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PRIMARY RESEARCH PAPER

Uncovering *Lampris* species (Actinopterygii, Lampridae) in the southwestern Atlantic Ocean: a molecular and morphometric approach

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Abstract Recent studies on the genus *Lampris* revealed five cryptic species within *Lampris guttatus*; however, the new phylogenetic structure of the globally distributed group claims more information from the South Atlantic Ocean. The present work, with a focus on the *Lampris* genus fishes from the Southwest Atlantic Ocean (SWAO), aims to (i) fulfill a gap in the distribution description of the recently uncovered species from the group (ii), making comparative analysis to molecular and morphological studies on the group and (iii) easily diagnose characters to facilitate identification in the field. The analysis of partial mitochondrial cytochrome c oxidase I gene sequences (705 pb) led to the identification of *Lampris australensis* and *Lampris megalopsis* and their genetic diversity. Compared with other oceanographic regions, both species presented high haplotypic diversity and low population structure. Principal component analysis using morphometric data revealed two distinct morphotypes with a slight overlap. PC-LDA analysis also showed two well-separated groups, with a high percentage of correctly classified fish for *L. australensis* and *L. megalopsis*. We also demonstrate that both species can be morphologically distinguished. This study confirms *L. megalopsis* as a globally distributed species and expands the distribution of *L. australensis*, which can now be considered circumglobal along the subtropical waters of the southern hemisphere. Understanding species captured as bycatch in fisheries based on their correct identification is fundamental for establishing conservation and management initiatives for marine fish populations.

Keywords Opah · Bycatch · COI · Conservation genetics · Fish morphology · Fisheries management

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Introduction

Opah species from the genus *Lampris* are caught as bycatch in all oceans by longline fisheries targeting large pelagic fish such as swordfish, tunas, and sharks (e.g., Runcie et al., 2009; Huang & Liu, 2010; Hawn & Collete, 2012; Underkoffler et al., 2018).

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However, the commercial interest in its capture has increased due to the quality of its meat, which has sparked growing culinary interest (Underkoffler et al., 2014). Despite this interest, the biology of opahs is still poorly studied, and their intra and interspecific relationships are still unclear (Hyde et al., 2014), as well as many aspects of their distribution (Underkoffler et al., 2018).

Until recently, the genus *Lampris* was thought to be a taxon comprising two species of opah: The opah *Lampris guttatus* (Brünnich, 1788) and the Southern opah *Lampris immaculatus* Gilchrist, 1905. *Lampris gutattus* was known as an epi-mesopelagic global species of easy visual identification by its white spots, which in *L. immaculatus* is absent (Hawn & Collete, 2012). However, DNA barcoding revealed five cryptic monophyletic lineages within *L. guttatus* species (Hyde et al., 2014), further taxonomically described by Underkoffler et al. (2018). More recently, Kukuev (2021) proposed an update of the genus *Lampris*, in which the spotless species, *L. immaculatus*, was allocated in the subgenus *Paralampris*, while the five spotted species were grouped in the subgenus *Lampris.*

Based on morphometric measurements of fresh opahs from Hawaiian fish markets, preserved samples from museums, and photographic records, the distribution of the newly described species was proposed by Underkoffler et al. (2018) as *L. guttatus*, restricted to the North Atlantic Ocean (NAO); *L. lauta* Lowe, 1838, in the Eastern Atlantic Ocean; *L. incognitus* Underkoffler et al. 2018, in Central and Eastern North Pacific; *L. australensis* Underkoffler et al. 2018, known only in the southern hemisphere, with records in Australia, Chile, South Africa, and Tasmania; and *L. megalopsis* Underkoffler et al. 2018, presumably a cosmopolitan species with records from the western Central-North Pacific to American Samoa, Australia, Indonesia, and South Africa. However, for the South Atlantic Ocean (SAO), the authors included samples only from the South African coast (Hyde et al., 2014; Underkoffler et al., 2018), which leaves a considering gap in the description of the potential distribution of the newly described species. In the Southwestern Atlantic Ocean (SWAO), records of the *Lampris* genus refer to *L. guttatus* lato sensu in southeast Brazil (Figueiredo & Menezes, 1980; Lopes & Oliveira-Silva, 2017), south Brazil (Piacentino & Muguetti, 1994), Uruguay (Nion et al., 2002) and Argentina (Piacentino & Muguetti, 1994); since these records are previous to the recent taxonomical changes, a review is needed.

Longline fishing fleets from more than seven countries operate in the SWAO, targeting swordfish *Xiphias gladius* Linnaeus, 1758, pelagic sharks (mainly blue shark *Prionace glauca* (Linnaeus, 1758), and several tuna species [bigeye *Thunnus obesus* (Lowe, 1839), yellowfin *T. albacares* (Bonnaterre, 1788), and albacore *T. alalunga* (Bonnaterre, 1788)] throughout the year (Tuck et al., 2003; Hazin et al., 2008; Jiménez et al., 2011). Little is known about the magnitude of opah bycatches in the region. Two years of longline landings monitoring the Brazilian fishing fleets operating in the SWAO recorded 3.6 tons in 2018 and 4.8 tons in 2019 (UNIVALI/EMCT/LEMA, 2020). If we consider that fishing fleets of at least six other countries also fish in the region, this amount is likely much higher. Catches in the SWAO are recorded only as a single species, and it is unknown if they are comprised of one or more species of the genus *Lampris*.

The erroneous and/or imprecise identification of exploited species is one of the main problems for fisheries management (Lleonart et al., 2006). Species misidentification causes uncertainty in population assessments, hindering its sustainable exploitation and potentially precluding accurate information on population changes for conservation purposes (Beerkircher et al., 2009; Garcia–Vazquez et al., 2012). For example, important species such as the white marlin *Kajikia albida* (Poey, 1860) and the roundscale spearfish *Tetrapturus georgii* Lowe, 1841 have unknowingly been assessed and managed as a single species, which raised concern as to the stock assessments and the conservation status of both species in the western North Atlantic (Shivji et al., 2006; Beerkircher et al., 2009). A high misidentification of carcharhinid sharks [*Carcharhinus leucas* (Müller & Henle, 1839), *C. amboinensis* (Müller & Henle, 1839), *C. tilstoni* (Whitley, 1950), *C. sorrah* (Müller & Henle, 1839) and *C. brevipinna* (Müller & Henle, 1839)] has also been reported in a fishery in northern Australia, which could result in incorrect estimates of fisheries mortality that are used for modeling stock resilience (Tillett et al., 2012). Another example of misidentification is for the highly vulnerable batoid fish *Dipturus intermedius* (Parnell, 1837) and *D. batis* (Linnaeus, 1758), formerly known as the "common ray". The extinction risk is likely significantly higher

than previous estimates that treated both species as a single homogeneous unit, and the correct identification of these species is necessary for the proper designation/implementation of conservation measures (Bache-Jeffreys et al., 2021). Thus, the accurate identification of commercially landed fish at the species level is essential for validating reported catches and collecting biological data, which are necessary for modeling population status and managing stocks. In fact, the United Nations Food and Agriculture Organization (FAO) program has highlighted the importance of species identification in fishery sciences since the 1960s (Lleonart et al., 2006). Considering the influence of humankind on nature through environmental disturbances, this information is becoming more relevant as many ecosystems suffer from severe deterioration (Pacifici et al., 2015).

Considering that the current distribution information is insufficient for identifying genus *Lampris* species present in the SWAO, hindering an adequate assessment of the distribution and biology of these fishes in this part of the globe, the goal of this study was to identify which *Lampris* species occur in the SWAO. This was achieved through a combination of molecular and morphometric analyses, providing new information on the distribution range and the morphological diagnosis of the species found in the region.

Materials and methods

Eighty-eight opahs were sampled opportunistically between July 2018 and March 2020 (see Table S1). Sampling the surface longline fleets operating in SWAO was conducted during landings in Rio Grande, RS, Brazil (Fig. 1). Fragments of muscle tissue from 20 individuals were stored in 95% alcohol until DNA extraction; these samples were chosen based on general morphological differences between specimens to attempt to include all possible morphotypes. Photographic identification was made for 79 individuals, and morphometric measurements of 65 samples were obtained based on Underkoffler et al. (2018). To guarantee the measurement of mature fish, we chose those with fork lengths close to 760–800 mm. As far as we know, there are no maturity studies for the recently studied species of opah, but a comprehensive study of *L. guttatus* lato sensu (Francis et al., 2008) indicates that sexual maturity is reached between 760 and 800 mm for males and females.

DNA extraction and amplification

Total genomic DNA from tissue samples was extracted through a salt-extraction protocol adapted from Aljanabi and Martinez (1997): instead of using the salt homogenizing buffer followed by SDS (2% final concentration), we used a lysis buffer already containing SDS and with the addition of β-mercaptoethanol (200 mM NaCl, 50 mM Tris–HCl pH 8.0, 10 mM EDTA pH 8.0, SDS 1% and β-mercaptoethanol 1%). The concentration (ng) of extracted DNA was measured in a Biodrop® 2000 spectrophotometer. Amplification of 705 bp fragments of the mitochondrial cytochrome c oxidase I gene (COI) gene was performed by polymerase chain reaction (PCR), with specific primers designed by the authors (opah Atl-S F: 5′ CCC TAC CTG TGG CAA TCA CTC G 3′ and opah Atl-S R: 5′ GGG AGA TTA TTC CAA AGC CAG G 3′). Primers were designed using the complete mitochondrial genome of *L. guttatus* available in the National Center for Biotechnology Information GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/, Accession NC_003165.1).

PCRs were carried out with PCR buffer (10%), $MgCl_2$ (3 mM), dNTPs (0.4 mM), primers (0.2 µM), Taq DNA polymerase (0.5 units—Ludwig Biotechnology), 4 µl of DNA (150 to 200 ng/ml), and ultrapure water up to 25 µl. PCR cycling conditions consisted of (1) one cycle of 94 °C for 1 min; (2) 35 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min; and (3) a final cycle at 72 °C for 5 min. To evaluate PCR success, products were submitted to electrophoresis on a 1% agarose gel with GelRed® nucleic acid stain and photographed in a UV UVP® M20 transilluminator. PCR products were then purified by precipitation with PEG 8000 at 20% (polyethylene glycol PM 8000), following Hartley & Bowen (1996), and resuspended in buffer TE pH 7. Negative controls were performed on all PCR reactions to check for contamination, and samples were sequenced in both directions at Macrogen, Seoul, Korea (http://www.macrogen.com). Sequences were visually evaluated and corrected with BioEdit 7.2.5 (Hall, 1999) and aligned using CLUSTAL W (Thompson et al., 1994).

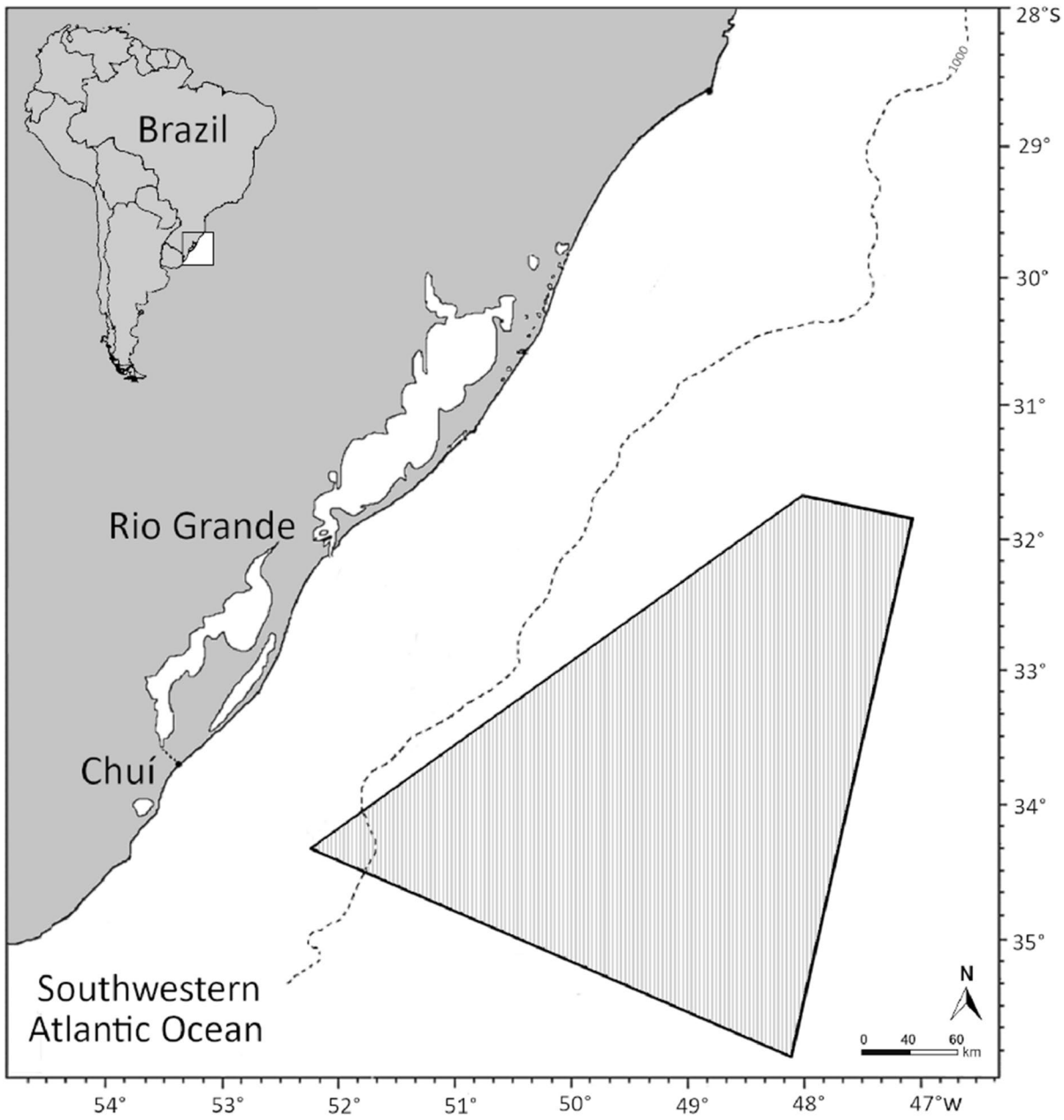

Fig. 1 Map of the sampling region along the Brazilian coast. The gray shape indicates the area where longline vessels captured *Lampris* spp. between 2018 and 2020

Molecular data analysis

Molecular identification of *Lampris* sequences (GenBank accession) was made in the Barcode of Life Data System (Ratnasingham & Hebert, 2007). All sequences achieved at least 98.92% of similarity with sequences already in this database, ensuring correct identification. The number of COI haplotypes was calculated using DnaSP 4.10.9 (Rozas & Rozas, 1999), and haplotype and nucleotide diversities were calculated using ARLEQUIN 3.5 (Excoffier & Lischer, 2010). The distribution of pairwise interspecific and intraspecific genetic distances (gap analysis) was evaluated in the statistical environment R, using

the 'barcodingR' package (Zhang et al., 2017). For this analysis, the Kimura-2-parameter (K2P) model was applied due to its common application in DNA barcoding literature (Čandek & Kuntner, 2015). A Maximum Likelihood Tree was constructed using MEGA X (Kumar et al., 2018) to evaluate phylogenetic relationships with the Tamura–Nei model of nucleotide substitution and rooted with a *L. immaculatus* COI sequence available in GenBank (Accession DQ108066.1).

To compare with other previously analyzed *Lampris* populations, we also conducted genetic diversity and gap analyses using sequences from the same species identified in this study, reported in Hyde et al. (2014) (datadryad.org/stash/dataset/https://doi.org/10.5061/dryad.71m33). For these comparative analyses, sequences were grouped according to the oceanographic region: Southwestern Atlantic Ocean (SWAO), North Pacific Ocean (NPO), South Pacific Ocean (SPO), SAO, South Indian Ocean (SIO), and the region between South Atlantic and South Indian Ocean (South Africa coast) (SAO–SIO) for *L. megalopsis*; and SWAO, SAO, SIO and Indian Ocean (IO) for *L. australensis*. The sequences obtained for the SWAO were truncated to 655 bp to compare with the previously available sequences.

We used all available sequences to construct median-joining (Bandelt et al., 1999) haplotype networks with POPART v1.7 (Leigh & Bryant, 2015) and evaluate the overall genetic structure of the two species between regions. Structure was evaluated through analysis of molecular variance (AMOVA) using the Tamura–Nei model of nucleotide substitution (Tamura & Nei, 1993), as determined in JModelTest 2.1.10 (Darriba et al., 2012), with significance tests based on 1,000 permutations (Excoffier et al., 1992). Resulting F_{st} values were classified as low, moderate, high, and very high when ranging from 0–0.05, 0.05–0.15, 0.15–0.25, and > 0.25, respectively (Wright, 1978).

Morphometric and meristic analysis

Measurements were taken to the nearest mm with a 1.5 m tree caliper when larger than 10 cm and with a 15 cm analogical hand caliper when smaller. Circumference measurements were taken with a 2 m measurement tape. From the set of 45 measures and counts, the 13 most informative were used in the analyses since the number of predictor variables (p) is required to be less than the sample size (n). The two filtering criteria we used were: (1) the formula $n \geq 5 \times p$ (James et al., 2013) and (2) the most informative morphometric measurements, in an exploratory PCA analysis (see Supplementary Material). Prior to the analyses, the morphometric data were standardized by dividing their individual values by the fork length.

The measurements and counts used for analysis were: greatest body depth (BD), head length (HL), head depth (HD), pre-pectoral distance (PPecD), pre-anal distance (PAD), vertical orbital diameter (vOD), horizontal orbital diameter (hOD), snout length (SnoutL), pectoral-fin height (PecFinH), maximum diameter (MD), operculum diameter (OD), caudal peduncle depth (CPD), and number of pelvic fin rays (*V*) (Fig. 2). Caudal fin rays were not counted as they were extremely packed. Measurements and counts followed Underkoffler et al. (2018), except for HD, which was measured as the vertical distance from the top of the head to the abdomen on a line crossing the center of the orbit, and SnoutL, represented by the premaxillary frontal height (Fig. 2). To reduce the dimensionality and better visualize the morphometric data, a second principal component analysis (PCA) was conducted with the 13 selected measurements and counts, using the 'factoextra' package (Kassambara & Mundt, 2020). The 'missMDA' package (Josse & Husson, 2016) was used to fill in missing values between variables.

Photographs were collected whenever possible to identify the opahs captured in the SWAO and detect some easy-to-diagnose characteristics. The general shape of the body and its appendages, coloration, in addition to the shape, distribution, and size of the spots on the body were observed according to the description of the species (Underkoffler et al., 2018) and also by comparison between samples. Subsequently, comparisons were made with the results of molecular and PCA analyses to identify the sampled opah. Complementarily, a linear discriminant analysis (LDA) was used to increase the classification efficiency and check our visual identification by photographs. For this, the first two principal components (PCs), which contained the maximum variance among the data, were used as input for LDA construction (PC-LDA). Two pre-defined groups for the PC-LDA were inserted based on the visual identification

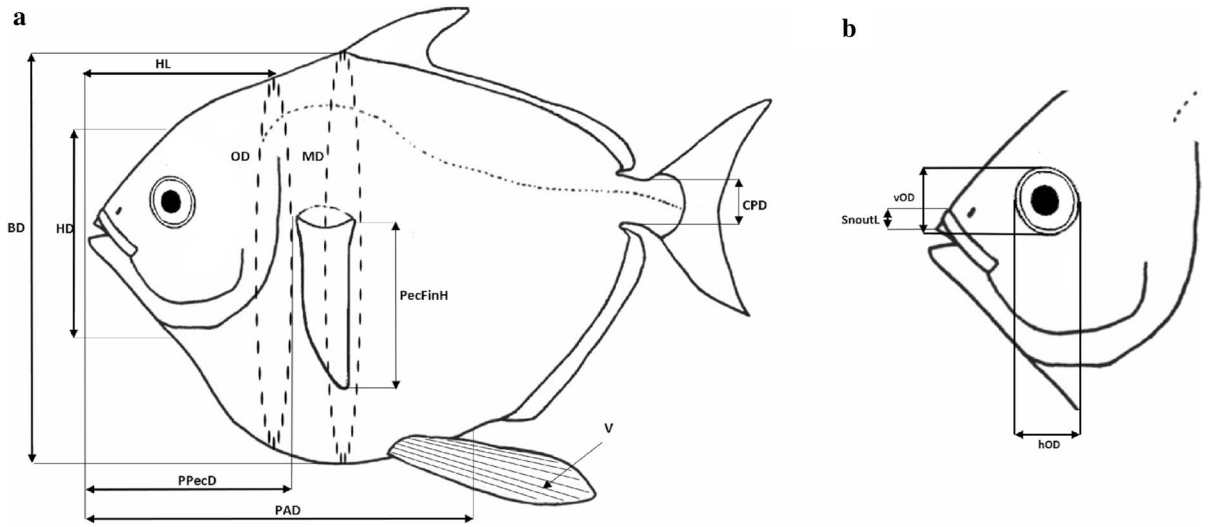

Fig. 2 Morphological measurements and count scheme (V) for subgenus *Lampris*. **a** Greatest body depth (BD), head length (HL), head depth (HD), pre-pectoral distance (PPecD), pre-anal distance (PAD), pectoral-fin height (PecFinH), maximum diameter (MD), operculum diameter (OD), pelvic fin rays (V), and caudal peduncle depth (CPD). **b** Vertical orbital diameter (vOD), horizontal orbital diameter (hOD), snout length (SnoutL)

of the samples. The PC-LDA model was validated by leave-one-out cross-validation. Distribution frequencies of landings by season and fork lengths (mm) were constructed to better visualize patterns among the sampled specimens, and significant differences were evaluated by one-way ANOVA. All analyses were performed in the statistical environment R (R Core Team, 2020).

Ethical statement

The research did not involve animal experimentation or harm and required no permits under Brazil animal welfare laws.

Results

Two species of the genus *Lampris*—*L. australensis* and *L. megalopsis*—were revealed for the first time in the SWAO, expanding the distribution range for both species. From the 88 sampled specimens, 26 were identified as *L. megalopsis,* and 62 were *L. australensis* either by photographs, molecular, or morphometric analysis (Table S1). The occurrence of *Lampris* species in the SWAO is shown in a worldwide context in Fig. 3, adapted from Hyde et al. (2014).

Of the 20 individuals with COI sequenced, thirteen were identified as *L. australensis* and seven as *L. megalopsis*. Eight haplotypes were found for *L. australensis*, four being described for the first time and four matching haplotypes previously reported by Hyde et al. (2014) for *L. australensis* in the SAO, IO, and SIO (Table 1; Fig. 3). Four haplotypes were found for *L. megalopsis*, of which one was new and three had been previously reported in several other regions (Hyde et al., 2014). Molecular diversity analyses showed high haplotypic and low nucleotide diversity for *L. australensis* and *L. megalopsis* from the SWAO (Table 1).

The median-joining haplotype networks for *L. australensis* and *L. megalopsis* (Fig. 4) from several geographic regions showed that one to three mutations separated haplotypes. Haplotypes 1 and 2 of *L. megalopsis* represent worldwide haplotypes present in all oceans. The barcode gap analysis showed that the percent difference of sequences ranged from 0 to 0.011 between individuals of the same species and from 0.066 to 0.080 between individuals of different species in the SWAO (Fig. 5). Mean values were, respectively, 0.0035 (s.e. 0.0024) and 0.0727 (s.e. 0.0288), resulting in an overall gap of 0.056. The results for this analysis using the sequences of these species available in Hyde et al. (2014), and with all data

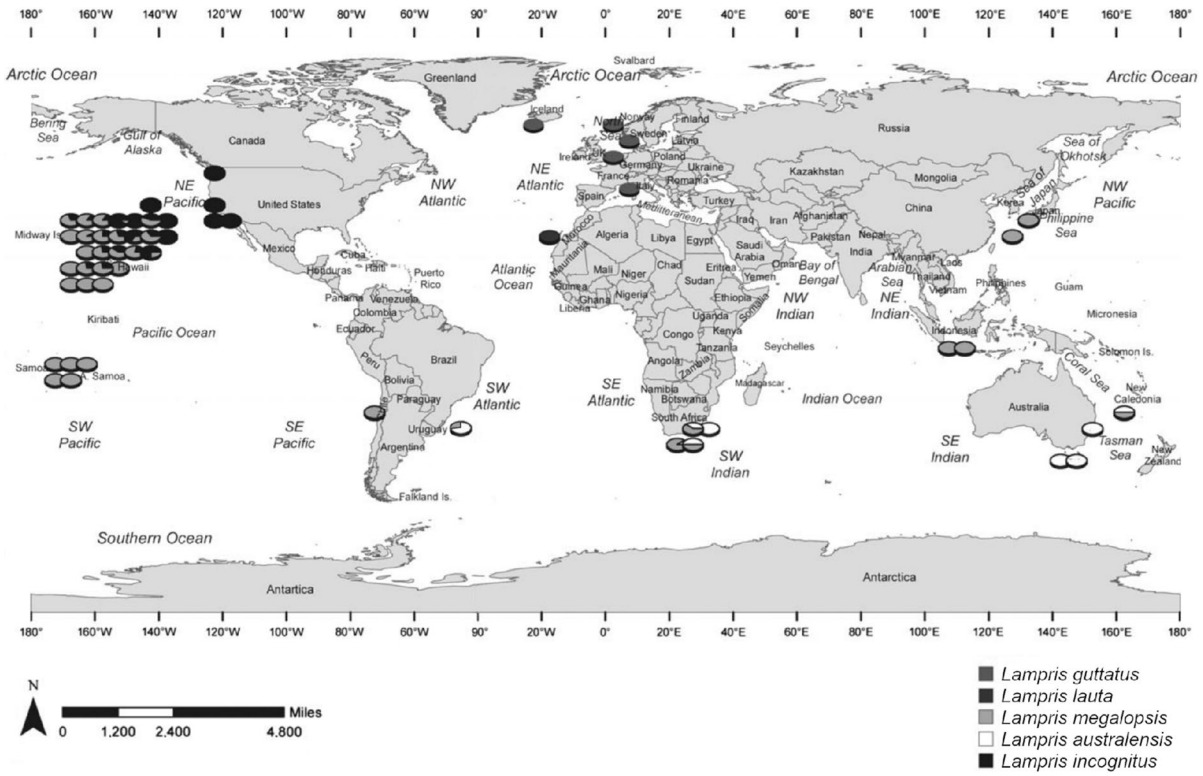

Fig. 3 Worldwide distribution of *Lampris* species described in Hyde et al. (2014) and the present study. The pie charts represent the proportion of each species described by Underkoffler et al. (2018), per location. Figure adapted from Hyde et al. (2014)

pooled, were similar. The phylogenetic tree produced two distinct clades containing and confirming the identified species (Fig. 6). AMOVA F_{st} values among regions were low, indicating no population structure for *L. australensis* ($F_{st}=0.02$; $P=0.41\pm0.012$). However, despite the low F_{st} found among *L. megalopsis* regions ($F_{st}=0.02$; $P=0.02\pm0.004$), the difference was significant.

PCA of morphometric data resulted in a plot showing two ellipses. The first principal component (PC1) explained 38.8% of the morphometric variation, and the second (PC2) explained 21.6% (Fig. 7). The other eleven components did not surpass 8.1% (Fig. S1). For *L. megalopsis*, PAD, HL, and PPecD were some of the measurements with the highest loadings (Table S2). These measurements are more longitudinally distributed along the body length than in *L. australensis*, which presented the highest values in measurements taken latitudinally, such as HD, greatest BD, and CPD, besides the OD and MD measurements. In the second principal component (PC2), PAD, OD, PecFinH, and HD were the main variables that explained the differences.

The PC-LDA analysis, built from two pre-defined groups by visual identification and 13 measurements from 65 individuals, resulted in a percentage of correctly classified (PCC) fish of 100% for *L. australensis* and 98% for *L. megalopsis*. These characters were noticed throughout sampling and photograph analysis, based on the description by Underkoffler et al. (2018) and subsequent genetic and morphometric confirmation.

Lampris australensis presents larger white spots, mainly in the anterior ventral region below the head, which can approximate the size of the fish's pupil and gives the impression of a darker outline (Fig. 8). The spots become smaller and sparser as they approach the posterior and dorsal regions. Eyes are smaller than in *L. megalopsis*, and the general body shape is rounder. The top of the head can sometimes be distinctly arched. In *L. megalopsis*, the spots are smaller and circular but often have long, irregularly shaped

Table 1 Molecular diversity indexes of *Lampris australensis* and *L. megalopsis* in the SWAO (This study) and from Hyde et al. (2014)

Species	References	*n*	*H*	uH	$h \pm SD$	$\pi \pm SD$	T_s	T_v	*V*	$P \pm SD$	*%C*	*%T*	*%A*	*%G*
L. australensis	This study	13	8	4	0.923 ± 0.050	0.003 ± 0.002	6	1	7	2.784 ± 1.573	30.61	28.18	21.61	19.60
L. australensis	Hyde et al. (2014)	7	6	2	0.952 ± 0.095	0.004 ± 0.003	6	1	7	2.969 ± 1.761	30.86	27.68	21.98	19.48
L. megalopsis	This study	7	4	1	0.809 ± 0.129	0.002 ± 0.001	4	1	5	1.631 ± 1.088	31.59	27.09	21.99	19.33
L. megalopsis	Hyde et al. (2014)	277	49	46	0.795 ± 0.001	0.002 ± 0.001	42	5	46	1.371 ± 0.848	31.73	26.73	22.42	19.12

Species sample sizes (*n*), total number of haplotypes (*H*), number of unique haplotypes (uH), haplotype diversity (*h*), nucleotide diversity (π), number of transitions (T_s), number of transversions (T_v), number of variable sites (*V*), uncorrected average pairwise differences between samples (*P*), and % nucleotide composition for cytochrome c oxidase I gene data

spots between them. Pectoral fins are usually longer than in *L. australensis*, and the eyes are notably bigger.

The body color of both species may vary over the time of death. Animals that were recently caught lose their vivid red and orange colors, but remain with red fins and a pinkish color on the body, especially if the scales are still present. After some time, *L. megalopsis* tends to maintain a pinkish or present a gray metallic color while *L. australensis* can remain pink (Fig. 8) or present a metallic blue color, as described in Underkoffler et al. (2018). The tongues of both species occasionally presented purple coloration spots, varying in size, intensity, and shape; sometimes the whole tongue was purplish. In most fish sampled, the fins had a vivid red color. However, when the fish had been stored for a few days on the fishing vessel or frozen in the laboratory for later processing, the tips of the caudal and pectoral fins sometimes had a yellowish color and dryness. It was not possible to identify SWAO specimens with the proposed key by Underkoffler et al. (2018) as most of the proportions did not match the description (Table 2).

Based on the multiple identification methods used, we observed that the frequency of each species differed among seasons, with *L. megalopsis* presenting higher frequency in summer months while *L. australensis* were more frequent throughout the rest of the year (Fig. S2). The size composition indicates that *L. megalopsis* are generally larger than *L. australensis* (Fig. S3), which was confirmed with a one-way ANOVA [$F(1.79) = 6.306$, $P = 0.01$].

Discussion

At least two species of the genus *Lampris* occur sympatrically in the Southwestern Atlantic Ocean: *L. megalopsis* and *L. australensis* were identified for the first time in the SWAO through molecular and morphological analyses, amplifying the distribution range of both species. Until now, *L. australensis* had been recorded only in the waters of Australia, Chile, South Africa, and Tasmania (Underkoffler et al., 2018; Kukuev, 2021), and with the results of this study, it is possible to affirm that the species is circumglobally distributed in subtropical southern hemisphere waters. Regarding *L. megalopsis*, this species was known to occur only in the Central-North Pacific,

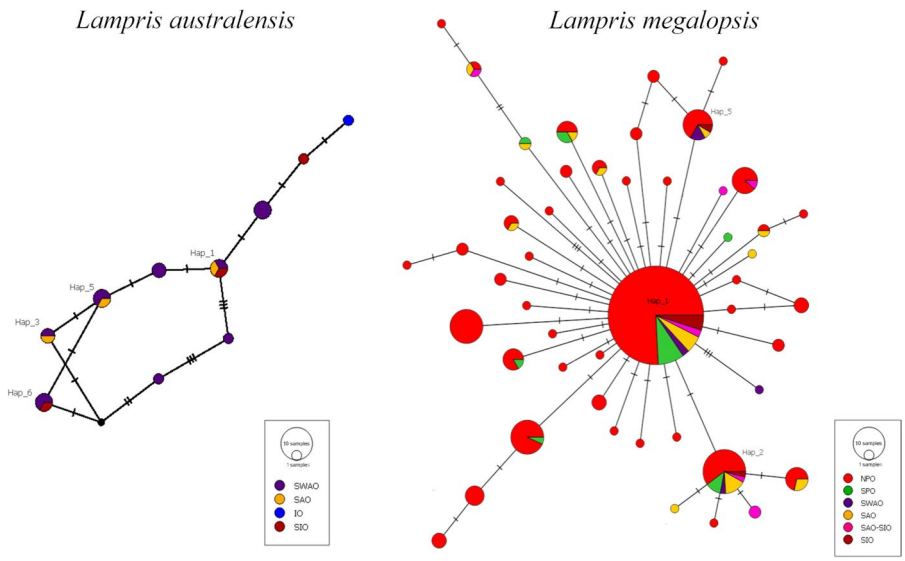

Fig. 4 Median-joining network of *Lampris australensis* (left) and *L. megalopsis* (right) haplotypes from different geographical regions. The size of the nodes is proportional to the number of individuals with that haplotype, and each dash mark indicates the number of mutations between haplotypes. *NPO* North Pacific Ocean, *SPO* South Pacific Ocean, *SWAO* Southwestern Atlantic Ocean, *SAO* South Atlantic Ocean, *SAO–SIO* region between South Atlantic and South Indian Ocean (South Africa coast), *SIO* South Indian Ocean, *IO* Indian Ocean

American Samoa, Australia, Indonesia, and South Africa (Underkoffler et al., 2018); with our study, it now presents a cosmopolitan distribution.

The average K2P distance of individuals within species was 0.35% compared to 7.2% for the *Lampris* genus in the SWAO. These values produced an overall gap of 5.6%, which is similar to the gap obtained for *L. australensis* and *L. megalopsis* by Hyde et al. (2014). These values are consistent with the distances between species and genera and are also corroborated by several other DNA barcoding studies on fishes (e.g., Ward et al., 2005; Ward, 2009; Lakra et al., 2011).

Diversity data showed high genetic diversity for the COI region for both species in the SWAO, which agree with the results obtained with the pooled data from Hyde et al. (2014). The similarity between the haplotype and nucleotide diversities between the sites, along with the low F_{st} values found in AMOVA, may indicate the presence of gene flow in both species along with their distribution ranges. We suggest that this be evaluated with more variable markers to obtain a fine-scale understanding of the connectivity of *Lampris* populations. *Lampris guttatus* lato sensu has a powerful swimming system (Rosenblatt & Johnson, 1976; Wegner et al., 2015), allowing this species to travel large distances and possibly explaining its widespread occurrence and the low differentiation values we found. The six recently identified *Lampris* species have similar pectoral anatomy (Davesne et al., 2018), which allows the assumption that endothermy and pectoral muscle swimming function occurs in all (Davesne et al., 2018). Furthermore, Underkoffler et al. (2018) report that the species have similar ecology, external morphology, and behavior.

The haplotype network built for *L. megalopsis* with our sequences, along with those from Hyde et al. (2014), suggests the presence of ancestral haplotypes from the NPO. Haplotype number 1 is in the middle of the network and is present in all sampled regions, with several haplotypes deriving from it (see Fig. 6). Therefore, it is possible to infer that this species could have originated in the NPO. For *L. australensis*, the network relations are less clear as the sample number is much smaller, and few relations are shown (Fig. 6). Despite this, the network of 10 haplotypes is enough to evidence a historical gene flow between locations.

Morphological analysis demonstrated the presence of two morphological groups of the genus *Lampris* in SWAO. In the PCA plot (Fig. 7), samples identified

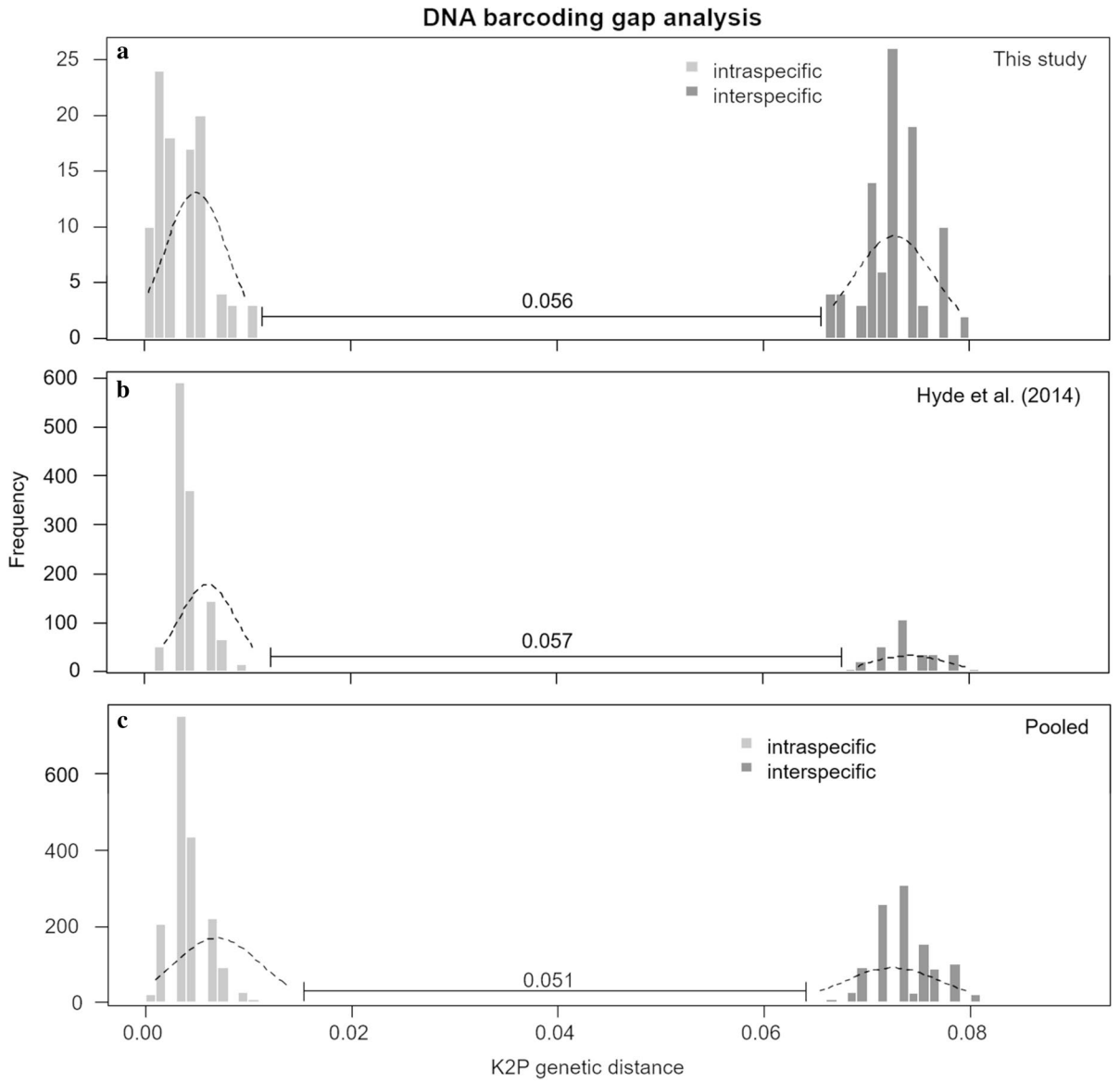

Fig. 5 Intra and interspecific DNA barcoding gap analysis of *Lampris australensis* and *L. megalopsis* from COI gene sequences. **a** SWAO, **b** Hyde et al. (2018) pooled data set from SAO–SIO, SIO, IO, SPO, and NPO, and **c** pooled data from both studies

with barcode analysis confirmed the correct classification of the species *L. megalopsis* and *L. australensis*, according to Underkoffler et al. (2018). In addition, the efficiency of the measurements chosen for morphometric analyses was confirmed, and we suggest they be adopted for distinguishing species. Furthermore, the successful classification was also observed with PC-LDA analysis, which had a PCC of 100% for *L. australensis* and 98% for *L. megalopsis*. These analyses showed high classification efficiency, demonstrating that *L. australensis* and *L. megalopsis* can be visually distinguished. We propose that the evaluation of pectoral fins and eye size (larger in *L. megalopsis*), spot pattern (larger and sparser in *L. australensis*), and body shape (generally rounder in *L. australensis*) should be conducted for species differentiation in the SWAO. These characteristics combined are relatively easy to diagnose and can be used

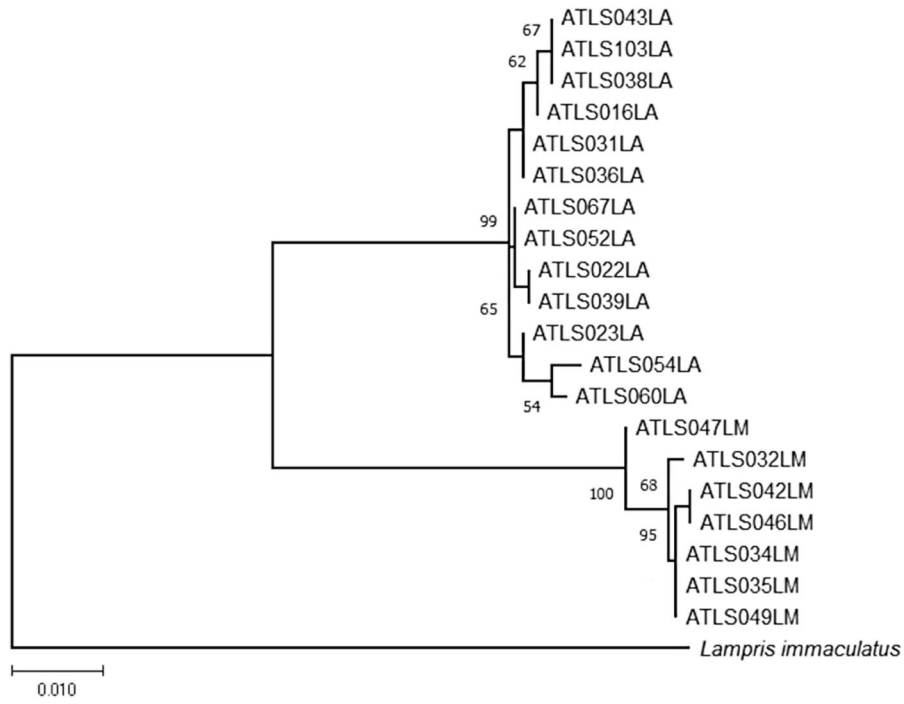

Fig. 6 Maximum likelihood tree based on *Lampris australensis* (ATL-LA) and *L. megalopsis* (ATL-LM) data. Bootstrap values are shown for each node. Nodes without numbers indicate support below 50. A single sequence from *L. immaculatus* sequence (GenBank accession DQ108066.1) is used as an outgroup

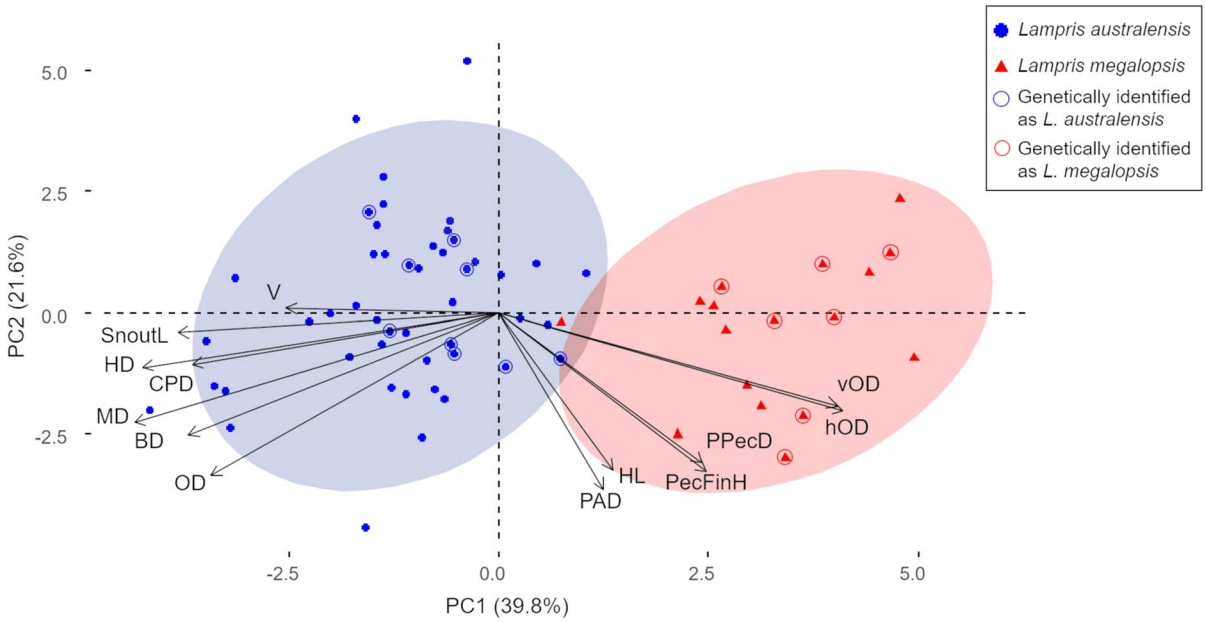

Fig. 7 Plot of principal components one and two from the principal component analysis of 13 morphometric variables of 65 specimens of *Lampris australensis* (blue) and *L. megalopsis* (red). Ellipses represent 95% confidence interval and circles represent samples with molecular identification by COI sequences analysis. The measurements and counts used for analysis were greatest body depth (BD), head length (HL), head depth (HD), pre-pectoral distance (PPecD), pre-anal distance (PAD), vertical orbital diameter (vOD), horizontal orbital diameter (hOD), snout length (SnoutL), pectoral-fin height (PecFinH), maximum diameter (MD), operculum diameter (OD), caudal peduncle depth (CPD), and number of pelvic fin rays (*V*)

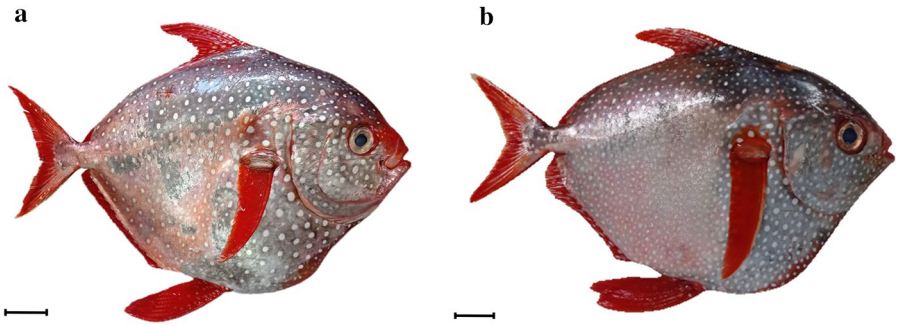

Fig. 8 Species of the subgenus *Lampris* with visible general differences between spot patterns, eyes, pelvic fins size of the head, and caudal peduncle. Specimens collected in the Southwestern Atlantic Ocean: **a** male *Lampris australensis*, 926 mm TL, 35 kg, and **b** male *L. megalopsis*, 1010 mm TL, 23.6 kg. Scale represents 100 mm

Table 2 Counts and measurements obtained from *Lampris* specimens in the Southwestern Atlantic Ocean

Measurements represent how many times one measurement fits in standard or head length. Values for the features according to Underkoffler et al. (2018) are shown in square brackets for *L. australensis* and *L. megalopsis*

Counts and measurements	*L. australensis*	*L. megalopsis*
Dorsal-fin rays	48–52 [50–52]	44–51 [50–51]
Anal-fin rays	38–44 [40–42]	37–42 [38]
Pectoral fin rays	22–25 [22–23]	20–26 [22–23]
Pelvic fin rays	5–18 [13–15]	13–17 [14–15]
Body laterally compressed and rounded, its greatest depth slightly anterior to pelvic fins and contained, on average, in standard length	1.5 [1.4]	1.6 [1.5]
Head length in standard length	3.1 [2.8]	3.1 [2.8]
Vertical eye diameter in head length	4.5 [5.2]	4.0 [4.7]
Dorsal-fin base length contained in standard length	3.6 [1.8]	2.1 [1.8]
Dorsal-fin height contained in standard length	2.2 [2.7]	4.0 [3.2]

on fishing vessels by fishers and on-board observers to distinguish *Lampris* species that are by caught at the sampled region.

Some of the general characteristics presented here, such as spot pattern, longer pectoral fin, and large eyes in *L. megalopsis*, are also cited by Underkoffler et al. (2018) as diagnostic characteristics. In *L. australensis*, the spot pattern and small eyes were the most concordant characteristics. However, there were also some discordant features: a distinctly arched head profile was not always present and was also observed in some samples of *L. megalopsis* in the SWAO. Yellow-tinged median fins, as described in Underkoffler et al. (2018), was only visible in samples kept frozen for a few days and are probably a consequence of storage conditions before sampling, as they were also dry and rigid after defrosting. However, fresh samples observed in this study did not present this characteristic. The freshness of the sample may also interfere with the presence of a purple color in *L. megalopsis* tongues, which could occur due to blood coagulation. Therefore, the use of tongue color as a diagnostic character for *L. megalopsis* should be done with caution. It was also not possible to identify SWAO *Lampris* species using the key proposed by Underkoffler et al. (2018) since the means of the proposed proportions overlapped between *L. australensis* and *L. megalopsis* (e.g., HD and BD) and because the proportions in our samples did not fully match *L. australensis*, *L. megalopsis* nor *L. incognitus* (which was the alternative to *L. megalopsis*). Thus, the construction of a new identification key for the genus is advised, using a higher number of individuals of each species from various locations worldwide, preferably involving other characteristics (i.e., gill-raker counts).

Considering the present distribution of *L. australensis* in the subtropical waters of the southern hemisphere and the cosmopolitan distribution of *L.*

megalopsis, previous records of *L. guttatus* in the southeast Brazilian (Figueiredo & Menezes, 1980; Lopes & Oliveira-Silva, 2017) and Argentine coasts (Piacentino & Muguetti, 1994), were likely actually *L. megalopsis*, while in Uruguay (Nion et al., 2002) and South Brazil (Piacentino & Muguetti, 1994) the presence of *L. australensis* is also possible since subtropical oceanic waters also border these regions.

Lampris species are receiving increasing commercial interest (Underkoffler et al., 2018), and it is crucial that studies on growth, reproduction, and diet be carried out for the definition of possible management actions, such as minimum legal sizes and closed fishing seasons, if necessary. These actions depend on the reproductive biology of fish stocks (Morgan, 2008) and can only be stipulated based on the correct identification of species. The species-specific data presented here are incipient but already indicate differences in landings by seasons (Fig. S2) and in maximum sizes (Fig. S3), which could also suggest possible differences in the life histories of *L. australensis* and *L. megalopsis* in the SWAO, which must be unveiled. Species with faster body growth generally support higher fishing mortality than those with slower growth (Reynolds et al., 2001), and the smaller size composition in *L. australensis* could indicate slower body growth and lower fishing mortality tolerance when compared to *L. megalopsis*; if this is indeed the case, separate management initiatives are required.

The results presented here elucidate which species of the genus *Lampris* occur in the SWAO, providing information on their biology and a detailed comparative description of their morphology, which contributes toward a better understanding of their distribution and allows their easy identification in the region. Our results also add to the global knowledge on the distribution and biology of the *Lampris* genus, which is fundamental for understanding the potential impacts of fisheries on these species and for creating conservation and management initiatives aimed toward a more sustainable use of these valuable resources.

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Author contributions GT conceptualized, conducted sample collection, laboratory and data analyses, interpreted data, and wrote the manuscript. LSR supported sample collection, provided input to morphometric data and molecular analyses, conducting part of these analyses and advising on their interpretation. EK provided historical context, supported sample collection, assisted with laboratory analyses, advised on the interpretation of analysis, and reviewed and edited the original draft, including figures and graphs. FVA provided historical context, conducted sample collection, assisted with laboratory analyses, and reviewed and edited the original draft. CB conducted sample collection and assisted with laboratory analyses, and review and edit the original draft. MAF conducted sample collection, assisted with laboratory analyses, and review and edit the original draft. MCFS supervised molecular analyses, data analyses, and interpretation, and review and edit the original draft. MCP oversaw the molecular experimental setup, the methodology, and proofread and refined the manuscript. LGC devised the study, wrote funding applications, supervised the student, and assisted with writing the manuscript, and review and edit the original draft.

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Data availability Mitochondrial sequence data generated as part of this project are stored in FASTA format and will be uploaded to the NCBI/GenBank Nucleotide Sequence Repository. All morphometrical data used for analysis are available in Supplementary Material.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose, and permission from each author has been granted. No other institutions hold copyright over this work. We have no conflict of interest to clarify.

Ethical approval The research did not involve animal experimentation or harm and required no permits under Brazil animal welfare laws. Accepted principles of ethical and professional conduct have been followed.

Informed consent Not applicable.

Consent for publication Not applicable. All authors gave consent for submission.

Ethical responsibilities of authors All responsibilities cited in Author Guidelines are followed by all authors.

Authorship clarified All authors agreed with the content of this manuscript and gave explicit consent for submission.

References

Aljanabi, S. M. & I. Martinez, 1997. Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25: 4692–4693.

Bache-Jeffreys, M., B. L. C. de Moraes, R. E. Ball, G. Menezes, J. Pálsson, C. Pampoulie, J. R. Stevens & A. M. Griffiths, 2021. Resolving the spatial distributions of *Dipturus intermedius* and *Dipturus batis*—the two taxa formerly known as the 'common skate.' Environmental Biology of Fishes 104: 923–936.

Bandelt, H. J., P. Forster & A. Röhl, 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48.

Beerkircher, L., F. Arocha, A. Barse, E. Prince, V. Restrepo, J. Serafy & M. Shivji, 2009. Effects of species misidentification on population assessment of overfished white marlin *Tetrapturus albidus* and roundscale spearfish *T. georgii*. Endangered Species Research 9: 81–90.

Čandek, K. & M. Kuntner, 2015. DNA barcoding gap: reliable species identification over morphological and geographical scales. Molecular Ecology Resources 15: 268–277.

Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. JModelTest 2: more models, new heuristics, and parallel computing. Nature Methods 9: 772–772.

Davesne, D., F. J. Meunier, M. Friedman, R. B. Benson & O. Otero, 2018. Histology of the endothermic opah (*Lampris* sp.) suggests a new structure–function relationship in teleost fish bone. Biology Letters 14: 20180270.

Excoffier, L. & H. E. Lischer, 2010. ARLEQUIN suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Excoffier, L., P. E. Smouse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Figueiredo, J. L. & N. A. Menezes, 1980. Manual de peixes marinhos do sudeste do Brasil. III. Teleostei. Museu de Zoologia da Universidade de São Paulo, São Paulo.

Francis, M. P., L. Griggs & C. Ó. Maolagáin, 2004. Growth Rate, Age at Maturity, Longevity and Natural Mortality Rate of Moonfish (*Lampris guttatus*). Final Research Report for Ministry of Fisheries Research Project TUN2003-01 Objective 1. National Institute of Water and Atmospheric Research, New Zealand [available on internet at http://fs.fish.govt.nz/Page.aspx?pk=113&dk=22484]. Accessed 5 August 2021.

Garcia-Vazquez, E., G. Machado-Schiaffino, D. Campo & F. Juanes, 2012. Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks. Fisheries Research 114: 52–55.

Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hartley, J. L. & H. Bowen, 2003. PEG precipitation for selective removal of small DNA fragments. Focus 25: 18.

Hawn, D. R. & B. B. Collette, 2012. What are the maximum size and live body coloration of opah (Teleostei: Lampridae: *Lampris* species)? Ichthyological Research 59: 272–275.

Hazin, F. H. V., M. K. Broadhurst, A. F. Amorim, C. A. Arfelli & A. Domingo, 2008. Catches of pelagic sharks by subsurface longline fisheries in the South Atlantic Ocean during the last century: a review of available data with emphasis on Uruguay and Brazil. In Camhi, M., E. Pikitch & E. Babcock (eds), Sharks of the Open Ocean Blackwell, New York: 213–229.

Huang, H. W. & K. M. Liu, 2010. Bycatch and discards by Taiwanese large-scale tuna longline fleets in the Indian Ocean. Fisheries Research 106: 261–270.

Hyde, J. R., K. E. Underkoffler & M. A. Sundberg, 2014. DNA barcoding provides support for a cryptic species complex within the globally distributed and fishery important opah (*Lampris guttatus*). Molecular Ecology Resources 14: 1239–1247.

James, G., D. Witten, T. Hastie & R. Tibshirani, 2013. An Introduction to Statistical Learning, Springer, New York:, 6–18.

Jiménez, S., A. Domingo, M. Abreu & A. Brazeiro, 2011. Structure of the seabird assemblage associated with pelagic longline vessels in the southwestern Atlantic: implications for bycatch. Endangered Species Research 15: 241–254.

Josse, J. & F. Husson, 2016. missMDA: a package for handling missing values in multivariate data analysis. Journal of Statistical Software 70: 1–31.

Kassambara, A. & F. Mundt, 2017. Factoextra: extract and visualize the results of multivariate data analyses. R package version 1:337-354.

Kukuev, E. I., 2021. Juvenile individuals of opahs (Lampridae) from the Atlantic and Pacific Oceans. Notes on the systematics and distribution of opahs, including the description of a new subgenus *Paralampris* subgen. nov. Journal of Ichthyology 61: 182–189.

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across

computing platforms. Molecular Biology and Evolution 35: 1547.
Lakra, W. S., M. S. Verma, M. Goswami, K. K. Lal, V. Mohindra, P. Punia, A. Gopalakrishnan, K. V. Singh, R. D. Ward & P. Hebert, 2011. DNA barcoding Indian marine fishes. Molecular Ecology Resources 11: 60–71.
Leigh, J. W. & D. Bryant, 2015. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6: 1110–1116.
Lleonart, J., M. Taconet & M. Lamboeuf, 2006. Integrating information on marine species identification for fishery purposes. Marine Ecology Progress Series 316: 231–238.
Lopes, P. R. D. & J. T. de Oliveira-Silva, 2017. Primeiro registro de *Lampris guttatus* (Brünnich, 1788) (Actinopterygii: Lampridae) no litoral de ilhéus (Estado da Bahia, Nordeste do Brasil). Revista Mosaicum (25).
Morgan, M. J., 2008. Integrating reproductive biology into scientific advice for fisheries management. Journal of Northwest Atlantic Fishery Science 41: 37–51.
Nion, H., C. Rios & P. Meneses, 2002. Peces del Uruguay: Lista sistemática y nombres comunes [available on internet at https://www.yumpu.com/it/document/view/14716803/peces-del-uruguay-direccionnacional-de-recursos-acuaticos]. Accessed 13 May 2021.
Pacifici, M., W. B. Foden, P. Visconti, J. E. M. Watson, S. H. M. Butchart, K. M. Kovacs, B. R. Scheffers, D. G. Hole, T. G. Martin, H. Resit Akçakaya, R. T. Corlett, B. Huntley, D. Bickford, J. A. Carr, A. A. Hoffmann, G. F. Midgley, P. Pearce-Kelly, R. G. Pearson, S. E. Williams, S. G. Willis, B. Young & C. Rondinini, 2015. Assessing species vulnerability to climate change. Nature Climate Change 5: 215–224.
Piacentino, G. & C. Mugetti, 1994. Registro de capturas de *Lampris immaculatus* Gilchrist 1904 y *L. guttatus* Brunich 1788 (Pisces, Lampriddidae) en el Mar argentino y comentarios sobre la distribucion de estas especies. Revista Del Museo Argentino De Ciencias Naturales-Hidrobiologia 7: 47–59.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna [available on internet at http://www.R-project.org/].
Ratnasingham, S. & P. D. Hebert, 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes 7: 355–364.
Reynolds, I. D., S. Lennings & N. K. Dulvy, 2001. Life histories of fishes and population responses to exploitation. In Reynolds, J. D., G. M. Mace, K. H. Redford & J. G. Robinson (eds), Conservation of Exploited Species Cambridge University Press, Cambridge: 148–168.
Rosenblatt, R. H. & G. D. Johnson, 1976. Anatomical considerations of pectoral swimming in the opah, *Lampris guttatus*. Copeia 1976: 367–370.
Rozas, J. & R. Rozas, 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174–175.
Runcie, R. M., H. Dewar, D. R. Hawn, L. R. Frank & K. A. Dickson, 2009. Evidence for cranial endothermy in the opah (*Lampris guttatus*). Journal of Experimental Biology 212: 461–470.
Shivji, M. S., J. E. Magnussen, L. R. Beerkircher, G. Hinteregger, D. W. Lee, J. E. Serafy & E. D. Prince, 2006. Validity, identification, and distribution of the roundscale spearfish, *Tetrapturus georgii* (Teleostei: Istiophoridae): morphological and molecular evidence. Bulletin of Marine Science 79: 483–491.
Tamura, K. & M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.
Tillett, B. J., I. C. Field, C. J. Bradshaw, G. Johnson, R. C. Buckworth, M. G. Meekan & J. R. Ovenden, 2012. Accuracy of species identification by fisheries observers in a north Australian shark fishery. Fisheries Research 127: 109–115.
Tuck, G. N., T. Polacheck & C. M. Bulman, 2003. Spatio-temporal trends of longline fishing effort in the Southern Ocean and implications for seabird bycatch. Biological Conservation 114: 1–27.
Thompson, J. D., D. G. Higging & T. J. Gibson, 1994. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.
Underkoffler, K. E., M. A. Luers, J. R. Hyde & M. T. Craig, 2018. A taxonomic review of *Lampris guttatus* (Brünnich 1788) Lampridiformes; Lampridae) with descriptions of three new species. Zootaxa 4413: 551–566.
UNIVALI/EMCT/LEMA. 2020. Estatística Pesqueira de Santa Catarina. Consulta On-line. Projeto de Monitoramento da Atividade Pesqueira do Estado de Santa Catarina [available on internet at http://pmap-sc.acad.univali.br/]. Accessed 13 May 2021.
Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last & P. D. Hebert, 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society b: Biological Sciences 360: 1847–1857.
Ward, R. D., 2009. DNA barcode divergence among species and genera of birds and fishes. Molecular Ecology Resources 9: 1077–1085.
Wegner, N. C., O. E. Snodgrass, H. Dewar & J. R. Hyde, 2015. Whole-body endothermy in a mesopelagic fish, the opah, *Lampris guttatus*. Science 348: 786–789.
Williams, S. M., J. G. Pepperell, M. Bennett & J. R. Ovenden, 2018. Misidentification of istiophorid billfishes by fisheries observers raises uncertainty over stock status. Journal of Fish Biology 93: 415–419.
Wright, S., 1978. Evolution and Genetics of Population: Theory of Gene Frequencies, University of Chicago Press, Chicago:
Zhang, A. B., M. D. Hao, C. Q. Yang & Z. Y. Shi, 2017. BarcodingR: an integrated R package for species identification using DNA barcodes. Methods in Ecology and Evolution 8: 627–634.