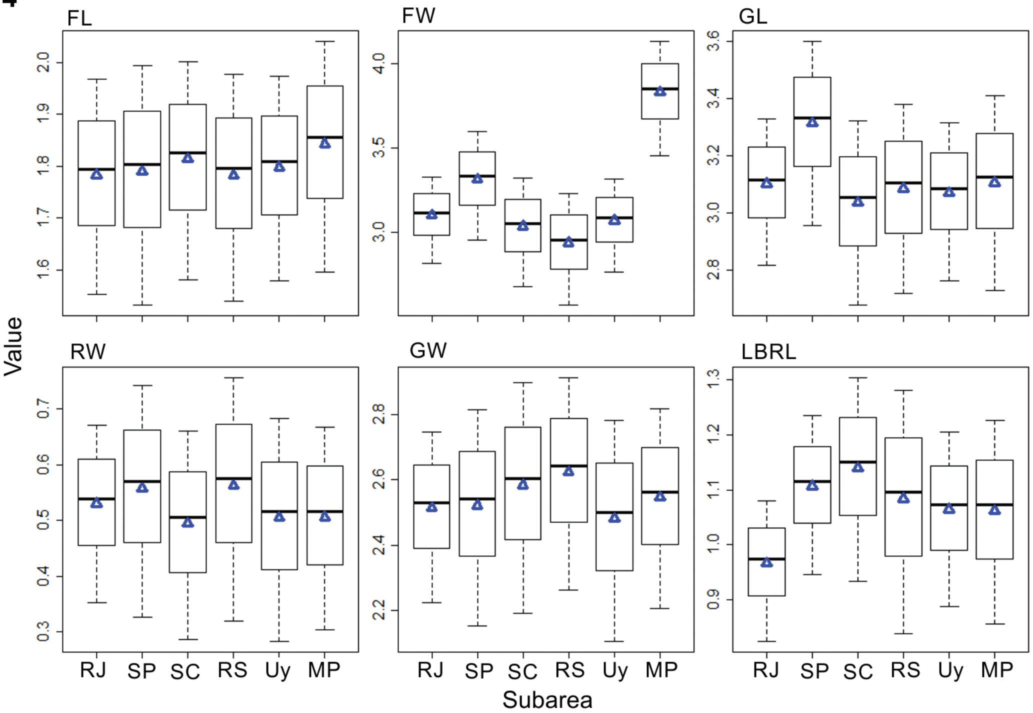

Figures 3–4. Box-Whisker plots of standardized values of attributes for the soft body parts of *Doryteuthis sanpaulensis* females (3) and males (4) from each region surveyed. Blue triangles represent mean values. FL: fin length, FW: fin width GL: gill length, RW: raquis width, GW: gladius width, LBRL: lower beak rostral length. RJ: Rio de Janeiro, SP: São Paulo, SC: Santa Catarina, RS: Rio Grande do Sul, Uy: Uruguay, MP: Mar del Plata.

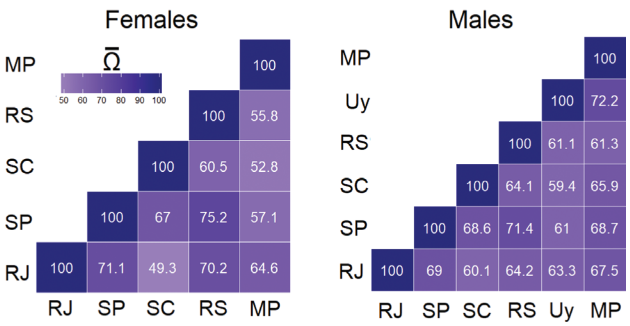

Figure 5. Heatmaps depicting the morphological overlap ($\bar{\Omega}$) among geographic areas in *Doryteuthis sanpaulensis*. RJ: Rio de Janeiro, SP: São Paulo, SC: Santa Catarina, RS: Rio Grande do Sul, Uy: Uruguay, MP: Mar del Plata.

size distribution of juvenile squid was unimodal regardless of the geographic area, but squid from the Patos Lagoon estuary were smaller than squid found north of it (which encompassed a similar size range) (Fig. 6).

The largest and smallest female squid were sampled south of 29°S (at Patos Lagoon estuary and off Uruguay, respectively), and in all cases, their size distribution was unimodal (Figs 7, 8). Females from Santa Catarina were larger than their male counterparts (Fig. 6).

In males, the average ML size and modes tended to decrease from Rio de Janeiro to Santa Catarina, increasing in squid sampled south of 29°S (Rio Grande do Sul) except at the Patos Lagoon estuary, where squid were <40 mm ML (Fig. 7). The largest average ML and modes were found off the Uruguayan coast, decreasing again in squid from Mar del Plata (Fig. 8). The size distribution of male squid from all remaining areas was unimodal, except for exemplars caught off Uruguay (Figs 6–8). Male-biased sex ratios were found in Rio de Janeiro (1 female: 2.2 males), São Paulo (1 female: 1.3 males), Santa Catarina (1 female: 1.9 males) and Mar del Plata (1 female: 1.4 males), and females outnumbered males in Uruguay (1 female: 0.5 males) ($\chi^2 = 2.7–19.6$, $p < 0.05$). Regarding bathymetry, the sex ratio was biased toward males in shallow (<31 m) bottoms (1 female: 1.4 males, $\chi^2 = 21.67$, $p = 3.24 \times 10^{-6}$) and toward females in deeper (>55 m) bottoms (1.3 females: 1 male, $\chi^2 = 4.06$, $p = 0.04$).

The proportion of *D. sanpaulensis* in each maturity stage in relation to the geographic subareas is given in Table 2. Immature females were the most frequent stage in all areas (≥74%), except off Uruguay (Table 2). Mature and spent females were frequent off Santa Catarina (42%) and off Uruguay (89.7%) (Table 2). Spent individuals were mostly recorded off the coast of Santa Catarina (Table 2). Maturing and mature males predominated in all subareas (>67.7%), except at the Patos Lagoon estuary (31.9%) (Table 2). Considering all subareas pooled, implanted

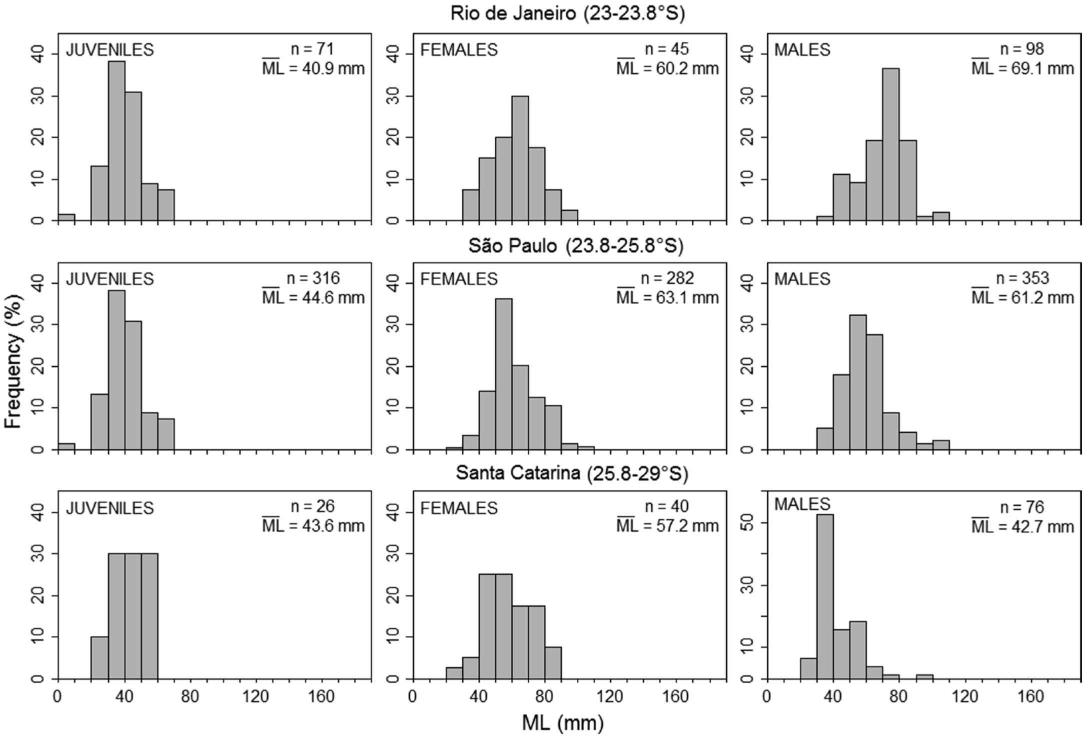

Figure 6. Size structure of *Doryteuthis sanpaulensis* according to the subareas nested within the SBB (22–29°S). Note de different y-axis.

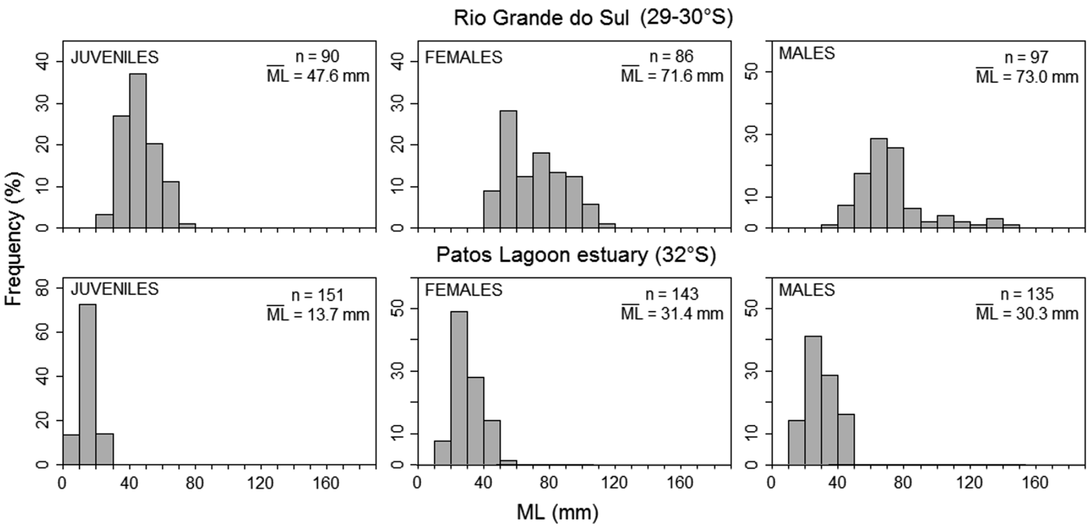

Figure 7. Size structure of *Doryteuthis sanpaulensis* according to the subareas nested within the Rio Grande do Sul coast (29–33.7°S). Note de different y-axis.

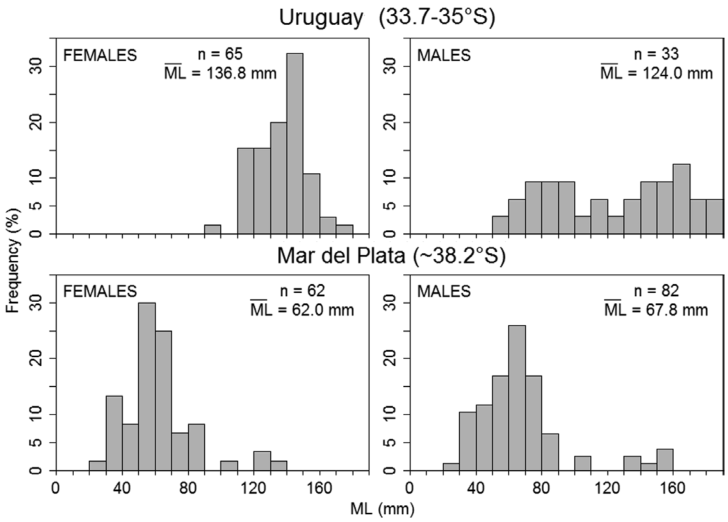

Figure 8. Size structure of *Doryteuthis sanpaulensis* according to the subareas nested within the Uruguayan-Argentinean coast (33.7–38.2°S).

spermatophores in the buccal receptacle occurred more frequently in mature females (n = 19), followed by spent (n = 6) and maturing squids (n = 1).

The size structure of *Doryteuthis pleii* is depicted in Fig. 9. The length of the mantle ranged from 30 to 247 mm. Since the morphological analysis had detected distinct groups (see above), two histograms were plotted separately (Fig. 9). Female squid between Cabo Frio and Paranaguá (23–26°S) had a normal size distribution with a distinct mode around 90–100 mm ML, whereas those off Santa Catarina (26–28°S) had a mode at smaller sizes (80–90 mm ML) (Fig. 9). The size distribution of male squid from both areas was skewed to the left, with a clear mode at 90–100 mm ML, although individuals between Cabo Frio and Paranaguá encompassed a wider size range (Fig. 9). The size range of juvenile squid was similar in both areas, but the mode obtained for squids sampled from Cabo Frio to Paranaguá was greater (Fig. 9).

In the two areas, the sex ratio of squid was significantly biased toward males (0.78 female: 1 male; $\chi^2 = 4.09$, p = 0.026). There were, however, no differences regarding bathymetric sex ratio (<30 m: 0.9 female: 1 male, $\chi^2 = 1.57$, p = 0.209 and >49 m: 0.88 female: 1 male, $\chi^2 = 1.10$, p = 0.292).

Of the 326 squid sampled for maturity assessment (143 females and 183 males), most (58% of females and 65.6% of

Table 2. Number of females and males of *Doryteuthis sanpaulensis* of each maturity stage per geographical area.

Geographic area	Maturity stages	Females		Males	
		n	%	n	%
Rio de Janeiro (23–23.8°S)	A	36	90.0	25	25.8
	B	1	2.5	21	21.6
	C	1	2.5	51	52.6
	D	2	5.0	–	–
	Total	40	100.0	97	100.0
São Paulo (23.8–25.8°S)	A	78	78.0	62	32.3
	B	5	5.0	58	30.2
	C	14	14.0	72	37.5
	D	3	3.0	–	–
	Total	100	100.0	192	100.0
Santa Catarina (25.8–29°S)	A	18	47.4	17	22.7
	B	4	10.5	23	30.7
	C	11	28.9	35	46.7
	D	5	13.2	–	–
	Total	38	100.0	75	100.0
Rio Grande do Sul (29–30°S)	A	57	74.0	39	43.8
	B	4	5.2	17	19.1
	C	13	16.9	33	37.1
	D	3	3.9	–	–
	Total	77	100.0	89	100.0
Patos Lagoon estuary (32°S)	A	118	82.5	92	68.1
	B	23	16.1	15	11.1
	C	2	1.4	28	20.7
	D	0	0.0	–	–
	Total	143	100.0	135	100.0
Uruguay (33.7–35°S)	A	2	2.9	2	6.5
	B	5	7.4	4	12.9
	C	60	88.2	25	80.6
	D	1	1.5	–	–
	Total	68	100.0	31	100.0
Mar del Plata (~38.2°S)	A	45	72.6	42.0	51.2
	B	12	19.4	29.0	35.4
	C	5	8.1	11.0	13.4
	D	0	0.0	–	–
	Total	62	100.0	82.0	100.0

males) were in an advanced stage of maturity (i.e. stages B, C and D). Nearly 44.1% of the females were mature, whereas 14% were maturing. Only 13.3% of the females were found spent. Immature squid comprised 34.4% and 42% of all males and females, respectively. No spent males were recorded. Mature females had the highest incidence of implanted spermatophores (n = 31), followed by spent individuals (n = 7) and maturing squid (n = 3).

The size of *Lolliguncula brevis* varied between 16 and 56 mm ML, females being larger than males, with a wider size interval. Two distinct modes were observed in female squid, 15–20 and 25–30 mm ML, whereas a well-defined mode at 25–30 mm ML (Fig. 10) was obtained for males. Only four females were found with implanted spermatophores, all of which were inside the mantle cavity.

All mated females were mature (stage C). Maturing and mature females (stages B and C) comprised little more than half of all individuals sampled (53.5%), followed by immature individuals (stage A) (43.6%). Only three spent females were found. Most mature females were caught in September 1979 at depths shallower than 18 m (FAUNEC III survey). In contrast, most male squid were mature (83.1%), and the bulk of them (n = 64) were caught in deeper waters (20–30 m). No spent males were found. The sex ratio was biased towards females (2.24 female: 1 male), although no significant difference was detected ($\chi^2 = 3.71$, p = 0.053).

The sizes of the three loliginids at maturity (ML_{50}) are given in Table 3. Note that calculations were made according to the availability of maturing/mature squid and, in some cases, not all stages were found in reasonable numbers. Size at maturity in female and male *D. sanpaulensis* varied according to the latitudinal gradient, with the largest and smallest ML_{50} found in Rio Grande do Sul and Santa Catarina, respectively, for both sexes

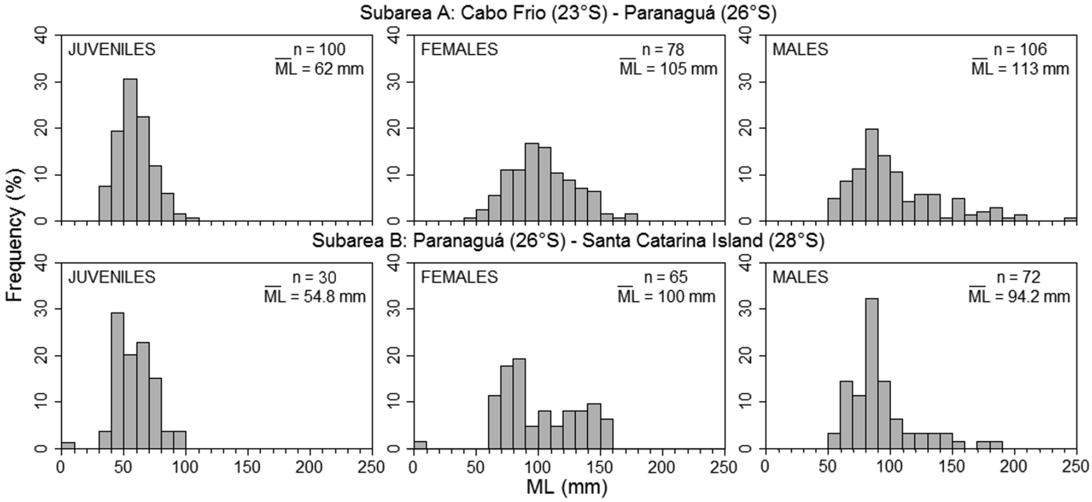

Figure 9. Size structure of *Doryteuthis pleii* caught off southern Brazil.

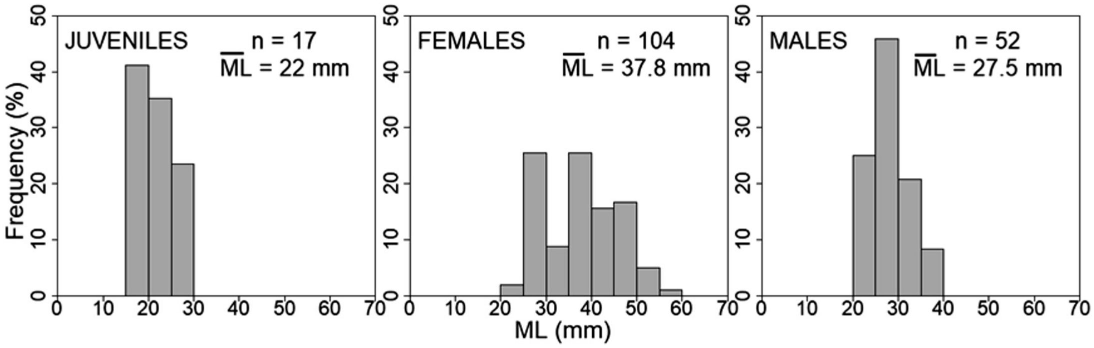

Figure 10. Size structure of *Lolliguncula brevis* caught off southern Brazil (24–26°S).

Table 3. Size-at-maturity (ML_{50}) of the three loliginid squids. Capital letters within brackets indicates the maturity stages used in the calculations (see Table 1). n = sample size.

Species	ML_{50} (mm)				Area
	n	females	n	males	
Doryteuthis pleii	22	110.16 (C + D)	30	86.85 (B + C)	22–29°S
Lolliguncula brevis	5	36.58 (B + C + D)	–	–	24–26°S
	–	–	10	66.66 (C)	Rio de Janeiro (23–23.8°S)
	8	81.56 (C + D)	16	56.55 (B + C)	São Paulo (23.8–25.8°S)
Doryteuthis sanpaulensis	9	61.11 (C + D)	10	56.55 (C)	Santa Catarina (25.8–29°S)
	9	82.52 (C + D)	14	78.17 (C)	Rio Grande do Sul (29–30°S)
	8	71.71(B +C + D)	8	66.66 (B + C)	Mar del Plata (~38.2°S)

(Table 3). Sizes at maturity of 104.66 mm ML and 86.85 mm ML were estimated for females and males of *D. pleii*, respectively, pooled for the whole study area (Table 3). A ML_{50} of 36.58 mm ML was estimated for female *L. brevis*, but in this case, sample size was very small (n = 5), which means that our results should be interpreted with caution. The number of mature males of *L. brevis* was not sufficient for a similar calculation.

Spatial and temporal patterns and response to environmental variables

The three loliginids were present on the SBB (22–29°S). *Doryteuthis sanpaulensis* and *D. pleii* were also recorded up to the southernmost portion of the study area (~38°S, Mar del Plata). The southernmost occurrence of *D. pleii* was based on four large male squid (>200 mm ML) sampled off Mar del Plata in 1972 and deposited at the malacological collection of the National Museum. *Lolliguncula brevis* occurred exclusively nearshore (<30 m) between 24 and 26°S (Fig. 2).

On the wider continental shelf (up to 130 m deep), *Doryteuthis* spp. had patchy distributions (Fig. 2), with mean catch rates of 21 ± 95.3 (SD) squid haul^{-1} for *D. sanpaulensis* (range 0–812 squid haul^{-1}) and 6.23 ± 23.2 (SD) squid haul^{-1} for *D. pleii* (range 0–167 squid haul^{-1}).

An analysis of the catches by depth showed that *D. sanpaulensis* was disproportionally more abundant in shallower depths (<40 m, 1210 squid caught, catch rate: 39 squid haul^{-1}) and moderately abundant at the deepest depth strata (100–130 m), although the highest catch rate was found between 70 and 100 m deep (993 squid haul^{-1}) (Fig. 11). *Doryteuthis pleii* was abundant on midshelf (395 squid caught, catch rate: 15.8 squid haul^{-1}), and the highest catch rate was recorded in the shallowest depth strata (<40 m, 369 squid haul^{-1}) (Fig. 11).

Regarding temporal occurrence, *D. sanpaulensis* was more abundant during mid-afternoon and in the first third of the night (between 3:00 pm and 9:00 pm). In contrast, the highest abundance of *D. pleii* was recorded between late night and the first third of the morning (3:00–9:00 am) (Fig. 11).

Doryteuthis sanpaulensis occurred more often in colder waters (T <19.5 °C), a pattern particularly evident for both bottom and surface temperatures. Likewise, the species was frequent in fresher waters (S <34.5), in both surface and the bottom, although it was found in high bottom salinities as well (Fig. 12). Interestingly, a reasonable number of *D. sanpaulensis* (n = 311) was also caught inside the Patos Lagoon estuary.

The patterns were less clear for *Doryteuthis pleii*, but apparently the species commonly occurs in warmer surface temperatures (T >21.5 °C), sharing with *D. sanpaulensis* the same bottom temperature (i.e. T ~19.0 °C). The response of *D. pleii* to water salinity signatures was also poorly discernible, but the species was apparently more frequent in saltier water (Fig. 12).

Doryteuthis sanpaulensis, *D. pleii* and *L. brevis* occurred together in the same haul only in four oceanographic stations

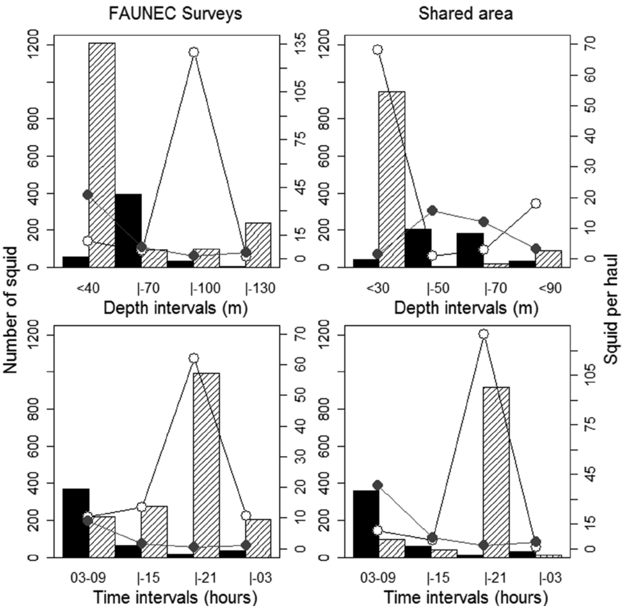

Figure 11. Total number and catch rates of *Doryteuthis sanpaulensis* and *D. pleii* collected in different depths and time of day during FAUNEC surveys and within the shared area off southern Brazil. Both metrics were calculated for stations where at least one of the two species was present (FAUNEC surveys) or where both species were present (shared area). Bars: number of squid. Circles: squid per haul. Crosshatched bars and empty circles: *D. sanpaulensis*. Black bars and dark grey circles: *D. pleii*.

conducted at a depth of <15 m. The Fager's affinity indexes calculated for *D. sanpaulensis*–*L. brevis* and *D. pleii*–*L. brevis* pairs were 0.28 and 0.31, respectively, indicating a poor association between *L. brevis* and the two *Doryteuthis* species.

Spatial and temporal patterns and response to environmental variables within the shared area

The two species of *Doryteuthis* co-occurred in only 12 out of the 78 oceanographic stations during the FAUNEC III and IV surveys. Those 12 stations were between 23.5 and 27°S ("shared area", comprising 32 oceanographic stations, see Fig. 2). A Fager affinity index of 0.7 was calculated for the two co-ocurrying species, suggesting a strong positive relationship between them. However, we believe that it is possible that this index does not reflect a strong affinity, since it was common to collect many exemplars of one species and only a few of the other in the same batch (Fig. 13).

Catches by depth strata within the shared area mirrored the general pattern for both species (Fig. 11). Catches by time of day differed between the two species: *Doryteuthis pleii* peaked between 3:00 am and 9:00 am (361 squid caught, 40 squid haul^{-1}), whereas *D. sanpaulensis* took peaked between 3:00 pm and 9:00 pm (915 squid caught, 130 squid haul^{-1}) (Fig. 11).

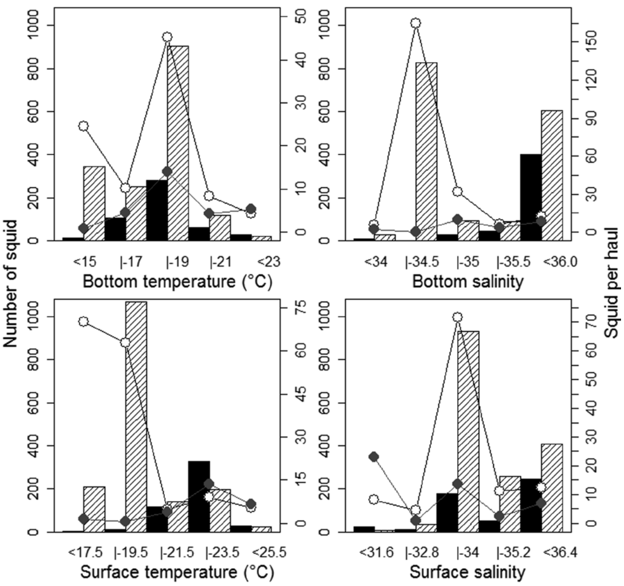

Figure 12. Total number and catch rates of *Doryteuthis sanpaulensis* and *D. pleii* collected in different temperatures and salinities during FAUNEC surveys off southern Brazil. Both metrics were calculated for stations where at least one of the two species was present. Bars: number of squid. Circles: squid per haul. Crosshatched bars and empty circles: *D. sanpaulensis*. Black bars and dark grey circles: *D. pleii*.

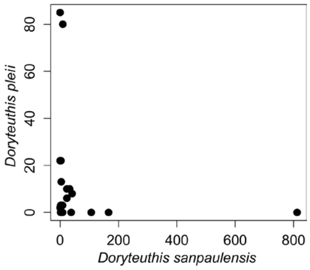

Figure 13. Catches of *Doryteuthis sanpaulensis* and *D. pleii* within the shared area.

Both species were caught in very similar environmental conditions within the shared area, except for the fact that *D. sanpaulensis* was caught in a slightly lower average salinity (surface: 34.90, bottom: 33.98) than *D. pleii* (surface: 35.15, bottom: 33.99). The temperature at the time of capture was the same for the two species (surface: 21.6 °C, bottom: 18.5 °C).

Feeding schedule in *Doryteuthis pleii*

The feeding activity of *D. pleii* was clearly concentrated between late evening and mid-morning (3:00–9:00 am) regardless of the stage of maturity stage and sex of the specimen. There was also an elevated number of empty stomachs found for the same period (Fig. 14).

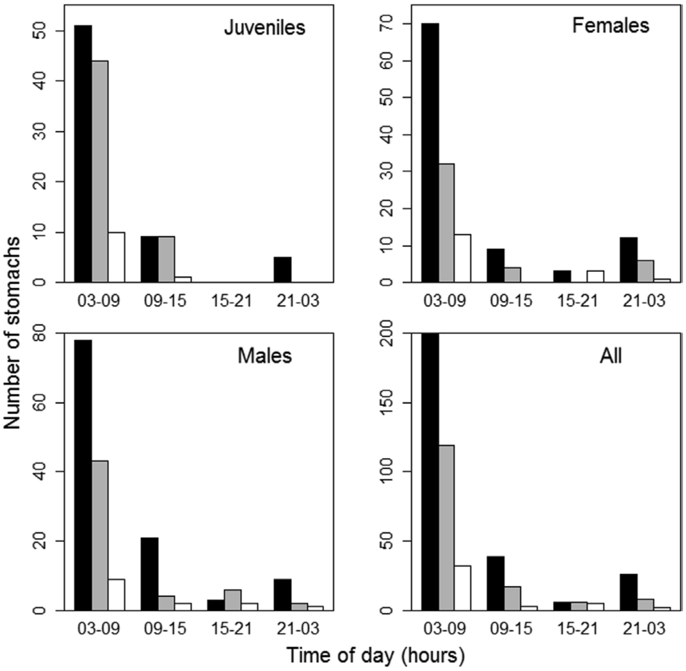

Figure 14. Stomach fullness frequency of *Doryteuthis pleii* collected during FAUNEC surveys per period of the day. Black bars: empty stomachs, grey bars: 50% full stomachs, white bars: 100% or distended stomachs.

DISCUSSION

This study has pioneered squid biology and ecology research in Brazil. For the first time ever, the population biology and ecology of the three most common shelf loliginids (*D. sanpaulensis*, *D. pleii* and *L. brevis*) were surveyed, along with early data on their spatial and bathymetric distributions and responses to environmental variables. The circadian levels of activity were described for the two species of *Doryteuthis*, along with the feeding schedule for *D. pleii*. In addition, a discriminant morphological analysis identified a latitudinal phenotypic variability for *D. sanpaulensis* and *D. pleii*, more remarkable in the first species. Hence, this survey may be regarded as a framework for all subsequent studies on loliginid squid biology and ecology off the southern Brazilian coast (e.g., Perez and Zaleski 2013, Vidal et al. 2013, Arkhipkin et al. 2015).

Geographic variation in *Doryteuthis* spp.

Ideally, studies on morphologic variations in squid populations must be conducted with samples collected simultaneously, to avoid mixed stock effects (Pierce et al. 1994). Even though this recommendation was followed for FAUNEC samples, we were not able to take simultaneous samples from Uruguay and Argentina. Despite the fact that Uruguayan and Argentinean squid were sampled in the same season (austral summer), their samples were obtained on different years, which may have introduced temporal biases to our analysis. Notwithstanding the latter, it was most instructive to keep these samples for comparison purposes, as they expanded our study area southward in nearly six degrees of latitude.

In addition to the limitations evoked above, it is also important to recognize that there were some other issues with our data that deserve mention. Most of our samples were obtained during a very narrow window of time, and since both *Doryteuthis* species produce at least two cohorts per year (Vidal et al. 2013, Arkhipkin et al. 2015), it is unlikely that we ended up with a representative set of environmentally driven morphological variability.

Cryptic squid species are difficult to separate on the basis of morphological analyses only (Herke and Foltz 2002) because squid display great phenotypic plasticity in response to environmental conditions (Pelc and Jackson 2008). Furthermore, even though it is unlikely that the morphological differences found in the squid from FAUNEC result from an artefact in the selectivity of the fishing gear (e.g. Martins and Perez 2007), since the same net was employed in all surveys, the same may not be the case of the samples collected from Uruguay and Argentina. This certainly must be considered when interpreting the results.

In our data, latitudinal morphological variability was very evident in *D. sanpaulensis*, as the dimensions of both soft and hard parts diverged significantly among geographically separated groups. This suggest that *D. sanpaulensis* populations experience different environmental conditions throughout the sampled area. This is in line with the restricted loliginid larval dispersal potential along the SBB (see Martins et al. 2014), which would expose paralarvae and early juveniles to distinct biotic and abiotic conditions. Environmental variability results in different growth patterns, and thus different final dimensions of body parts as squids mature (Pelc and Jackson 2008). In addition, the morphological variability among geographical groups suggests a strong homing behavior in adult squid.

The morphological variability of body parts along with the morphological overlap analysis and size at maturity data found for *D. sanpaulensis* in the study area suggests that there are at least six distinct groups scattered between Cabo Frio (23°S) and Mar del Plata (37.8°S). On the SBB (23–29°S), squid inhabiting ~26–28°S (Santa Catarina coast) had shorter mantle lengths and matured at smaller sizes than squid to the north and to the south of this region. In addition, males were significantly smaller than females, in contrast with the typical sexual dimorphism described for this species (i.e., males larger than females; Vidal et al. 2013). Actually, the squid from the Santa Catarina coast differed from others in almost all morphological characters investigated.

Doryteuthis sanpaulensis dwelling to the north and to the south of the coast of Santa Catarina were very similar in morphology. This suggests that these geographically divided groups experienced similar environmental conditions, at least during some part of their lifetime. Latitudinal gradients in water temperature may be a major driver of phenotypic divergence in *D. sanpaulensis*, since temperature has a direct effect in growth-related parameters (Forsythe 2004). However, variability in other environmental conditions, such as dissolved oxygen and food availability, may also play a role (Jackson and Moltschaniwskyj 2001, van der Vyver 2016).

The most distinct geographic group of *D. pleii* was also found off the coast of Santa Catarina. Nevertheless, the species had just two distinguishable groups, and these were much less distinct than those of the co-generic species. Thus, it is suggested that the phenotype of *D. pleii* in Santa Catarina also results from local environmental conditions. However, due to the subtle morphological differences between those two groups, we hypothesise that *D. pleii* individuals move more between latitudes than *D. sanpaulensis*, perhaps "homogenizing" the environmental conditions experienced during the squid's lifetime, since they actively track suitable temperature fields (see Haimovici and Perez 1991b). In addition, these latitudinal movements may promote strong gene flow among different stocks at the SBB. This hypothetical scenario is consistent with a recent genetic analysis that failed to find any relevant genotypic differences between squid from this area and their counterparts on the rest of the South American Atlantic coast (Sales et al. 2017).

Spatial patterns resulting from environmental variability

In our data, *Doryteuthis* spp. have specific thermal preferences. *Doryteuthis sanpaulensis* was found more often in colder waters, whereas *D. pleii* was associated with tropical (warmer) waters. The two species occurred together only when the water was thermally stratified (when both cold and warm water were present in the same location but in different strata) in the shared area. This ability to actively select water strata that are suitable for them has allowed these two species of squid to exploit locations where they would not normally occur.

We found small and mostly immature representatives of *D. sanpaulensis* at the Patos Lagoon estuary in January, February and March 1979. The presence of these squid inside the estuary during three consecutive summer months does not seem to be by chance. It is worthy of note that *D. sanpaulensis* had not been recorded in estuaries (at least in Brazilian waters), and to the best of our knowledge, this is the first record of a loliginid squid other than *L. brevis* (which has well-described physiologic mechanisms to tolerate low salinities, see Hendrix et al. 1981) inside an estuary.

These small *D. sanpaulensis* may enter the estuary during events of physiologically tolerable environmental conditions to exploit the food-rich, productive estuarine areas. This could be related to landward intrusion of the salt edge into the upper estuary, a near-bottom, high-salinity tongue of seawater, which is particularly strong during austral summer months, when the freshwater discharge is less (Möller et al. 2001). Off the coast Rio de Janeiro, juvenile *D. sanpaulensis* were found entering the warm Guanabara Bay (22°43'S), during flood tide, tracking bottom landward intrusions of cold SACW. In this case, squid seems to use the physiologically suitable near-bottom temperature field to exploit the abundant food resources inside this eutrophized tropical coastal bay (Moraes and Lavrado 2017).

When this study was conducted, our data extended the southernmost distribution range of *D. pleii* (29°S, Palacio 1977) by at least nine degrees of latitude, owing to some specimens obtained at ~38°S (Mar del Plata, Argentina). In fact, latter studies found that *D. pleii* occurs during spring and summer to the south of its normal range over the shelf break between 29 and 34°S, tracking the warm, fast flowing superficial Brazil Current (BC) (Haimovici and Perez 1991b). Recently, *D. pleii* was recorded as far as Península Valdez, Argentina (42°S) (Augusto Crespi-Abril 2017, personal communication). Thus, it is clear that stronger poleward BC penetration in some years may carry vagrant individuals toward higher latitudes during austral warmer seasons.

Competition avoidance between *Doryteuthis* spp.

According to our results, the two species of *Doryteuthis*, which are morphologically similar, employ several mechanisms to avoid direct competition in the wider continental shelf. For instance, the diel activity pattern was inverse, with *D. pleii* being more active during daylight, whereas *D. sanpaulensis* was more active at night. Furthermore, both species had clearly different thermal preferences: *D. sanpaulensis* was found more often in colder temperatures than *D. pleii*. In addition, the most common depths where each species was found were different. The feeding schedule in *D. pleii* peaks between late night and early morning, whereas *D. sanpaulensis* feeds more intensively between mid-morning and late afternoon (Andriguetto and Haimovici 1997). This temporal mismatch between feeding activity schedules may be regarded as an additional mechanism that prevents direct competition.

Concluding remarks

This study revisits data from a time when climate change had not yet been perceived as an agent of significant environmental variability in the ocean (Abran et al. 2016). Thus, the information reported here may be regarded as a "snapshot" on the ecology of sympatric squids in a colder and less acidic marine environment (see Nagelkerke and Connell 2015). It seems that the abundance of the commonest loliginid squids off the southern Brazilian coast has shifted in the last few decades. *Doryteuthis pleii* apparently replaced *D. sanpaulensis* as the most abundant squid in some areas where the latter species used to be dominant, such as off the coast of northern Rio de Janeiro (~23°S) (e.g., Costa et al. 2017). Whether this apparent shift was caused by climate change or not is currently unknown. Accordingly, our results may be particularly important for a better understanding of more recent studies on the biology and ecology of loliginid squids in the southwestern Atlantic Ocean and elsewhere.

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Supplementary material 1

Details of the material analyzed
Authors: Rodrigo Silvestre Martins, Marcelo Juanicó.
Data type: species data
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zoologia.35.e23176.suppl1

Supplementary material 2

The Modified Royce Method (Juanicó 1978)
Authors: Rodrigo Silvestre Martins, Marcelo Juanicó
Data type: statistical data
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