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ECOMORFOLOGIA DOS OTÓLITOS *SAGITTAE* DE PEIXES DA FAMÍLIA LUTJANIDAE NA COSTA NORDESTE DO BRASIL

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Dissertação apresentada ao Programa de Pós-graduação em Oceanografia da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Mestre em Oceanografia.

Área de concentração: Oceanografia Biológica.

Orientadora: Prof. Dr. Beatrice Padovani Ferreira.

Coorientador: Jonas Eloi de Vasconcelos-Filho.

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Ao meu avô e avó materna Manoel e Creuza, meus pais Viviane e Emerson, e aos meus irmãos.

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RESUMO

A análise de imagens tem sido amplamente utilizada para a caracterização morfológica dos otólitos e do *sulcus acusticus*, desempenhando um papel significativo na biologia das espécies. Espécies da família Lutjanidae são importantes espécies-modelo para estudos tanto de diferenças intra como interespecíficas que possam contribuir com seu conhecimento ecológico e consequentemente sobre os efeitos da pesca em suas populações. Devido a isto, este estudo objetivou caracterizar morfologicamente a forma dos otólitos sagittae e seus sulcus acusticus para oito espécies da família Lutjanidae distribuídas no Atlântico Tropical Oeste, relacionando tais características de forma com a profundidade, ao crescimento e à ecologia das espécies. Ao total, foram analisados 1135 otólitos, amostrados ao longo do Nordeste do Brasil por diversos projetos, dentre eles o REVIZEE. As imagens dos otólitos foram processadas e todas as métricas e índices de formas foram realizadas com auxílio do Software R. A forma dos otólitos variaram entre as espécies, enquanto a dos *sulcus acusticus* não. As regressões significativas sugerem modificações no formato dos otólitos ao longo do desenvolvimento dos indivíduos. O *O. chrysurus* aprensentou diferença entre sexo para a relação ao peso-comprimento e para as demais métricas e índices. O *sulcus relative surface* (SRS) diferiu significativamente entre as espécies, onde *O. chrysurus* e *L. synagris* se destacaram com as maiores proporções de área do sulcus acusticus, enquanto *L. vivanus* e *L. bucanella* tiveram as menores. Através do teste de Mann-Whitney verificou-se que as espécies de raso apresentam maior SRS que as de fundo. De forma geral, os otólitos dos indivíduos de águas mais profundas apresentaram otólitos menores e mais alongados, com *sulcus acusticus* menores, mais irregulares e mais alongados. Estas duas abordagens combinadas resultaram em uma maior eficiência para discriminar as espécies, tendo uma média de 73.7% de classificação. Com isso, é plausível concluir que através das características morfométricas e morfológicas dos otólitos obtidas para esta família é possível caracterizar suas espécies e habitats utilizados.

Palavras-chave: imagens; Sagittae; forma; Atlântico Ocidental; Lutjanidae.

ABSTRACT

Image analysis has been widely used for the morphological characterization of otoliths and *sulcus acusticus*, playing a significant role in species biology. Species of the Lutjanidae family are important model species for studies of both intra- and inter-specific differences that may contribute to their ecological knowledge and consequently to the effects of fishing on their populations. Because of this, this study aimed to morphologically characterize the shape of otolith sagittae and their *sulcus acusticus* for eight species of the family Lutjanidae distributed in the western tropical Atlantic, relating such shape characteristics to the depth, growth and ecology of the species. A total of 1135 otoliths, sampled along the Northeast of Brazil by several projects, among them REVIZEE, were analyzed. The otolith images were processed and all metrics and shape indices were performed with the help of Software R. The shape of otoliths varied among species, while that of *sulcus acusticus* did not. The significant regressions suggest modifications in otolith shape throughout the development of the individuals. *O. chrysurus* showed a sex difference for the length-weight relationship and for the other metrics and indices. The *sulcus relative surface* (SRS) differed significantly among species, where *O. chrysurus* and *L. synagris* stood out with the highest proportions of *sulcus acusticus* area, while *L. vivanus* and *L. bucanella* had the lowest. Using the Mann-Whitney test, it was found that shallow water species have a higher SRS than deep water species. On average, otoliths of individuals from deeper water had smaller and more elongated otoliths, with smaller, more irregular and more elongated *sulcus acusticus*. These two approaches combined resulted in a higher efficiency to discriminate the species, having an average of 73.7% classification. With this, it is plausible to conclude that through the morphometric and morphological characteristics of otoliths obtained for this family it is possible to characterize its species and habitats used.

Keywords: images; Sagittae; shape; Tropical Atlantic; Lutjanidae.

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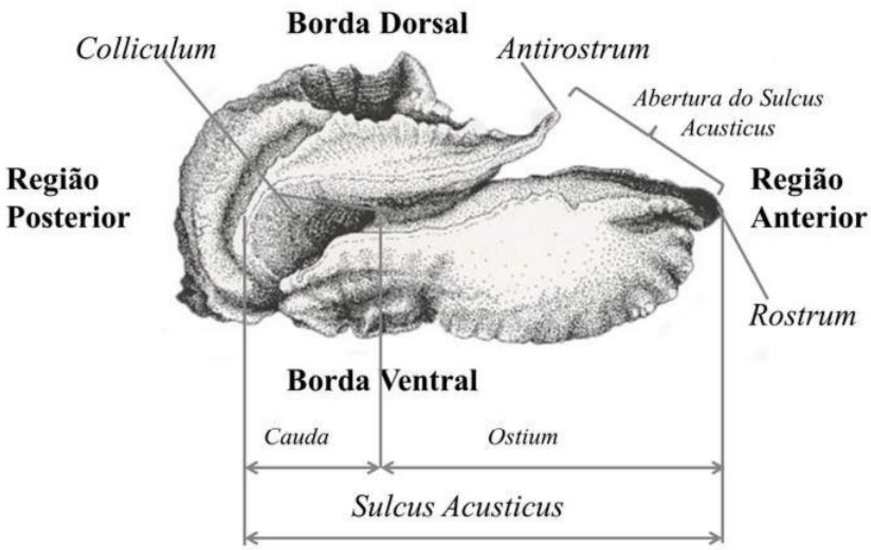
1 INTRODUÇÃO

Os otólitos são concreções calcárias presentes na cápsula auditiva dos peixes ósseos constituídas de 95% de carbonato de cálcio e 3-5% de matérias orgânicas e inorgânicas, que fazem parte do sistema relacionado a mecanismos de equilíbrio e audição (Campana, 2004). Os peixes têm uma variedade de receptores sensoriais diferentes que lhes permitem reunir uma riqueza de informações de seu ambiente sensorial (Popper & Lu, 2000). Destes sistemas sensoriais, o ouvido interno é mais útil na detecção de fontes sonoras e captura de informações quando a iluminação é limitada (Popper & Lu, 2000). Em outras palavras, o ouvido interno de um peixe é uma estrutura que pode detectar a fonte sonora e até mesmo detectar a direção do emissor de som. Durante a evolução do sistema auditivo dos peixes, esse compartimento se tornou importante para diversos fins, como, para localizar presas e seus respectivos predadores, como também para encontrar seus pares para o acasalamento (Popper & Lu, 2000).

O sistema vestibular dos peixes ósseos é constituído por três pares de otólitos denominados de *sagitta*, *lapillus* e *asteriscus* (Reichenbacher *et al*., 2009). O *sagitta* é o mais utilizado em estudos de forma dessas estruturas devido ao seu tamanho e robustez (Begg *et al*., 2005). A sua variação específica o converteu em uma ferramenta útil para estudos sobre taxonomia, filogenia (Assis, 2003), arqueologia (Disspain; Ulm; Gillanders, 2015), paleontologia (Reichenbacher *et al*., 2007), variação geográfica das espécies (Rossi-Wongtschowski *et al*., 2015), identificação de estoque (Duarte-Neto *et al*., 2008) entre outros.

Os otólitos constituem uma das mais importantes estruturas para o conhecimento do ciclo de vida dos peixes (Campana, 2004). Além disso, possuem diversas estruturas anatômicas que são utilizadas para descrever e classificar os otólitos (Figura 1). Através dos estudos dessas estruturas é possível chegar ao conhecimento sobre o nascimento, crescimento, idade e mortalidade das espécies. Além disso, por apresentarem alta especificidade morfológica, estas estruturas são consideradas úteis para a identificação taxonômica dos peixes (Torno, 1976; Hecht,1987). Os otólitos também estão relacionados com a profundidade em que os indivíduos habitam, como demonstrado por Lombarte (1992), o qual relacionou a diminuição do tamanho do otólito e o aumento do tamanho do *sulcus acusticus* com o crescimento do peixe e a profundidade em que o mesmo habita.

Figura 1 – Estruturas anatômicas dos otólitos *sagittae*.



Fonte: Rossi-Wongtschowski *et al*. (2016).

Segundo Lychakov & Rebane (2000), a frequência e amplitude da emissão sonora do peixe são características intrínsecas de cada espécie e tamanho do peixe. Os obstáculos são características mais comuns próximo ao substrato. Então, os peixes que habitam este nicho possuem uma audição aguçada em relação aos demais peixes distribuídos em outras regiões. Segundo estes autores, os peixes pequenos emitem sons graves, enquanto os grandes, sons agudos. Portanto, devido a isso, os otólitos *sagittae* de peixes menores e de fundo tendem a serem maiores e ser mais pesados.

A forma do otólito é uma característica altamente espécie-específica (Gaemers, 1984) e exibe, frequentemente, variações relacionadas a fatores bióticos e ambientais (Cardinale *et al*., 2004). Gauldie (1988) descreveu como a forma do otólito pode ser controlada por vários fatores, incluindo variações e descontinuidades no crescimento, controlados pelo epitélio sensorial. Consequentemente, esses estímulos são induzidos por variações nas condições ambientais, como fotoperíodo, temperatura e hábitos alimentares (Lecomte-Finiger, 1999).

Os otólitos existem em pares iguais, e qualquer assimetria na forma ou no peso entre eles afeta a audição e/ou equilíbrio do peixe (Oxaman *et al.*, 2007). Lychakov *et al.* (2006) mostraram que, baseado em um modelo matemático, a assimetria do peso dos otólitos pode afetar as suas funcionalidades acústicas, como a sensibilidade, o processamento temporal e a localização sonora. Além disso, estudos anteriores mostraram que, a maioria das espécies de peixes possuem assimetria dos pesos dos otólitos na faixa de 20% (Lychakov, 1992; Lychakov *et al.* 1988; Lychakov & Rebane, 2004, 2005; Taka Bayashi & Ohmura-Iwasaki, 2003).

Os otólitos apresentam grande plasticidade fenotípica. A forma dos otólitos é determinada geneticamente e reflete a relação filogenética entre as espécies, cada uma das quais exibe mudanças conformacionais que distinguem cada espécie uma da outra. Essas mudanças variam, em menor grau, por fatores ambientais. Os otólitos têm uma forma específica para os táxons a que o peixe pertence e, do ponto de vista filogenético, mais evidente são as diferenças morfológicas entre as diferentes espécies (Assis, 2004). Devido a isso, as análises de imagens desempenham um papel significativo no estudo suas formas, pois melhoram a coleta de dados, fornecem uma melhor descrição da estrutura e têm potencial para o desenvolvimento de novos métodos analíticos aplicados à biologia pesqueira (Cadrin & Friedland, 1999). Algumas dessas aplicações são: identificar estoques pesqueiros, táxons e descrever suas variações com inferências ecológicas da vida dos peixes (Duarte-Neto *et al*., 2008; Stransky *et al.*, 2008ª; Campana & Calsseman, 1993; Tuset, 2003).

Para isso, algumas metodologias podem ser empregadas para a análise da forma dos otólitos, como a análise Fourier, análise de Wavelet e os índices de forma. Os índices de forma são razões entre as medidas básicas dos otólitos, como o comprimento, a altura, a área e o perímetro. Esses índices auxiliam na análise de padrões dentro de um mesmo grupo taxonômico, além de permitir outras inferências ecológicas, assim como Volpedo & Echeverría (2003), que usaram a morfometria dos otólitos ajuda a entender os efeitos da disposição na coluna de água e dos diferentes tipos de substratos onde as espécies vivem. De acordo com Capoccioni *et al*. (2011), os otólitos crescem e mudam de formato juntamente com o aumento do tamanho do animal. Além disso, ainda segundo eles, essas modificações ocorrem de maneiras diferentes em cada ambiente. Portanto, é interessante relacionar os índices de forma com o comprimento do indivíduo por modelos de regressão e considerar o efeito dos fatores abióticos.

Vignon e Morat (2010), utilizando morfometria geométrica em *Lutjanus* sp., descobriram que, mesmo que o padrão geral do otólito possa ser modificado por condições ambientais, as variações genéticas intra-específica afetam apenas o *rostrum* e *antirostrum*. Segundo Campana *et al.* (1993), a forma do otólito é uma característica específica da espécie, cuja diferença dos estoques está associada à heterogeneidade genética e ao meio ambiente. Já

Reichenbacher *et al*. (2009), usando morfometria linear dos otólitos sagitais, concluíram que os fatores ambientais tiveram maior influência na divergência populacional.

Os peixes da família Lutjanidae estão entre os recursos pesqueiros mais importantes nas regiões tropicais e subtropicais (Duarte & Garcia, 1999). Essa família é constituída por 113 espécies distribuídas em quatro subfamílias: (Apsilinae, Etelininae, Lutjaninae e Paradicichtinae) (William & Anderson, 1987). As subfamílias Lutjaninae e Etilininae são representadas por quatro gêneros (*Etelis, Lutjanus, Ocyurus e Rhomboplites*) onde 11 espécies representantes ocorrem em águas brasileiras (Allen, 1985; Anderson, 1987; Menezes & Figueiredo, 1980).

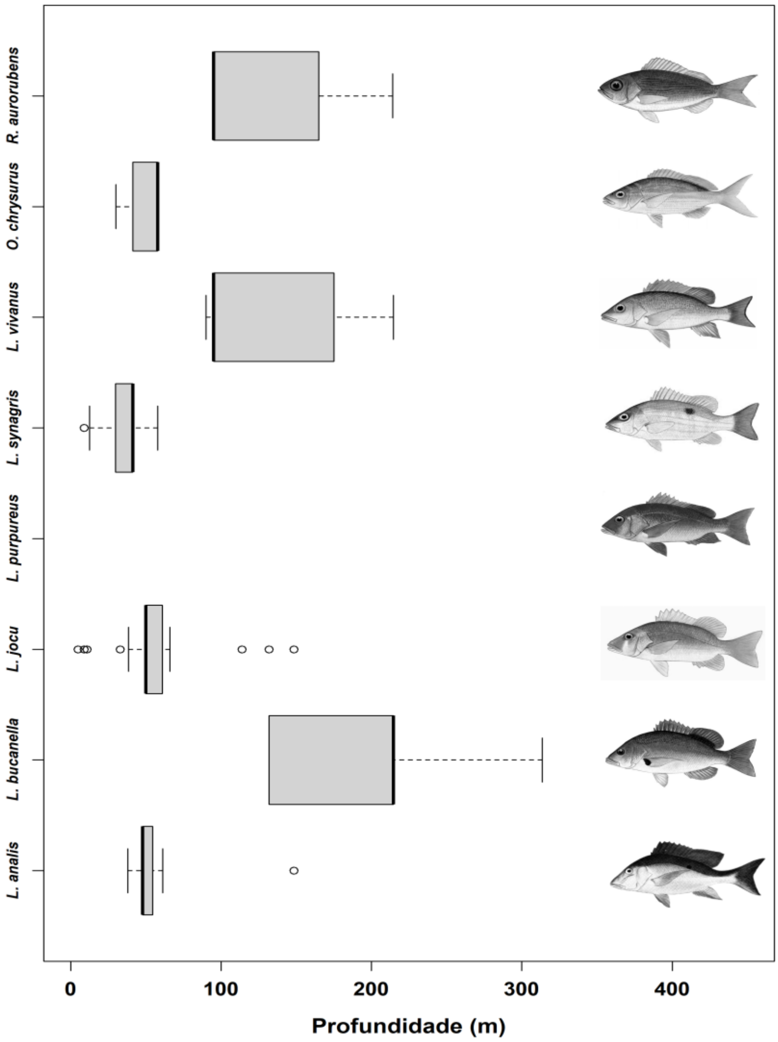
As espécies da família Lutjanidae estão entre os recursos pesqueiros mais importantes para as pescarias da costa brasileira e são responsáveis pela renda de milhares de famílias (SEAP, 2006). Em 2010, a produção total da pesca extrativista dessas espécies de Lutjanídeos ultrapassou as sete mil toneladas (MPA, 2010). Atualmente, a família Lutjanidae, popularmente conhecidos como vermelhos, está entre as categorias de pescado mais valiosas no mercado, sendo capturadas em água costeiras até a plataforma. No entanto, de acordo com a União Internacional para a Conservação da Natureza (2022), seus estoques estão diminuindo.

Segundo Rezende e Ferreira (2003), as espécies dessa família apresentam crescimento lento (k<0,2) e altas longevidades (20 a 30 anos), que os tornam vulneráveis à sobrepesca. Estas espécies se distribuem desde águas rasas até ambientes com pouca luz, na costa e em ilhas oceânicas, apresentando diferenças ecológicas em relação à profundidade, hábito alimentar, posição na coluna d’água e habitats preferenciais ao longo da vida (Allen, 1985; Nelson, 2006; Sale, 1991).

Os Lutjanidae são um grupo de peixes predadores que se abrigam nos recifes durante o dia, e dispersam-se à noite para se alimentar quando estão menos visíveis e mais capazes de atacar suas presas (RANDALL, 1967; LOWE-McCONNELL, 1999). Alimentam-se principalmente de invertebrados, sendo a maioria: crustáceos e peixes. Como ocupam o topo da cadeia alimentar, espécies de Lutjanidae desempenham uma função de controle ecológico de suma importância no ambiente recifal (SALE, 1991). Esses peixes abrigam áreas distintas quando relacionado à fase de vida. Os jovens, por exemplo, vivem em áreas rasas, podendo ser encontrados em rios e até mesmo estuários em busca de alimento, enquanto que os adultos estão em águas mais profundas e geralmente fora da costa onde é possível para algumas espécies a agregação reprodutiva (SALE, 1991).

O comprimento médio dos grupos de lutjanídeos é geralmente entre 30-60 cm, sendo alguns superiores a 60 cm e superiores a 100 cm. Além disso, a família Lutjanidae possui corpo semicircular e alongado, o que facilita sua locomoção durante a alimentação e natação (Moura & Lindeman, 2007). Lutjanídeos são comuns em corpos d'água, desde a superfície até profundidades de mais de 500 m. Com base em informações obtidas em publicações sobre as profundidades máxima e mínima em que os pargos adultos são encontrados, há uma variação considerável na distribuição vertical entre as subfamílias (Martinez-Andrade, 2003). A amplitude de profundidade das espécies amostradas está sendo representadas na figura 2.

Figura 2 – Amplitude de profundidade para as espécies amostradas. Não consta no banco de dados informações a cerca de profundidade para a espécie *Lutjanus purpureus*.



Fonte: Barboza, M. G. (2022).

Desta forma, são importantes espécies-modelo para estudos tanto de diferenças intra- como inter-específicas que possam contribuir com seu conhecimento ecológico e, consequentemente, sobre os efeitos da pesca em suas populações.

1.1 OBJETIVO GERAL

Caracterizar morfologicamente o otólito *sagittae* e o seu *sulcus acusticus* para 8 espécies da família Lutjanidae que ocorrem no Atlântico tropical ocidental, relacionando tais características de forma com a profundidade, ontogenia e ecologia da espécie.

1.2 OBJETIVOS ESPECÍFICOS

a) Descrever a forma geral dos otólitos *sagittae* e dos seus *sulcus acusticus* para as espécies da família Lutjanidae usando Índices de forma
b) Relacionar a morfologia e morfometria dos otólitos e *sulcus acusticus* das espécies com a profundidade, ontogenia e ecologia.

2 ARTIGO 1 – MORPHOLOGICAL CHARACTERIZATION OF OTOLITHS AND *SULCUS ACUSTICUS* OF SNAPPER SPECIES OCCURRING ON THE NORTHEAST COAST OF BRAZIL

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Abstract

Image analysis has been widely used for the morphological characterization of otoliths and *sulcus acusticus*, playing a significant role in species biology. Because of this, this study aimed to morphologically characterize the shape of otolith sagittae and their *sulcus acusticus* for eight species of the family Lutjanidae distributed in the western tropical Atlantic, relating such shape characteristics to depth, growth, and species ecology. A total of 1135 otoliths, sampled along the Northeast of Brazil by several projects, among them REVIZEE, were analyzed. The otolith images were processed and all metrics and shape indices were performed with the help of Software R. Otolith shape varied among species, while that of *sulcus acusticus* did not. Significant regressions suggest otolith modifications throughout the development of the individuals. *O. chrysurus* showed a sex difference for the length-weight relationship and for the other metrics and indices. The *sulcus relative surface* (SRS) differed significantly among species, where *O. chrysurus* and *L. synagris* stood out with the highest proportions of sulcus acusticus area, while *L. vivanus* and *L. bucanella* had the lowest. The Mann-Whitney test indicated that shallow-water species have higher SRS than deep-water species. On average, otoliths of individuals from deeper water had smaller and more elongated otoliths, with smaller, more irregular and more elongated *sulcus acusticus*. These two approaches combined resulted in a higher efficiency to discriminate the species, with an average of 73.7% classification. With this, it is plausible to conclude that through the

morphometric and morphological characteristics of otoliths obtained for this family it is possible to characterize its species and habitats used.

Keywords: images; sagittae; shape; Tropical Atlantic, Lutjanidae.

1. Introduction

Otoliths are structures of great importance for the auditory system of bony fishes and are composed mainly of calcium carbonate in the form of aragonite and other minerals in smaller proportions, embedded in a protein matrix (Campana, 1999; Popper & Lu, 2000). They are present in the inner ear, precisely within labyrinthine compartments (sacculus, utricule, and lagena), where each accommodates a pair of otoliths called *sagittae*, *lapilli*, and *asterisci*, respectively (Assis, 2003; Popper & Lu, 2000).

The sagittae is the most commonly used pair for age determination (Monteiro *et al*., 2005) and also in otolith shape studies due to its larger size (Begg *et al*., 2005). In addition, its species-specific characteristics made it an useful tool for studies on taxonomy, phylogeny (Assis, 2003), archaeology (Disspain; Ulm; Gillanders, 2015), paleontology (Reichenbacher *et al*., 2007), geographic variation of species (Rossi-Wongtschowski *et al*., 2015), stock identification (Duarte-Neto *et al*., 2008), among others. Otolith characteristics reflect the habit and life history of the species, in addition to the composition of the environment in which they live (Baumann *et al*., 2006; Campana *et al*., 2000; Cardinale *et al*., 2004; Duarte-Neto *et al*., 2014). The *sulcus acusticus*, located on the inner side of the otolith and in direct contact with the sensory tissue (macula), is directly associated with the hearing capacity of fish. This function is related to the size of the otolith and the area of the sulcus acusticus in relation to the total area of the otolith (Lombarte, 1992). Thus, variation in the shape of the *sulcus* is a morpho-functional characteristic of otoliths (Platt & Popper, 1981).

Image analysis has been widely used for the morphological characterization of otoliths and sulcus acusticus, playing a significant role in fisheries biology. Its application led to significant advancements in data collection, to a better description of otolith shape, and the development of new analytical methods (Cadrin & Friedland, 1999). Image analysis techniques have been employed to identify fish stocks (Dos-Santos *et al*., 2022; Duarte-Neto *et al*., 2008; Stransky *et al*., 2008a) and taxa given the phenotypic plasticity of otoliths, as well as to describe shape variations with ecological inferences about fish life (Campana & Casselman, 1993; Tuset, 2003).

Among the techniques used are shape indices (Sadighzadeh, 2012; Tuset, 2021), Fourier analysis (Duarte-Neto *et al*., 2008; Tuset, 2013) and Wavelets (Lombarte & Tuset, 2015; Sadighzadeh, 2014). In addition, because otolith growth is directly related to fish growth, fish weight and length can be estimated from otolith metrics (Waessle *et al*., 2003). In some cases, it is possible to assess age by otolith weight, due to the high correlation between these parameters (Arrhenius; Cardinale, 2004; Lepak *et al*., 2012).

Among the various techniques, shape indices stand out for being simple to calculate and interpret, representing simple morphometric ratios of otoliths. They are successfully applied in the analysis of potential taxonomic patterns and/or other ecological inferences about shape, such as the effects of differing habitat occupation, including factors such as placement in the water column and types of substrates (Volpedo & Echeverría, 2003).

According to Campana *et al*. (1993), otolith shape is a species-specific characteristic, and differences between stocks are associated with genetic heterogeneity and environmental variability. In contrast, Reichenbacher *et al*. (2009), using linear morphometry of sagittal otoliths, concluded that environmental factors had the greatest influence on population divergence. Thus, model species are important for studies on both intra- and interspecific differences that can contribute to their ecological knowledge.

Morphological studies comparing otoliths of species of the Lutjanidae family are scarce, especially for South Western Atlantic Species (Ferreira *et al*. 2004), even though the family is among the most important fish resources in tropical and subtropical regions (Duarte & Garcia, 1999). The Lutjanidae includes 113 species distributed across four subfamilies: Apsilinae, Etelininae, Lutjaninae and Paradicichtinae (Allen, 1985; Nelson *et al*., 2016; William & Anderson, 1987). The subfamilies Lutjaninae and Etilininae, commonly known as snappers, are represented by four genera (*Etelis*, *Lutjanus*, *Ocyurus* and *Rhomboplites*), of which 11 species occur in Brazilian waters (Allen, 1985; Anderson, 1987; Menezes & Figueiredo, 1980).

These species can be divided into two main groups according to their ecology: the shallow water snapper species, which are found from the coast, where they use coastal environments as nurseries (Aschenbrenner *et al*., 2014), to deeper regions of the shelf, and the deeper water species that preferentially inhabit mesophotic environments distributed from the 30 m isobath to the outer shelf and slope break and on islands and seamounts, exhibiting ecological differences in terms of depth, feeding habit, position in the water column and preferred habitats throughout life (Allen, 1985; Nelson, 2006; Sale, 1991). In addition to its economic importance, such variability in habitat occupation, as well as biological and ecological characteristics among Lutjanidae species make the family a key source of information regarding the morphofunctional relationships of otoliths.

In this context, the present study aimed to morphologically characterize the sagittae otolith and its *sulcus acusticus* for 8 species of the family Lutjanidae occurring in the western tropical Atlantic, relating such shape characteristics to the depth, growth and ecology of the species.

2. Material and methods

2.1. Study area and sample

The sample used in this study consisted of 1135 otoliths from 8 species: *Lutjanus synagris* (Linnaeus, 1758) (n = 152), *L. analis* (Curvier, 1828) (n = 244), *Ocyurus chrysurus* (Bloch, 1790) (n = 171), *L. jocu* (Bloch and Schneider, 1801) (n = 171), *L. bucanella* (Curvier, 1828) (n = 69), *Rhomboplites aurorubens* (Curvier, 1829) (n = 56), *L. vivanus* (Curvier, 1828) (n = 198), and *L. purpureus* (Poey, 1866) (n = 74), collected off the coast of Pernambuco (PE), Ceará (CE), Rio Grande do Norte (RN), and Bahia (BA) states, Northeastern Brazil (Figure 1), from the REVIZEE, Pró-arribada, Recifes Costeiros, and Repensapesca projects. Analyses were also done based on the sex of the species: *Lutjanus synagris* (Linnaeus, 1758) (n = 140; F = 91; M = 49), *L. analis* (Curvier, 1828) (n = 154; F = 71; M = 83), *Ocyurus chrysurus* (Bloch, 1790) (n = 144; F = 73; M = 71), *L. jocu* (Bloch and Schneider, 1801) (n = 140; F = 64; M = 76), *L. bucanella* (Curvier, 1828) (n = 56; F = 32; M = 24), *Rhomboplites aurorubens* (Curvier, 1829) (n = 30; F = 13; M = 17), *L. vivanus* (Curvier, 1828) (n = 111; F = 71; M = 40), and *L. purpureus* (Poey, 1866) (n = 20; F = 13; M = 7). They are part of the collection and database of the Laboratory of Coastal and Reef Ecosystems (LECOR), located in the Department of Oceanography of the Federal University of Pernambuco - UFPE. The database also contains specimens' weight (W, g), total length (TL, cm), standard length (SL, cm), fork length (FL, cm), and sex. Species that are not observed in shallow environments (above 10 meters) in their juvenile stage were classified as "shallow-water", and those that inhabit shallow environments in their juvenile stages were classified as "deep-water", including coastal ecosystems such as seagrass beds, mangroves, and coral reefs (Aschenbrenner *et al*, 2014; Fredou & Ferreira, 2005). The deep-water species group included *R. aurorubens*, *L. purpureus*, *L. bucanella*, and *L. vivanus*, while the shallow-water one included *L analis*, *L. jocu*, *L. synagris*, and *O. chrysurus*. The possible occurrence of the same population for the species was not evaluated.

2.2. Image acquisition and morphometric measurements

Whole otoliths were photographed using a Leica S9D stereomicroscope, with an attached MC190 HD camera. Plasticine was used as a background to aid in the positioning of the otoliths due to its smooth, opaque texture. The left otoliths were used, because they are in larger quantities, but for those species whose sample size was small, images of the mirrored right otolith were used, since there is no difference between left and right otoliths for these species. Each otolith was photographed in a standardized manner, with the *sulcus acusticus* facing upwards. The morphological description of the otoliths of the studied species was based on the detailed definitions available in Tuset *et al*. (2008), considering: overall shape, anterior and posterior region; dorsal and ventral margins; the shape of the *rostrum* and *antirostrum* (Figure 2). The morphological description of the *sulcus acusticus* was based on definitions presented in Assis (2000), considering its shape, position, opening and orientation.

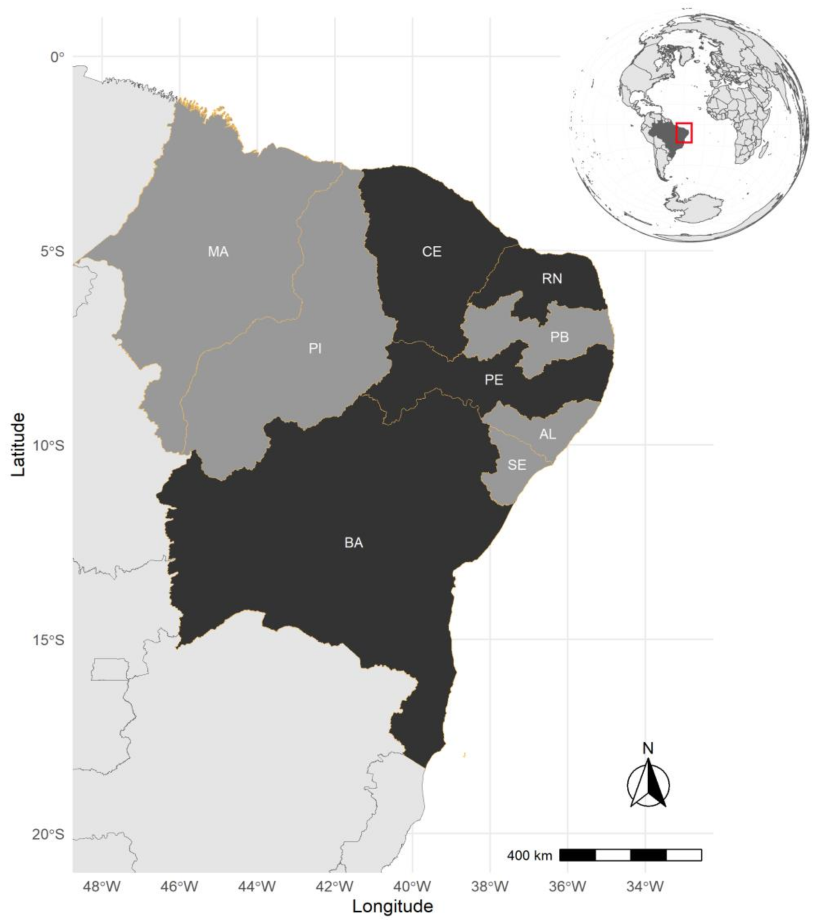

FIGURE 1 Study area in Northeast Brazil (light gray). Highlighted states (dark gray) indicate where samples were obtained.

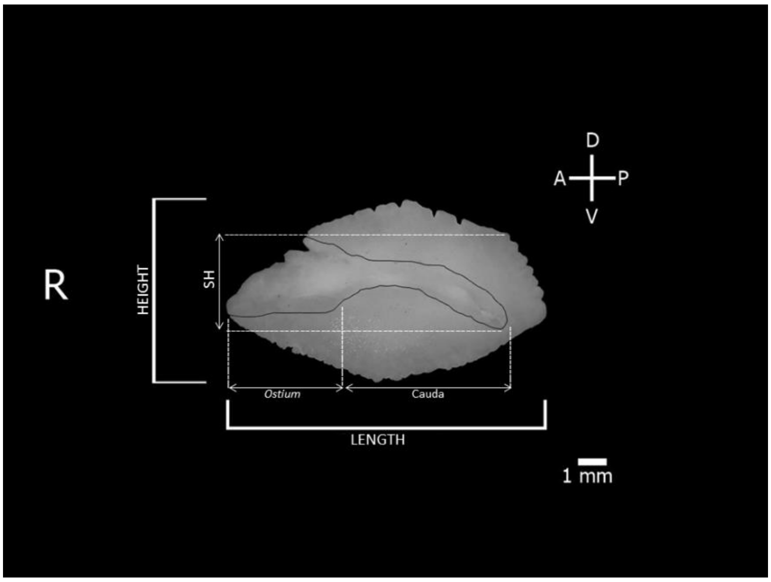

FIGURE 2 Internal surface of the saccular otolith of *Lutjanus vivanus* indicating the length and height measurements. The highlighted region is the sulcus acusticus. R = *rostrum* direction, D = dorsal, V = ventral, A = anterior and P = posterior. The *Ostium*, *cauda* and height of the *sulcus acusticus* (SH) are also shown.

Otolith weight (OW, g) was obtained using an analytical scale (± 0.0001 g) and all morphometric variables were obtained using the R Software (R Development Core Team, 2018). Length, height, area, perimeter and various shape indices such as form factor, circularity, roundness, rectangularity, ellipticity and aspect ratio were also measured, as shown in Table 1. Area was calculated based on the number of pixels within the outline, while perimeter was calculated by the number of pixels in the outline. The form factor is an estimator of surface area irregularity, assuming values between 0 for irregular and 1 for regular. Roundness and circularity compare objects to a perfect circle, assuming values ≥ 4π and ≥ 1, respectively. Rectangularity relates the object to a square, assuming values equal to 1 for a perfect square. Ellipticity indicates whether the changes in the axes are proportional. Finally, aspect ratio determines the circular or elongated aspect, assuming a value equal to 1 if it is a circle or a perfect square (Figure 2) (Assis *et al*., 2020; Russ, 1990; Tuset *et al*., 2003; Volpedo & Echevarría, 2003).

TABLE 1 Shape indices and their respective equations used for otoliths.

Shape Indices	Equation
Form Factor	$FF = \frac{4\pi * area}{perimeter^2}$
Circularity	$C = \frac{perimeter^2}{area}$
Roundness	$RD = \frac{4 * area}{\pi * length^2}$
Rectangularity	$R = \frac{area}{length * height}$
Ellipticity	$EL = \frac{length - height}{length + height}$
Aspect Ratio	$AR = \frac{height}{length}$

Linear ($y = a + bx$) and potencial regressions ($y = ax^b$) between the fork length of individuals (y) and the morphometric variables, and the weights of otoliths and individuals (x), respectively, were performed to evaluate the relationship between the otolith growth pattern and the development of individuals of each species. For dimensionless shape indices, linear regressions were used, while for quadratic and cubic ones (area and weight, respectively), we used non-linear regressions. Potential intra- and interspecific sex-based differentes were tested through an analysis of covariance (ANCOVA). In addition, non-linear regressions were fitted between otolith and individual weights, and otolith length (OL, mm) and individual fork length, respectively.

2.3. Morphometric Analysis

To reduce the confounding effects of growth-related morphological variability, a subsample composed only of adult individuals of all species was used for the following analyses, to better measure the interspecific and depth effects on otolith morphological characteristics. This subsample was composed of 1040 individuals, divided as follows: 127 *L. synagris*, 213 *L. analis*, 170 *O. chrysurus*, 166 *L. jocu*, 69 *L. bucanella*, 56 *R. aurorubens*, 186 *L. vivanus*, and 53 *L. purpureus*.

A Principal Component Analysis (PCA) was carried out to reduce data dimensionality, condense the information and eliminate possible collinearities. The number of principal components (PCs) retained was determined based on a segmented regression between the components and the variance explained by each of them, using the 'segmented' package version 0.5-3.0 (Muggeo, 2008). The estimation of the breakpoint, defined in the model composed of two linear models, makes the use of the scree plot less subjective. The calculated scores for the retained components were then used as shape variables in subsequent analyses.

To test the influence of species and depth (deep *vs* shallow), a multivariate analysis of variance (MANOVA) with the Hotelling-Lawley test (Thompson, 1992) was performed. When significant, an analysis of variance (ANOVA) was then employed to verify in a one-dimensional manner which principal components (PCs) were significant. Finally, a linear discriminant analysis (LDA) was used to measure the potential for correctly classifying individuals into their species or depth strata. A classification model was built using a 70% sample and tested on 30% of the database. The significance level was set at $\alpha = 0.05$ for all analyses.

2.4. *Sulcus acusticus* Analysis

From the sample of adult individuals, 30 individuals of each species were randomly selected, totaling 240 otolith images, from which the *sulcus* region was manually segmented. Then, the same morphometric measurements were made for the otolith contour. Additionally, the *sulcus relative surface* (SRS) was calculated for each individual (Lombarte, 1992), which represents the ratio between the *sulcus* surface area and the otolith surface area (SA/OA). By analyzing this surface area, we can draw a relationship between otolith morphology and the communication strategy of each species (Lombarte, 1992; Popper *et al*., 2005, Reichenbacher *et al*., 2009).

The Shapiro-Wilk and Levene tests were applied to this variable to evaluate the assumptions of normality and homogeneity of variance, respectively. As the distribution proved to be non-normal, the Kruskal-Wallis test was used to test for possible differences between species. The Nemenyi's post-hoc test was then used for multiple comparisons to investigate significant area differences between species. The Mann-Whitney test was also used to compare the two depth groups (deep and shallow) with respect to *relative sulcus surface* area. As for the otoliths, PCA and LDA were also performed for the *sulcus acusticus* sample and for the combined sample of otolith contour and *sulcus acusticus,* in order to compare the patterns of morphometric variations of these different parts of the otoliths separately and combined.

2.5. Hierarchical Clustering and Decision Tree Analysis

The groups were formed from the similarity between them and using the Euclidean distance as a measure. The principal components of all morphometric measurements were used in order to include all variables and remove collinearity among them.

Ward's method (WARD, 1963) was used to classify the groups, based on the loss of information resulting from the grouping of species and is measured by the sum of the squares of the deviations of individual plots from the means of the groups in which they are classified, presenting a high internal homogeneity (BARROSO; Artes, 2003). The recursive decision tree algorithm (BREIMAN, 1984) was used for classification with the "tree" package in Rstudio software (RIPLAY, 1984). This algorithm establishes networks that are related to each other by a hierarchy, similar to a dendrogram, where, from a dichotomous condition, it subdivides the data. Samples that satisfy these conditions are said to be a homogeneous group, to which side of the tree this network follows. A proportion for test database and training database of 70% and 30%, respectively, was used.

3. Results

A total of 1040 otoliths were measured from specimens with average fork lengths per species ranging from 31.74 to 53.92 cm (Table 2). In addition, the table also shows the minimum and maximum furcal lengths for the analyzed species. Tables 2 and 3 present the mean and standard deviation values, by species, of the morphometric measurements and shape indices of the otoliths and *sulcus acusticus*, respectively. The species *L. analis* presented the largest otoliths, followed by *L. purpureus*, both in length and area. The species *R. aurorubens* and *O. chrysurus* had smaller ones. While no considerable variation among species was observed for indices, significant differences in measurements were found. The main morphological characteristics of otoliths and *sulcus acusticus* were described for each lutjanidae species studied (Table 4). Despite the great morphological variability of the otoliths, the *sulcus* did not present differences in their characterizations. The dorso-ventral edge of the otoliths varied between species in crenulate, irregular and sinuous, while their anteroposterior region presented angular, rounded, or oblique features. The *sulcus acusticus* presented the same general characteristics for all species: heterosulcoid, median position, ostial opening, and horizontal orientation (Table 4).

TABLE 2 Descriptive measures of the indices (mean and standard deviation) of otoliths for the studied species. And simples measuremants (min – máx (mean)). N = Number of individuals; FL = Fork length; BW = Body Weight; OW = Otolith Weight.

Species/Variables	*L. analis*	*L. bucanella*	*L. jocu*	*L. purpureus*	*L. synagris*	*L. vivanus*	*O. chrysurus*	*R. aurorubens*
N	213	69	166	53	127	186	170	57
FL (cm)	37 - 76 (46.3)	28 - 50 (35.5)	31.7 - 95 (55.6)	41.5 - 71 (55.5)	22.8 - 48.5 (26.1)	23 - 56 (35.5)	20.5 - 53.6 (33.3)	20.5 - 46.5 (32.2)
BW (kg)	0.12 - 5 (2.4)	0.4 - 2.4 (1.16)	0.4 - 8.3 (1.9)	1.3 - 4.5 (3.7)	0.12 - 1.9 (0.7)	0.2 - 2.2 (0.85)	0,2 - 2.16 (0.82)	0.14 - 1 (0.33)
OW (g)	0.036 - 1.8 (0.63)	0.14 - 0.79 (0.26)	0.069 - 0.75 (0.24)	0.39 - 1.08 (0.55)	0.16 - 1.14 (0.48)	0.093 - 0.85 (0.28)	0.045 - 0.31 (0.12)	0.058 - 0.29 (0.15)
Area	129.15 ± 37.3	74.25 ± 16.75	77.91 ± 24.09	127.26 ± 18.81	96.35 ± 40.49	86.03 ± 27.19	48.85 ± 13.5	48.34 ± 11.99
Perimeter	50.76 ± 8.72	38.02 ± 5.25	40.8 ± 7.04	51.69 ± 4.52	41.64 ± 9.85	40.52 ± 6.83	32.22 ± 5.49	31.45 ± 4.6
Length	17.06 ± 2.49	13.24 ± 1.53	13.62 ± 2.1	17.24 ± 1.44	14.98 ± 3.47	13.99 ± 2.22	10.82 ± 1.59	10.66 ± 1.57
Height	10.7 ± 1.57	7.89 ± 0.88	8.27 ± 1.38	10.29 ± 0.78	8.49 ± 1.86	8.57 ± 1.31	6.38 ± 0.88	6.43 ± 0.75
Circularity	20.25 ± 1.86	19.6 ± 1.32	21.84 ± 1.8	21.11 ± 1.4	18.85 ± 1.01	19.48 ± 1.15	21.53 ± 1.91	20.72 ± 1.47
Rectangularity	0.7 ± 0.02	0.7 ± 0.01	0.68 ± 0.02	0.71 ± 0.02	0.72 ± 0.01	0.7 ± 0.02	0.69 ± 0.02	0.7 ± 0.02
Form Factor	0.63 ± 0.05	0.64 ± 0.04	0.58 ± 0.05	0.6 ± 0.04	0.67 ± 0.03	0.65 ± 0.04	0.59 ± 0.05	0.61 ± 0.04
Ellipticity	0.23 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.25 ± 0.02	0.28 ± 0.02	0.24 ± 0.02	0.26 ± 0.03	0.25 ± 0.03
Aspect Ratio	0.63 ± 0.03	0.6 ± 0.02	0.61 ± 0.03	0.6 ± 0.02	0.57 ± 0.02	0.61 ± 0.03	0.59 ± 0.03	0.61 ± 0.04
Roundness	0.56 ± 0.03	0.54 ± 0.02	0.52 ± 0.03	0.54 ± 0.03	0.52 ± 0.02	0.55 ± 0.03	0.52 ± 0.03	0.54 ± 0.04

TABLE 3 Descriptive measures (mean and standard deviation) of the *sulcus acusticus* for the studied species. N = Number of individuals; FL = Fork length; SRS = *Sulcus Relative Surface*.

Species/Variables	*L. analis*	*L. bucanella*	*L. jocu*	*L. purpureus*	*L. synagris*	*L. vivanus*	*O. chrysurus*	*R. aurorubens*
N	30	30	30	30	30	30	30	30
FL	53.54 ± 8.54	36.63 ± 5.28	54.33 ± 17.67	43.11 ± 12.39	28.02 ± 8.93	33.78 ± 9.74	28.23 ± 4.96	28.82 ± 7.53
SRS	0.33 ± 0.03	0.28 ± 0.02	0.3 ± 0.03	0.31 ± 0.03	0.38 ± 0.08	0.28 ± 0.02	0.48 ± 0.05	0.33 ± 0.04
Area	49.86 ± 13.31	22.48 ± 5.68	25.23 ± 10.73	30.54 ± 14.47	25.75 ± 15.4	24.95 ± 8.44	13.51 ± 4.11	16.08 ± 5.4
Perimeter	38.5 ± 4.95	28.3 ± 3.71	29.01 ± 6.31	31.48 ± 7.53	27.42 ± 8.18	29.57 ± 4.31	21.18 ± 3.05	23.04 ± 4.02
Length	14.54 ± 1.71	10.98 ± 1.25	10.39 ± 2.02	12.2 ± 2.85	10.66 ± 3.1	11.6 ± 1.75	8.25 ± 1.22	8.91 ± 1.52
Height	5.47 ± 0.86	3.6 ± 0.63	4.5 ± 1.18	4.16 ± 1.12	3.54 ± 1.09	3.66 ± 0.6	2.72 ± 0.41	3.12 ± 0.66

Circularity	30.42 ± 2.32	36.14 ± 2.19	34.83 ± 3.17	35.15 ± 3.36	31.74 ± 2.11	36.02 ± 1.94	34.12 ± 1.88	33.89 ± 2.33
Rectangularity	0.62 ± 0.04	0.56 ± 0.04	0.52 ± 0.03	0.56 ± 0.04	0.63 ± 0.03	0.57 ± 0.02	0.59 ± 0.03	0.57 ± 0.04
Form Factor	0.42 ± 0.03	0.35 ± 0.02	0.36 ± 0.03	0.36 ± 0.03	0.4 ± 0.02	0.35 ± 0.02	0.37 ± 0.02	0.37 ± 0.03
Ellipticity	0.45 ± 0.03	0.51 ± 0.03	0.4 ± 0.04	0.49 ± 0.04	0.5 ± 0.03	0.52 ± 0.03	0.5 ± 0.03	0.48 ± 0.04
Aspect Ratio	0.38 ± 0.03	0.33 ± 0.03	0.43 ± 0.05	0.34 ± 0.03	0.33 ± 0.02	0.32 ± 0.02	0.33 ± 0.02	0.35 ± 0.04
Roundness	0.29 ± 0.03	0.23 ± 0.02	0.28 ± 0.03	0.24 ± 0.02	0.27 ± 0.02	0.23 ± 0.01	0.25 ± 0.02	0.25 ± 0.02

TABLE 4 Morphological characterization of the main features of otoliths (shape; dorso-ventral edge; antero-posterior region; *cauda*; ostium and rostrum) and *sulcus acusticus* (shape, position, opening and orientation).

	Species / Variable	*L. analis*	*L. bucanella*	*L. jocu*	*L. purpureus*	*L. synagris*	*L. vivanus*	*O. chrysurus*	*R. aurorubens*
Otolith	**Shape**	Elliptical	Elliptical	Triangular	Elliptical	Elliptical	Elliptical	Triangular	Elliptical
	Dorsal edge	Irregular	Irregular	Sinuous	Irregular	Sinuous	Irregular	Irregular	Irregular
	Ventral edge	Sinuous	Sinuous	Crenulated	Sinuous	Sinuous	Sinuous	Irregular	Crenulated
	Anterior region	Angular	Round	Round	Angular	Round	Round	Angular	Angular
	Posterior region	Angular	Oblique	Oblique	Oblique	Oblique	Angular	Angular	Oblique
	Cauda	Moderately curved	Slightly curved	Strongly curved	Moderately curved	Moderately curved	Slightly curved	Moderately curved	Strongly curved
	Ostium	Funnel	Rectangular	Funnel	Rectangular	Rectangular	Funnel	Rectangular	Funnel
	Rostrum	Concordant	Concordant	Concordant	Concordant	Concordant	Concordant	Concordant	Concordant
Sulcus	**Shape**	Heterosulcoid	Heterosulcoid	Heterosulcoid	Heterosulcoid	Heterosulcoid	Heterosulcoid	Heterosulcoid	Heterosulcoid
	Position	Median	Median	Median	Median	Median	Median	Median	Median
	Opening	Ostial	Ostial	Ostial	Ostial	Ostial	Ostial	Ostial	Ostial
	Orientation	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal

3.1. Otolith Morphometry

All relationships between otolith weight and length were significant and showed good fits, with r^2 equal or greater than 0.70. The regression between fish weight and fork length also showed good fits in its majority, except for *L. bucanella*, which showed an r^2 of 0.58. No sex-based significant differences between otolith weight-length relationship (POE x CO) were found for most species, except for *R. aurorubens* (ANCOVA, df = 46, *t-test* = 2.89, *p-value* < 0.006). As for fish weight-length ratio (PT x FC), the exception was *O. chrysurus* (ANOVA, df = 107, *t-test* = -2.72, *p-value* < 0.008) (Figure 3, Table 5). There was no significant difference between the otolith weight-length ratio for the species *L. jocu* with the others (ANCOVA, *p-value* < 0.00), and, for *L. analis* with *L. bucanella*, *L. vivanus* and *R. aurorubens* (*p-value* < 0.00). Furthermore, there was no difference between *L. purpureus* with the other species, except with *L. analis*, *L. synagris* and *R. aurorubens* (ANCOVA, *p-value* >0.00). For fish length-weight relationship, there was no significant difference between *L. purpureus* and *L. synagris*, *L. vivanus*, and *O. chrysurus* (ANCOVA, *p-value* < 0.00), and for *L. jocu* and *R. aurorubens* (*p-value* < 0.00). There was also a difference for *L. analis* with *L. synagris*, *L. vivanus* and *O. chrysurus* (ANCOVA, *p-value* < 0.00), and for *L. bucanella* with *O. chrysurus* (*p-value* = 0.0001).

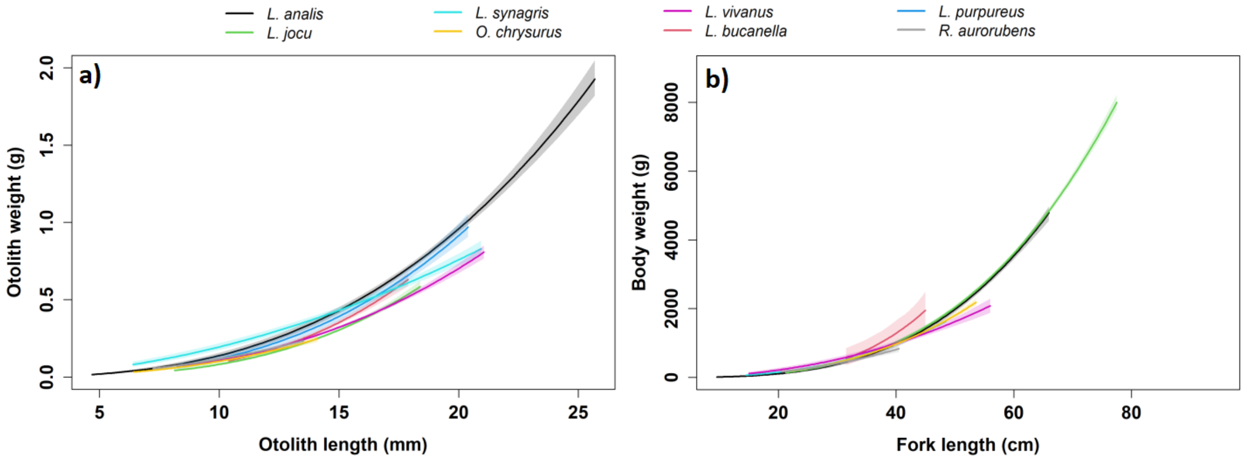

FIGURE 3 Regressions between (a) otolith weight and otolith length, and (b) fish weight and fish length (FL, cm) for each assessed species of the family Lutjanidae.

For the variables height (ANCOVA, df = 167, *t-test* = -2.5, *p-value* < 0.05) and area (ANCOVA, df = 167, *t-test* = -2.2, *p-value* < 0.05), significant differences between males and females were only observed for the species *O. chrysurus* (Figure 4, Table 6). There was no significant difference between species, except between *L. bucanella* and *L. purpureus*, for all measures (ANCOVA, *p-value* = 0.28; *p-value* = 0.79; *p-value* = 0.21; and, *p-value* = 0.086, respectively). A significant difference in area was found between *L. analis* and *L. vivanus* (*p-value* = 0.35). The species *L. purpureus* and *R. aurorubens* were similar regarding the length variable (*p-value* = 0.059).

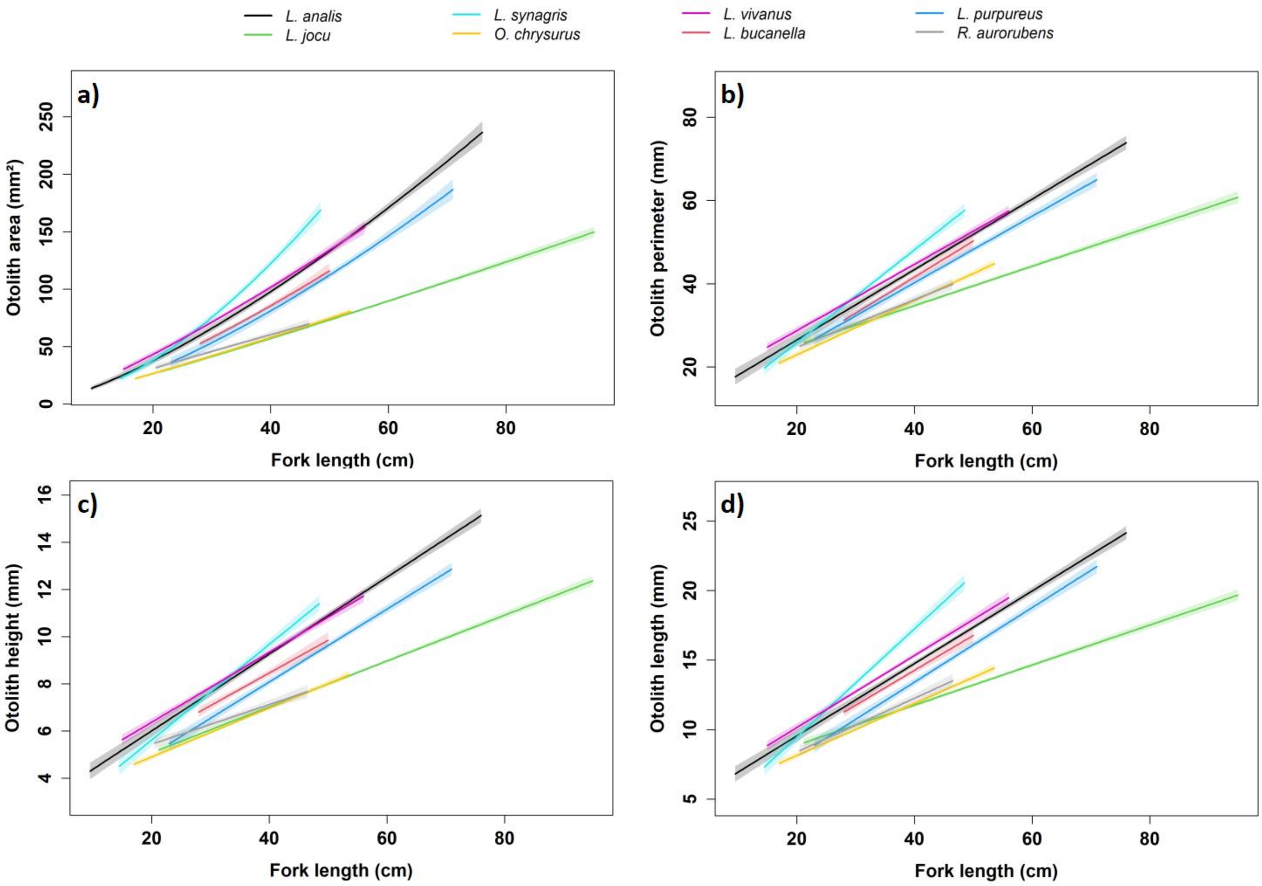

FIGURE 4 Regressions of area (a), perimeter (b), otolith height (c), and otolith length (d) measurements with respect to the fork length of individuals for each assessed species of the family Lutjanidae.

Significant sex-based differences in otolith roundness were found for *O. chrysurus* (ANCOVA, df =167, *t-test* = -2.07, *p-value* < 0.05). Likewise, only rectangularity was divergent for *L. jocu* (ANCOVA, df = 170, *t-test* = 2.8, *p-value* < 0.006). However, only female otoliths showed a significantly negative relationship with fork length (ANOVA, df = 169, *t-test* = -3.34, *p-value* < 0.05). That is, the otoliths of *L. jocu* females became less rectangular, while the otoliths of males did not change. Similarly, only males of *L. bucanella* showed ontogenetic variation for aspect ratio (ANOVA, df = 22, *t-test* = -2.18, *p-value* < 0.05), while only females exhibited variations in ellipticity (ANOVA, df = 67, *t-test* = 1.33, *p-value* < 0.05) (Figure 5, Table 6).

Overall, the significant regressions suggest the following modifications of otoliths throughout the development of the individuals. The otoliths of *L. jocu* become more eccentric and less elliptical. The otoliths of *L. purpureus* and *L. synagris*, on the other hand, lose eccentricity but gain on rectangularity and ellipticity. The otoliths of *L. analis* tend to become more eccentric and more circular/rounder. Finally, for *L. vivanus*, *L. bucanella*, *O. chrysururs*, and *R. aurorubens*, otoliths become more circular and elliptical (Figure 5). There was no significant difference between the species *O. chrysurus* and *R. aurorubens* for the variables circularity (*p-value* = 0.051), rectangularity (*p-value* = 0.84), form factor (*p-value* = 0.07) and roundness (*p-value* = 0.06). There was no significant difference between *L. jocu* and *R. aurorubens* for all variables except rectangularity (*p-value* < 0.00). *L. analis* and *L. vivanus* were similar in circularity (*p-value* = 0.81) and form factor (*p-value* = 0.70). *L. purpureus* and *L. vivanus* were also similar, but in ellipticity (*p-value* = 0.90) and eccentricity (*p-value* = 0.99).

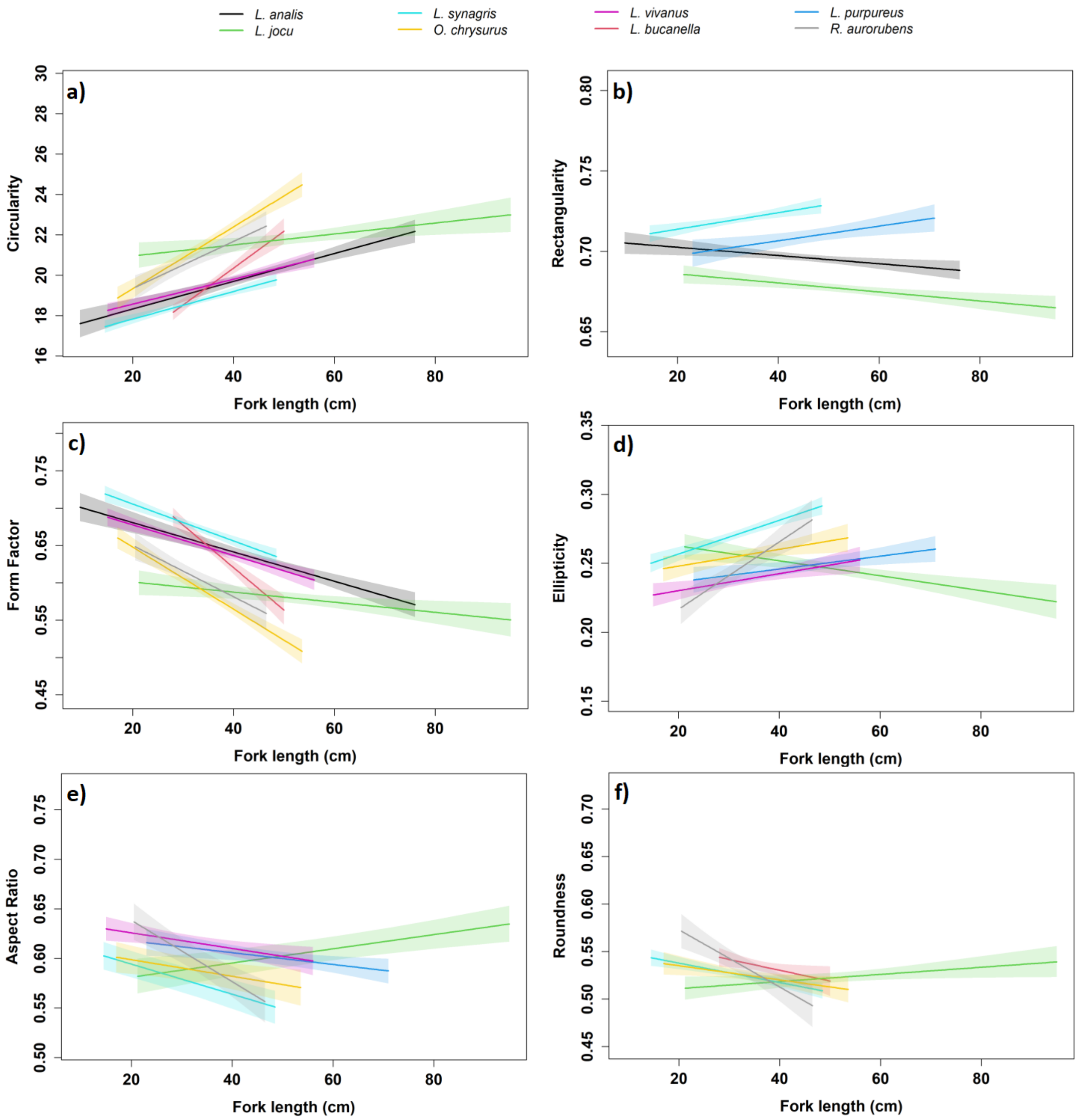

FIGURE 5 Regressions of the circularity (a), rectangularity (b), form factor (c), ellipticity (d), aspect ratio (e), and roundness (f) indices with respect to the fork length of individuals for each species of the Lutjanidae family. Only the significant linear regressions are represented in the graphs.

3.2. *Sulcus acusticus* analysis

The SRS differed significantly between species (KW = 156.16, df = 7, *p-value* < 0.001). Two shallow-water species, *O. chrysurus* and *L. synagris*, had the highest area proportion of the *sulcus acusticus*, while two deep-water species, *L. vivanus* and *L. bucanella*, had the lowest. No significant differences were found within each pair of species (Figure 6), either for the upper (*p-value* = 0.44) or lower proportions (*p-value* = 0.99). No differences were observed between *L. synagris* and *R. aurorubens* (*p-value* = 0.99) or *L. analis* (*p-value* = 0.70), and between *R. aurorubens* and *L. purpureus* (*p-value* = 0.97) (Figure 6). In addition, the median SRS was significantly higher for shallow-water species compared to the deep-water species (Mann-Whitney, W = 3350, *p-value* < 0.001) (Figure 7).

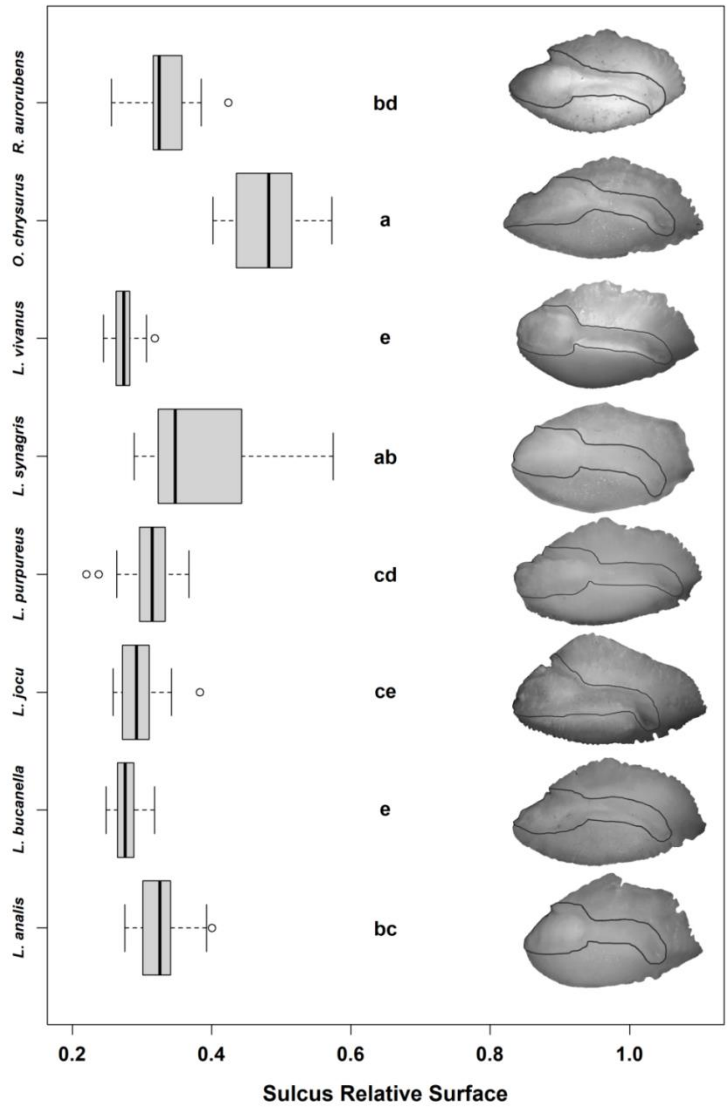

FIGURE 6 Relationship between otolith area and area of their respective *sulcus acusticus* (Sulcus Relative Surface) for each Lutjanidae species studied. Equal letters between species indicate no significant difference (*p-value* > 0.05 by Nemenyi's paired comparison test). On the right side, otolith images of the species are represented with demarcation of their respective *sulcus acusticus*.

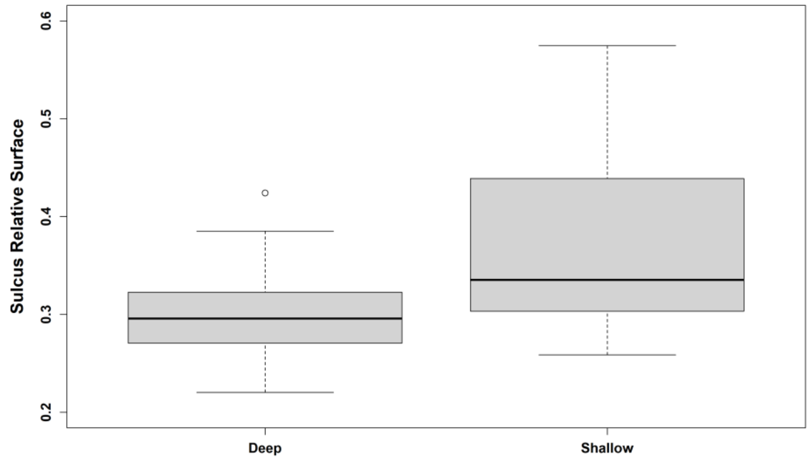

FIGURE 7 Relationship between otolith area and area of their respective *sulcus acusticus* (Sulcus Relative Surface) according to the depth strata of the studied Lutjanidae species.

3.3. Principal component analysis

The estimated breakpoint value in the fit of the segmented model to the screeplot points was 4.64, indicating that the retention of the first 4 components (Figure 8a), which explain almost all of the variation in the data (99.7%). Subsequent analyses were only applied to these components. The variables most correlated with PC1 were height (-0.99), perimeter (-0.98), area (-0.97), and length (-0.95), indicating that this first component is related to otolith size. In this case, individuals to the left of the axis (negative values) of this component have the largest structures, and to the left of the axis (positive values) the smallest. In the case of PC2, the highest correlations were for the variables roundness (-0.91), aspect ratio (-0.80), ellipticity (0.79), form factor (-0.72), circularity (0.71), that is, otoliths with negative scores are more circular and regular, while those with positive values are more irregular. The variables most correlated with PC3 are rectangularity (-0.66), from factor (-0.58), circularity (0.58), ellipticity (-0.54) and aspect ratio (0.54). PC3 is related to the circular/square or elongated shape of the otolith, and also refers to its irregularity. The more positive the values of this component, the more irregular and less elongated the otolith, and the more negative the more rectangular and elongated. From Figure 8b-d, it can be seen that the species have distinct morphological patterns, both in relation to size and the more elongated and irregular aspect of the otoliths of some species. There was a significant difference among all species (MANOVA, HL = 4.31, df = 7, F-test = 64.41, *p-value* < 0.001), and this difference was observed for all PCs analyzed (ANOVA, df = 7, *p-value* < 0.001). In Figure 9, these patterns of variability were not graphically identifiable when we considered the data labeled by depth. However, there was also a significant difference between deep and shallow depths (MANOVA, HL = 0.22, df = 1, F-test = 22.62, *p-value* < 0.001), and differences were observed in all PCs analyzed (ANOVA, df = 1, *p-value* < 0.05). On average, otoliths from individuals from deeper water had the smallest otoliths and the most elongated otoliths.

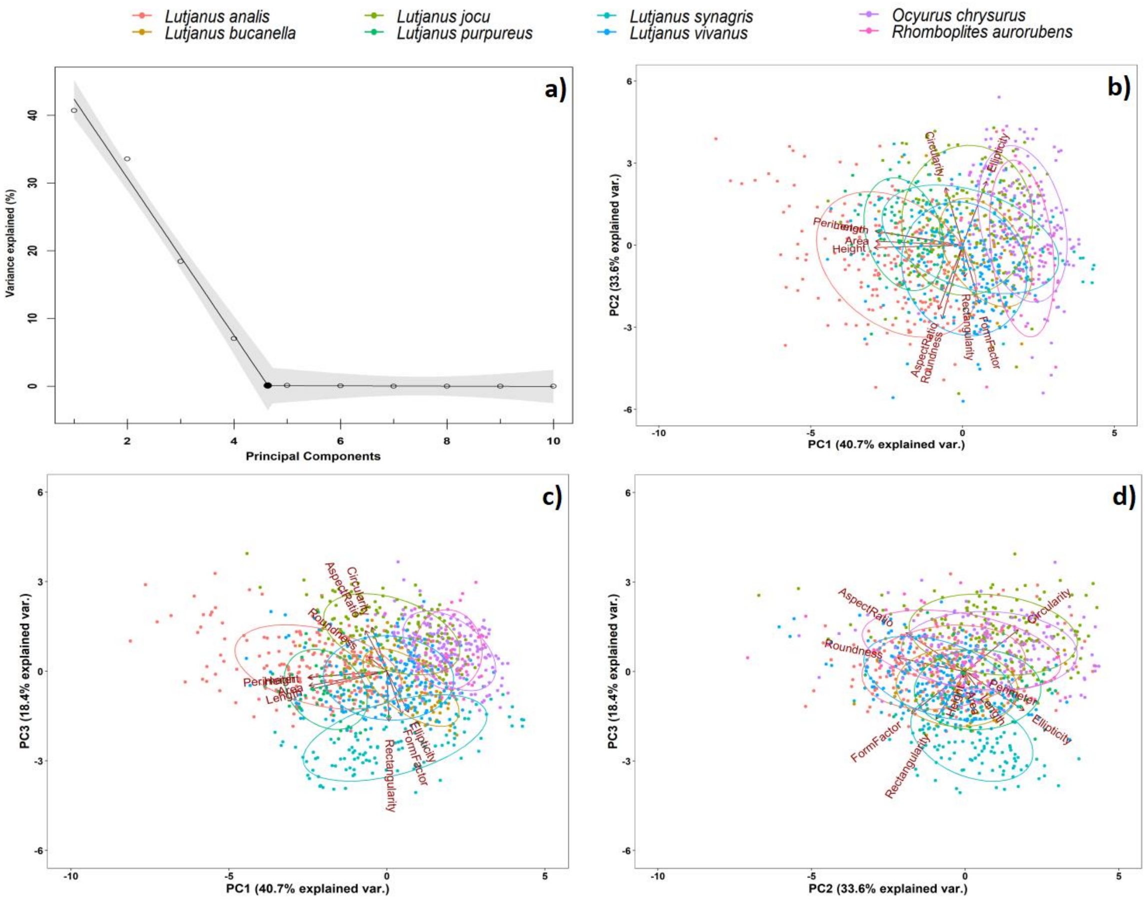

FIGURE 8 Graphical representation of the percentage of data explanation by the 10 Principal Components. Cumulative percentage of explanation of data variation indicated for the first four components (a). Scatter plots of the principal components for the species variable for otolith only and their combinations PC1xPC2 (b), PC1xPC3 (c) and PC2xPC3 (d), with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

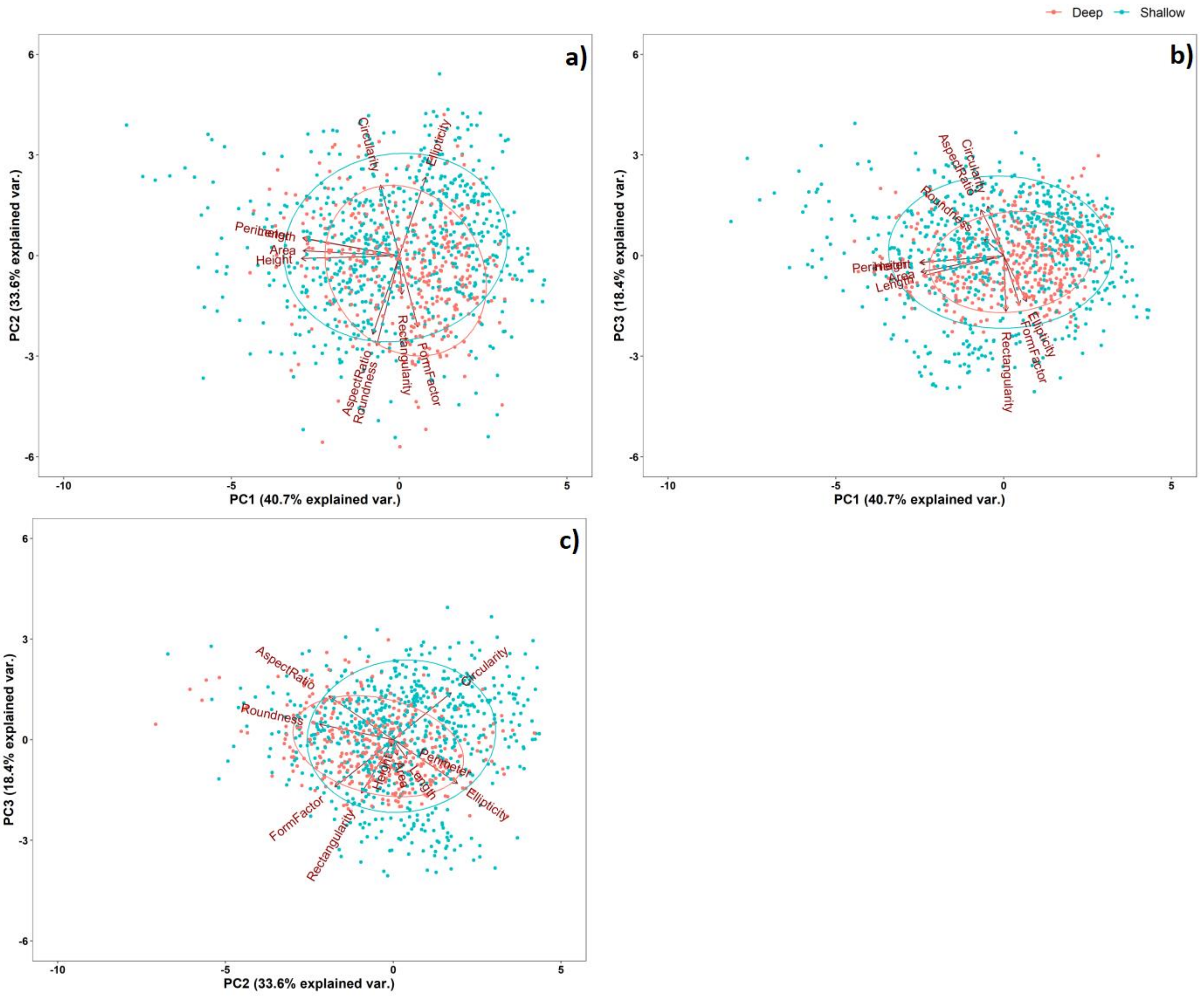

FIGURE 9 Scatter plots of the principal components for the depth variable and its combinations PC1xPC2 (a), PC1xPC3 (b) and PC2xPC3 (c) for the otolith, with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

In the sulcus analysis, the estimated breakpoint value was 4.39, also indicating retention of the first 4 components (Figure 8a), which explains 98.1% of the total sulcus variation. The variables most correlated with PC1 were height (0.92), area (0.89), perimeter (0.82), roundness (0.80), and length (0.77), indicating that this first component is most related to *sulcus* size. In this case, individuals to the left of the axis (negative values) of this component have the smallest structures, and to the left of the axis (positive values) the largest. In the case of PC2, the highest correlations were for the variables rectangularity (0.86), circularity (-0.75), form factor (0.74), and SRS (0.58), that is, *sulcus* with negative scores are more irregular, while those with positive values are more rectangular and regular. The variables most correlated with PC3 are ellipticity (-0.70) and aspect ratio (0.70). PC3 is related to the circular/square or elongated shape of the *sulcus*. The more positive the values of this component, the less irregular and elongated the sulcus is, and the more negative the more irregular and elongated. From Figure 10b-d, it can be seen that the species have distinct morphological patterns, both with respect to size and the more elongated and irregular appearance of their *sulcus acusticus*. Significant difference was observed among species (MANOVA, HL = 7.55, df = 7, F-test = 21.59, *p-value* < 0.001), and also for all PCs individually (ANOVA, df = 7, *p-value* < 0.001). In Figure 11, these patterns of variability were graphically identifiable when we considered the data labeled by depth. There was a significant difference between deep and shallow depths (MANOVA, HL = 0.99, df = 1, F-test = 20.53, *p-value* < 0.001), and for all PCs analyzed (ANOVA, df = 1, *p-value* < 0.001). In general, the *sulcus* of individuals from deeper water were smaller and more irregular and elongated.

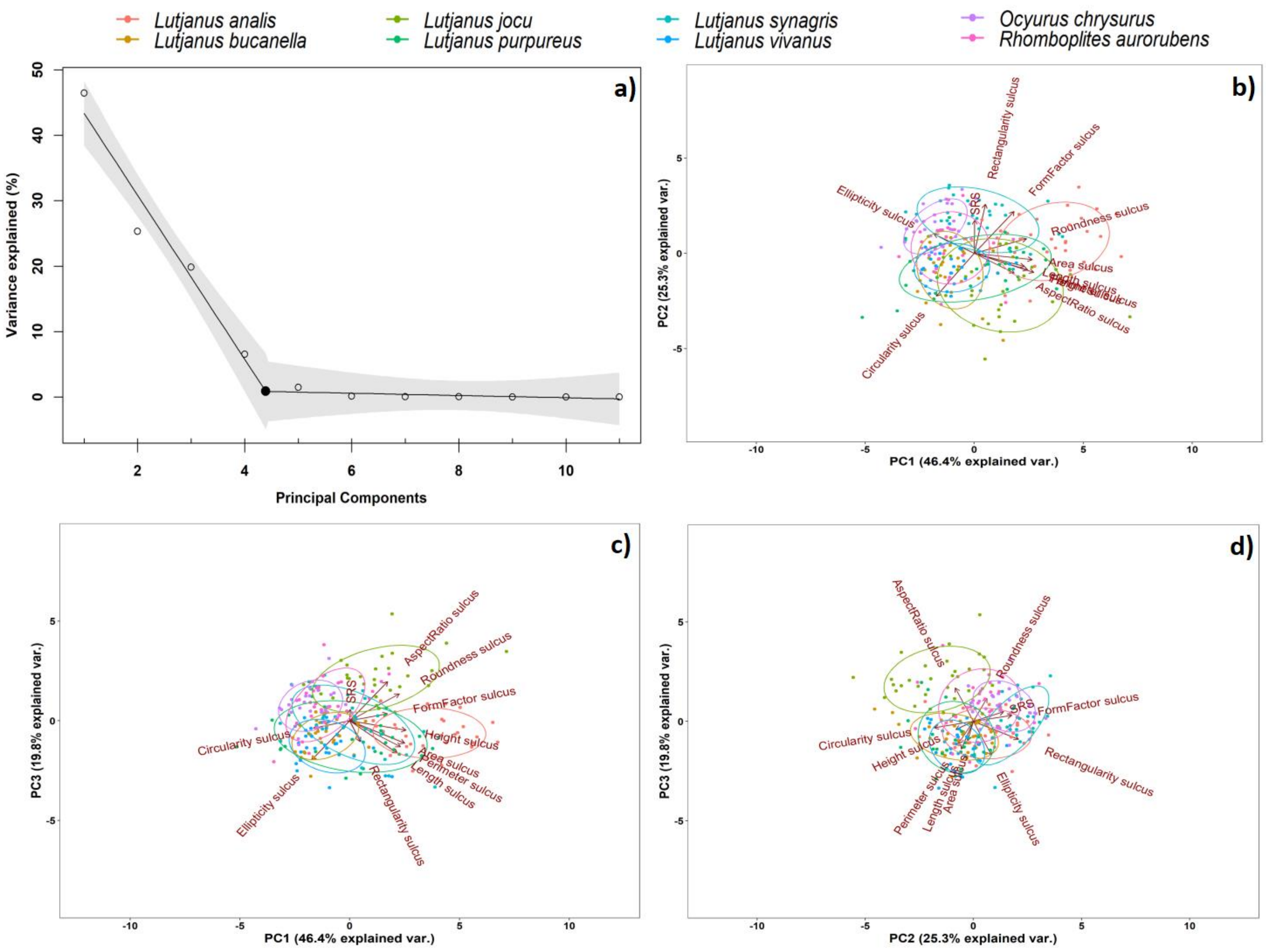

FIGURE 10 Graphical representation of the percentage of data explanation by the 10 Principal Components. Cumulative percentage of explanation of data variation indicated for the first four components (a). Scatter plots of the principal components for the species variable for *sulcus acusticus* alone and their combinations PC1xPC2

(b), PC1xPC3 (c) and PC2xPC3 (d), with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

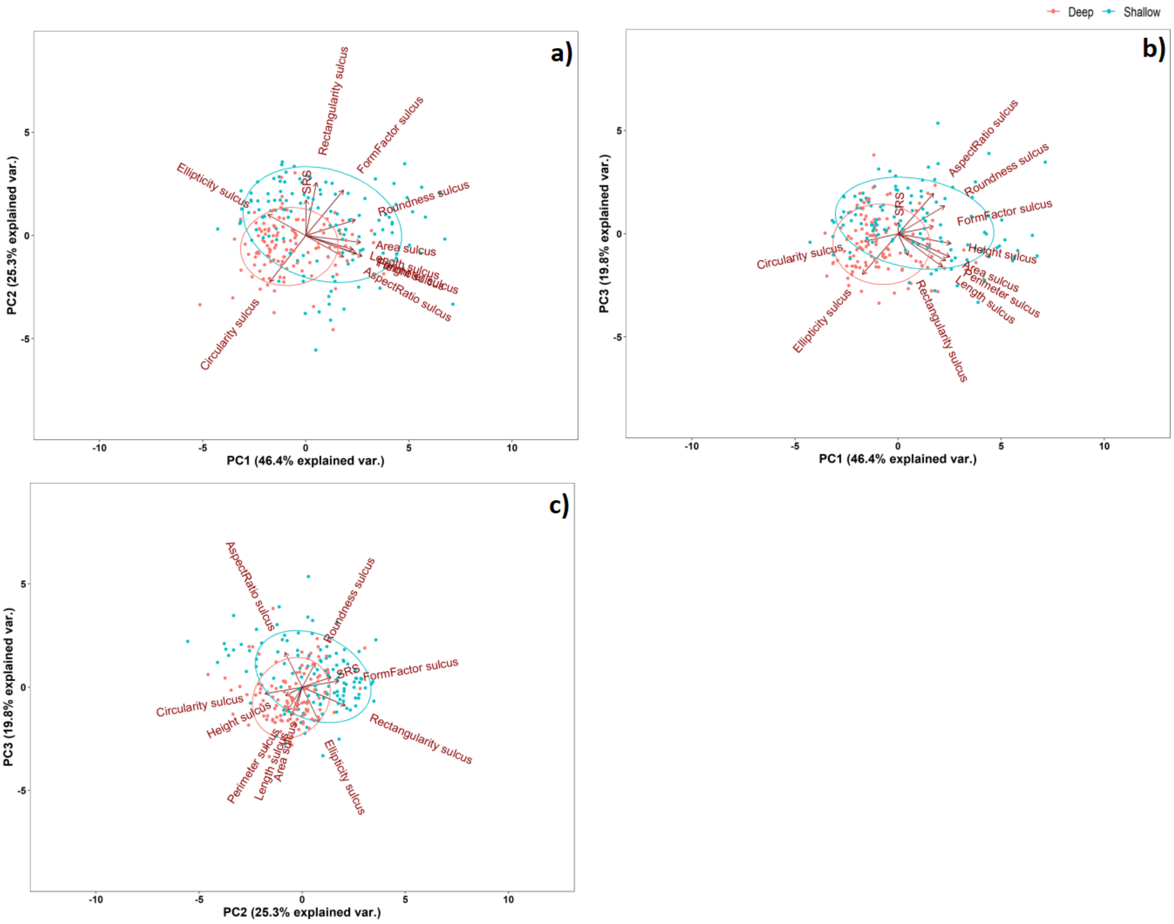

FIGURE 11 Scatter plots of the principal components for the depth variable and its combinations PC1xPC2 (a), PC1xPC3 (b) and PC2xPC3 (c) for *sulcus acusticus*, with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

The plots and statistics were applied only for the principal components that had high explained variance and were considered retained from the estimated break-point of 5.09 (Figure 12a). That is, the first five principal components (90.76%). The variables most correlated with PC1 were *sulcus* height (-0.95), *sulcus* area (-0.93), otolith height (-0.92), otolith area (-0.92), otolith perimeter (-0.91), *sulcus* perimeter (-0.90), otolith length (-0.88), and *sulcus* length (-0.86), indicating that this first component is related to otolith and sulcus size. In this case, individuals to the left of the axis (negative values) of this component have the largest structures, and to the left of the axis (positive values) the smallest. In the case of PC2, the highest correlations were for the variables otolith from factor (-0.79), otolith circularity (0.79), otolith rectangularity (-0.72), *sulcus* rectangularity (-0.61), *sulcus* form factor (-0.60), and *sulcus* circularity (-0.60). That is, otoliths and *sulcus* with negative scores are more circular and regular, while those with positive values are more irregular. The variables most correlated with PC3 are sulcus aspect ratio (-0.73), *sulcus* ellipticity (0.73), otolith aspect ratio (-0.65), and otolith ellipticity (0.65). PC3 is related to the circular/square or elongated shape of the otolith. The more positive the values of this component, the less elongated the otolith and sulcus are, and the more negative the more elongated. From Figure 12b-d, it can be seen that the species have distinct morphological patterns, both with respect to size and the more elongated appearance of the otoliths and *sulcus* of some species. There was significant difference among species (MANOVA, HL = 14.06, df = 7, F-test = 20.11, *p-value* < 0.001), and for all PCs analyzed (ANOVA, df = 7, *p-value* < 0.001). In Figure 13, these patterns of variability were not graphically identifiable when we considered the data labeled by depth. However, there was also a significant difference between deep and shallow depths (MANOVA, HL = 1.53, df = 1, F-test = 15.86, *p-value* < 0.001), and for all PCs analyzed (ANOVA, df = 1, *p-value* < 0.001), except for PC2, PC3 and PC5 (ANOVA, df = 1, *p-value* > 0.05). On average, otoliths from individuals from deeper water had the smallest otoliths and the most elongated otoliths.

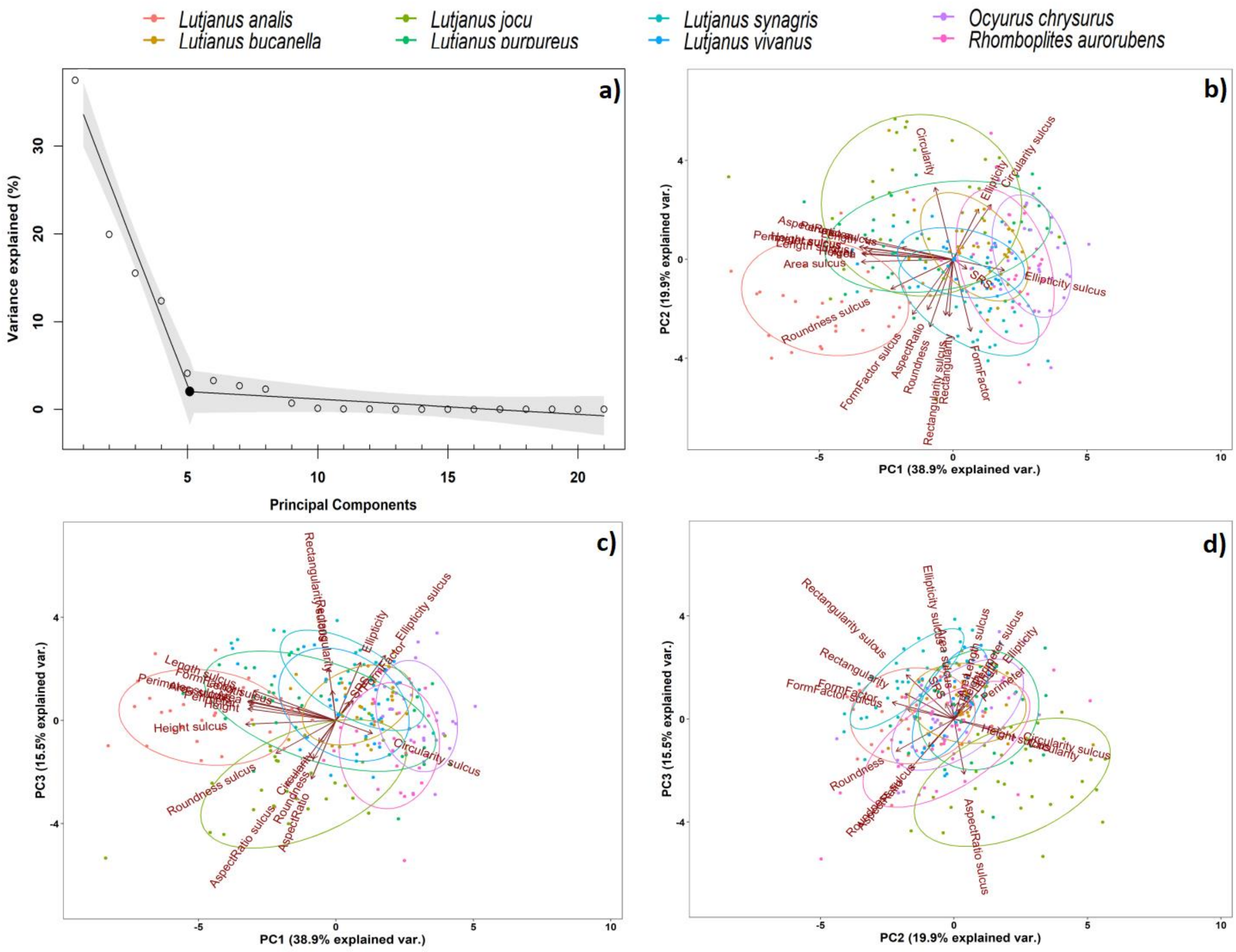

FIGURE 12 Graphical representation of the percentage of data explanation by the 10 Principal Components. Cumulative percentage of explanation of data variation indicated for the first four components (a). Scatter plots of the principal components for the species variable for otoliths and *sulcus acusticus* combined and their combinations PC1xPC2 (b), PC1xPC3 (c) and PC2xPC3 (d), with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

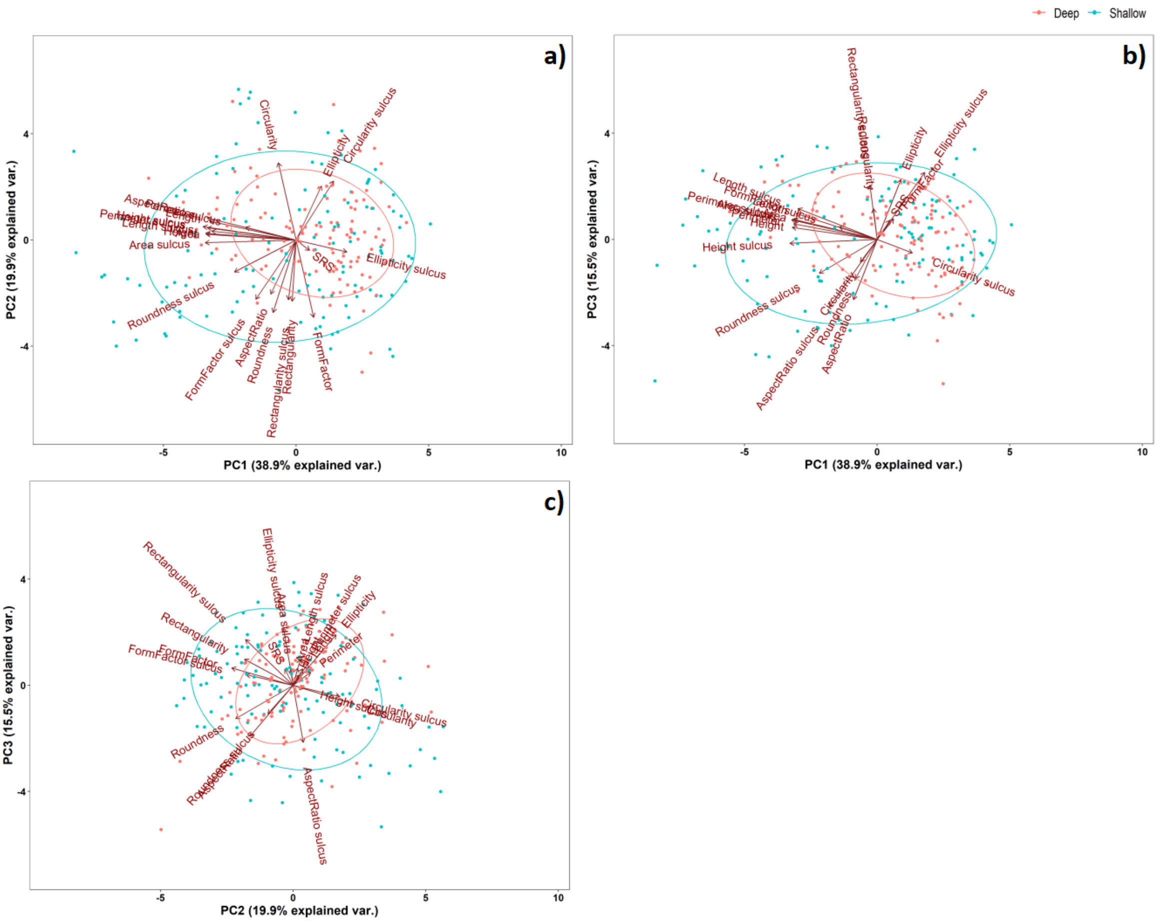

FIGURE 13 Principal component scatter plots for the depth variable and its combinations PC1xPC2 (a), PC1xPC3 (b) and PC2xPC3 (c) for otoliths and *sulcus acusticus* combined, with the 95% confidence ellipses, where the direction of the arrows indicate the direction of influence and the size of the arrow line indicates the magnitude of influence.

3.4. Linear discriminant analysis

In the LDA for otoliths, groupings were made by species and by depth (Figure 14). For the latter, there was a separation between deep and shallow with a classification percentage above 60%, and the deep class presented the highest percentage of success (81%) (Figure 14b). Among species, *O. chrysurus*, *L. purpureus* and *L. synagris* stood out (80%, 75% and 72% of success, respectively) (Figure 14a).

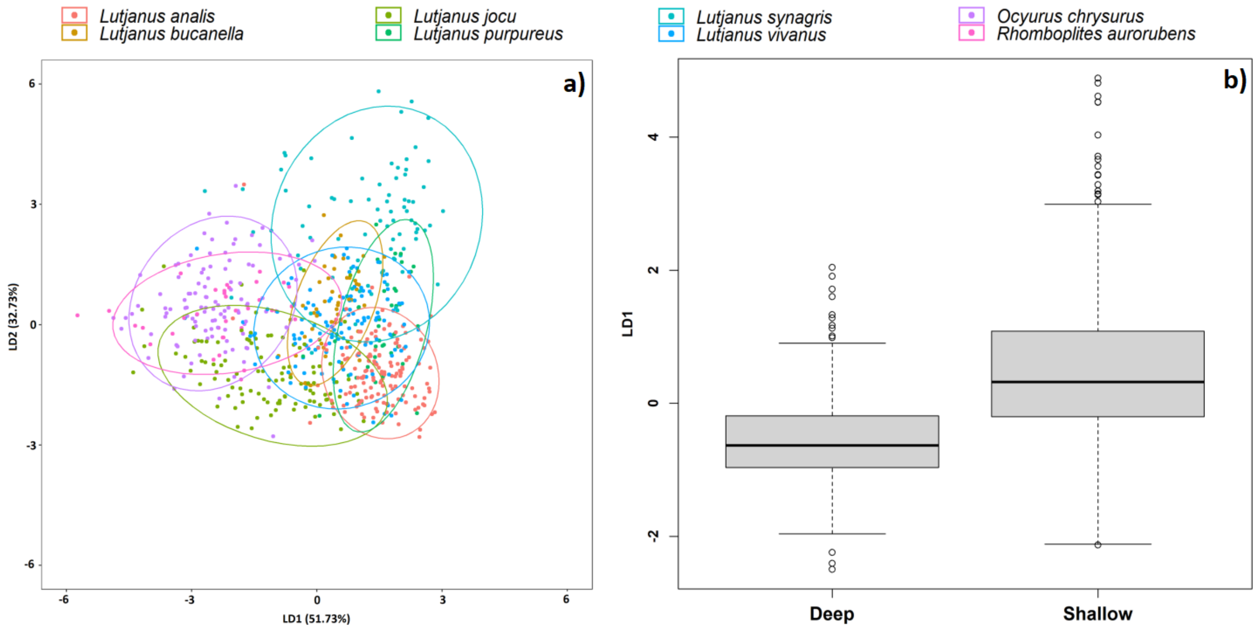

FIGURE 14 Scatter plot of LD1 with LD2 for the species variable with otoliths only, with confidence ellipses at 95%, where the direction of the arrows indicates the direction of influence and the size of the arrow line indicates the magnitude of influence (a). Boxplot of the linear discriminant 1 for the depth variable (b).

As in the LDA for otoliths, the linear discriminant analysis of *sulcus acusticus* classification by species and by depth were also performed (Figure 15). For the latter, there was a separation between deep and shallow with a percentage of correct classification above 85% (Figure 15b). As for the LDA between species, *O. chrysurus* and *L. jocu* stood out, both with 100% classification (Figure 15a).

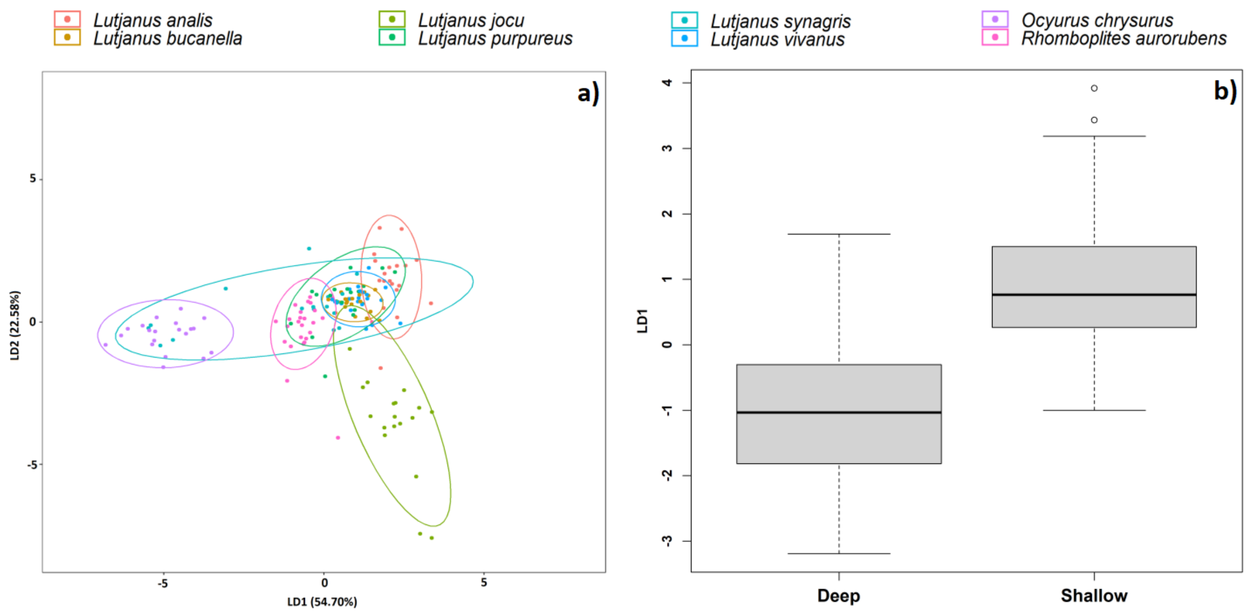

FIGURE 15 Scatter plot of LD1 with LD2 for the species variable with *sulcus acusticus* only, with the 95% confidence ellipses, where the direction of the arrows indicates the direction of influence and the size of the arrow line indicates the magnitude of influence (a). Boxplot of the linear discriminant 1 for the depth variable (b).

As with the other discriminant analyses performed, classification analysis was also performed both by species and depth for the otoliths and *sulcus acusticus* combined (Figure 16). For the latter, there was a separation between deep and shallow with a success percentage above 90% (Figure 16b). As for the LDA among species, *O. chrysurus* and *L. vivanus* stood out (100% and 89% success rate, respectively) (Figure 16a).

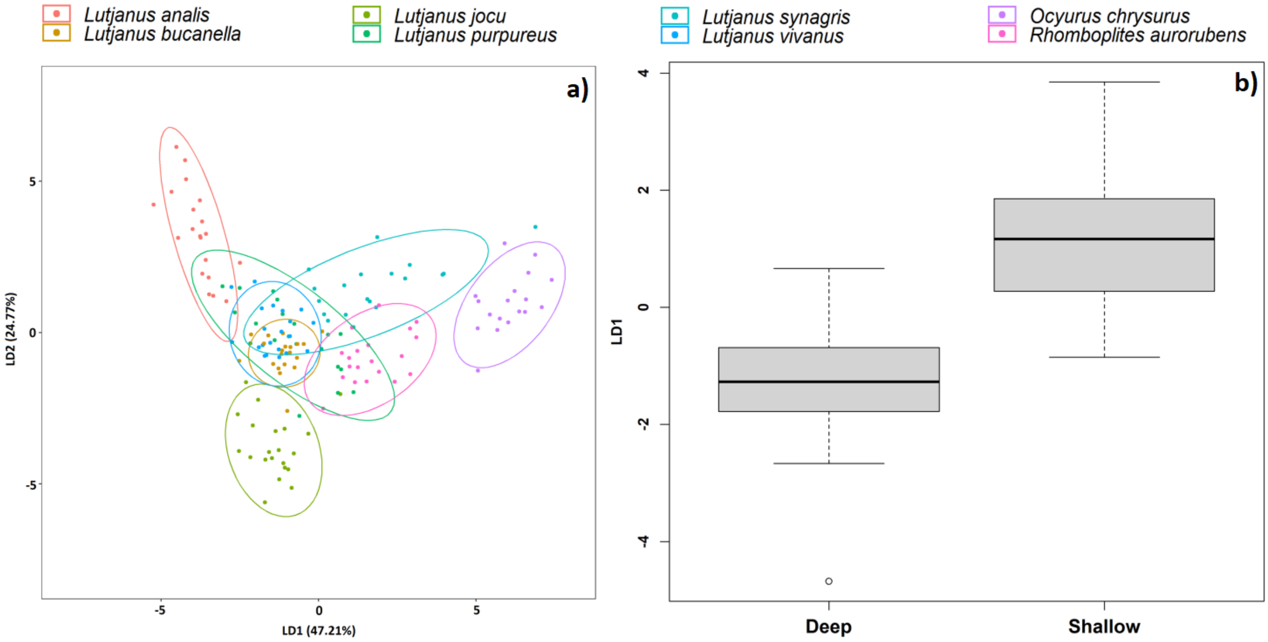

FIGURE 16 Scatter plot of LD1 with LD2 for the species variable with the otoliths and *sulcus acusticus* combined, with the confidence ellipses at 95%, where the direction of the arrows indicates the direction of influence and the size of the arrow line indicates the magnitude of influence (a). Boxplot of the linear discriminant 1 for the depth variable (b).

Comparing the discriminant analyses performed, it can be seen that the LDA for the combined otoliths and *sulcus acusticus* showed the highest efficiency to discriminate the species, with an average of 73.7% classification (Table 7). Also, for discrimination between depths with a percentage above 90% classification.

TABLE 7 Percentage hit grouping of species by linear discriminant analysis (LDA) for otolith only, *sulcus* only, and, otolith and *sulcus* combined.

	L. analis	***L. bucanella***	***L. jocu***	***L. purpureus***	***L. synagris***	***L. vivanus***	***O. chrysurus***	***R. aurorubens***	**Média**
Otolith	59%	14%	58%	75%	72%	67%	80%	24%	56.1%
Sulcus	86%	50%	100%	12%	50%	42%	100%	75%	64.4%
Combined	60%	78%	78%	55%	60%	89%	100%	70%	73.7%

3.5. Hierarchical Clustering Analysis

Principal component analyses provided a large amount of variation in individual form. However, three morphotypes were identified by cluster analysis. It was possible through clustering and decision tree analysis to identify possible otolith patterns and shapes for adult species. Of the 1037 otoliths analyzed, 57% had an area greater than or equal to 92 mm2, within this margin, 31% had a circularity smaller than 20, having as representative species mainly Ocyurus chrysurus (23.5%) and Lutjanus jocu (29.5%). Lutjanus synagris showed a wide range of otolith area as well as circularity. While 30% of the analyzed Lutjanus showed an otolith area greater than 92 mm2 with ellipticity less than 0.25 (Figure 17).

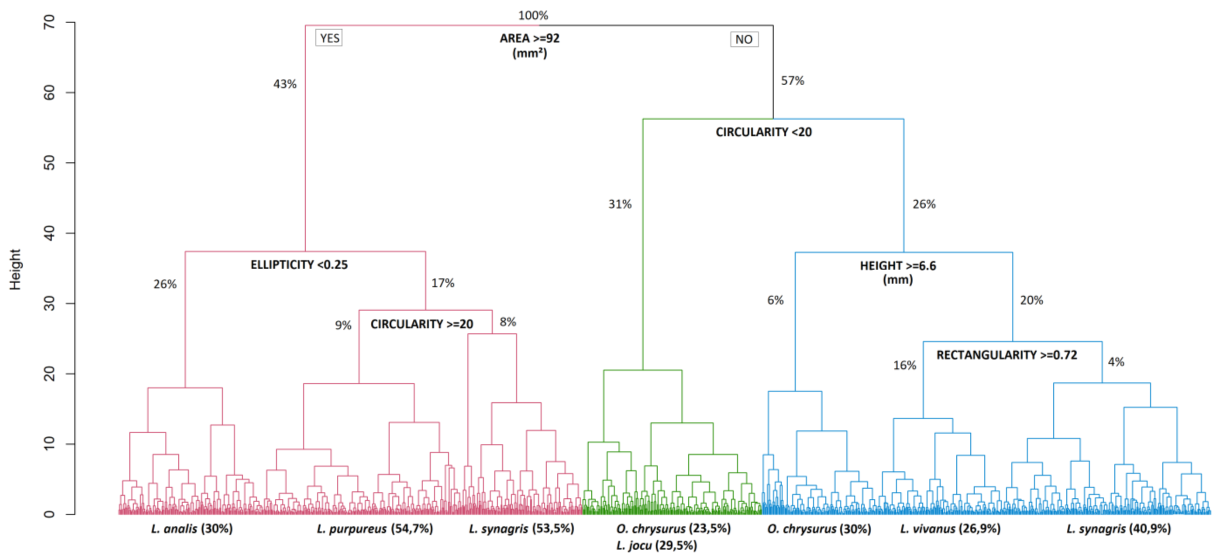

FIGURE 17 Dendrogram of cluster analysis on Euclidean distance and decision tree with PCA of all morphometric measurements and shape indices of otoliths from adults only. Different colors refer to morphotypes.

4. Discussion

Otolith size varies due to ontogenetic factors, reflecting the relationship between otolith and fish growth (Campana & Neilson, 1985). This variability of sagittal otoliths is not only related to intrinsic factors, such as sex and ontogeny (Lombarte *et al*., 2003), but is also linked to environmental factors such as depth, temperature and salinity (Cardinale *et al*., 2004; Lombarte & Lleonart, 1993). Therefore, relating otolith shape to environmental factors is fundamental to identify the causes of morphological variability (Gauldie, 1988; Platt & Popper, 1981; Volpedo & Echeverría, 2003). Lombarte & Cruz (2007), comparing otoliths of demersal species, found a trend for otolith size, where with increasing depth, the greater the average relative size of the sagitta. Therefore, species considered shallow-water in this study had relatively smaller otoliths than deep-water species, such as *Lutjanus analis*.

A clear trend was observed in otolith shape for the shape indices. In general, shallow-water species tend to have more circular and eccentric otoliths, while those of deep-water species tend to be more rectangular and elliptical (Figure 5). For the other indices, the regressions did not show a clear trend.

A greater diversity in otolith shape was observed for the shallow-water species in comparison with the deep-water species. Inhabiting the outer shelf slope of the continental shelf, those species have to adapt to abrupt variations in depth, since these areas have high declivity. *O. chrysurus*, despite being a shallow water species, is an exception, probably because this species has the habit of migrating in the water column, which brings it closer to the bottom species, which make similar movements. This habit can be confirmed by Fonseca (2009), who indicates that this species feeds more in the water column than on the substrate. In addition, shallow-water occupy a greater diversity of habitats during their life and ontogeny. That is, while bottom-dwelling species concentrate in small homogeneous areas, shallow-dwelling species live in large heterogeneous areas.

When analyzing each species separately, results indicated that the otoliths of *L. jocu* became more eccentric and less elliptical. The otoliths of *L. purpureus* and *L. synagris* became less eccentric, more rectangular, and more elliptical. *L. analis* tended to become more eccentric and more circular/rounder. The other species (*L. vivanus*, *L. bucanella*, *O. chrysurus* and *R. aurorubens*) became more circular and more elliptical. According to Avigliano *et al*. (2013), elliptical otoliths are correlated with species that inhabit more saline environments. However, mid and low latitude regions have a higher salinity at shallower depths, mainly due to evaporation, while at greater depths salinity decreases considerably (Moura, 1992; Silva, A.C. *et al*., 2001). In addition, many shallow-water species occur in estuaries in the juvenile phase, where salinity is lower (Rozas & Zimmerman, 2000). Considering this information, and that the otoliths of the species considered as bottom-dwelling presented a more elliptical shape, the results of the present study differ from the one reported in the aforementioned work.

According to Volpedo & Echeverría (2003), values in the shape aspect (OH/OL%) between 30-50 point to species associated with unconsolidated substrates, as in some species of sciaenidae. In the present work, all species in this study had values greater than 0.50, that is, 50%. Adult specimens of the Lutjanidae family inhabit sandy, rocky or coral reef areas in the marine environment, while juveniles inhabit mangrove estuaries (Allen, 1985), that is, consolidated environments, corroborating the work cited above.

In the linear discriminant analysis (LDA) by species, the best result obtained was for otoliths and sulcus acusticus combined. The species that showed the highest classification percentages were *O. chrysurus* (100%), followed by *L. vivanus* (89%). There was a clear separation between *L. analis*, *L. jocu*, *R. aurorubens* and *O. chrysurus* (Figure 16a). On the other hand, the LDA by depth showed a great distinction between bottom and shallow species, both for otoliths (81% and 60%, respectively), *sulcus acusticus* (85% and 85%, respectively) and both combined (92% and 95%, respectively). From these results, it can be stated that the best methodology for depth discrimination was the combined otolith and *sulcus acusticus*, which separated the groups with the highest percentage of correctness. This approach also showed better separation between species (73.7% correct), while the *sulcus acusticus* measurements separated 64.4% and the otolith measurements obtained only 56.1% correct.

Cruz & Lombarte (2004), in an ecomorphological study, identified a clear relationship between otolith sizes and communication strategies for four species of Perciformes, where species considered specialists in sound production, such as Sciaenidae and Haemulidae had relatively large otoliths, while those with small otoliths used other communication strategies, such as visual, chemical and electrical as a way to compensate for the lack of light (Buran *et al*., 2005; Lombarte & Aguirre, 1997). In the above mentioned study, specimens of Sciaenidae and Haemulidae with a total length of 20 cm had an average otolith area between 30 and 51 mm^2. The Lutjanids analyzed in this study in the same length range averaged between 17 and 39 mm^2. Thus, as there is a small intersection between these measurements and with the same reference, it is suggested that members of the family Lutjanidae are not known as sound producing specialists.

The Sulcus Relative Surface (SRS) index describes the proportion of the *sulcus acusticus* area in relation to the otolith area. That is, the closer to 1, the greater the area occupied by the *sulcus acusticus*, and from this relationship it is possible to infer the hearing ability of the fish (Volpedo *et al*., 2008). The species *O. chrysurus* possessed a higher median SRS (0.57), while *L. bucanella* and *L. vivanus* exhibited lower medians (0.32) (Figure 6). In addition, individuals with more developed otoliths have better hearing ability and balance, characteristic of neritic and coastal species, such as species of the genus Lutjanus (Volpedo, Vaz-dos-Santos, 2015). Among the species belonging to the genus Lutjanus, *L. synagris* had a higher SRS value, which may indicate that it has a greater hearing capacity. Both species with higher SRS values live at shallow depths. In other words, for the Lutjanidae, shallow species need more accurate hearing. It is expected that phylogenetically close species, such as *O. chrysurus* and *R. aurorubens*, would have similar otolith characteristics. In the present study, these species were not similar with respect to *sulcus acusticus*, but were similar with respect to otolith shape. Which suggests that this variation with respect to *sulcus* is related to the niche/habitat in which each species is associated. Therefore, the variation in otolith shape is related to phylogenetic aspects, while the variation in *sulcus* is related to depth.

For the studied species, only the species *R. aurorubens*, *O. chrysurus*, *L. jocu* and the *L. bucanella* showed sexual dimorphism in otolith. Male of the species *R. aurorubens* have a higher weight than females. Females of the species *O. chrysurus* have a higher total weight and have a larger otolith area and height than males. For *L. jocu*, males have more rectangular otoliths than females. In addition, males of *L. bucanella* have a more elliptical and less eccentric otolith than females.

Methodologies for morphometric characterization of the sagittal otolith have proven efficient in discriminating fish species (Bani *et al*., 2013; Tuset *et al*., 2003). However, phylogenetic inferences based on morphometry, biochemistry and molecular analysis show that these genera diverged from distinct lineages (Chow, Walsh, 1992; Gold *et al*., 2011; 2015). In addition, most of the distinct morphological features may represent differential adaptation to feeding, thus representing homoplasies (Nirchio *et al*., 2009).

Through the hierarchical grouping analysis and decision tree it was possible to elaborate a dichotomous key for some species regarding the main indexes and measurements, which can be used to identify taxa from these morphometries and indexes. The otoliths of L. analis, L. purpureus and L. synagris had the highest values for area, the first being less elliptical. L. synagris had less rectangular and more circular otoliths (40.9%). O. chrysurus, L. jocu and L. vivanus showed otoliths with smaller areas, the first two being less circular (31%). No other work on Lutjanidae has been found using this methodology linked to shape analysis. However, the results of this grouping analysis were consistent with the regressions of the morphometric indices developed in that same study.

In the Persian Gulf indicated that otoliths were very similar in morphological terms (shape, type of *sulcus acusticus*, *ostium* and *cauda*), but showed differences in the anterior and posterior edges of the otolith (Sadighzadeh *et al*., 2012). In this same study, several different methods were tested in the cross-validation procedures of the canonical discrimination function (DFA) analysis to determine the otolith shape of the species. However, only the combination of all methods provided strong results (Sadighzadeh *et al*., 2012).

The species *O. chrysurus* and *R. aurorubens* showed similarities to each other, which corroborates the work of Gold *et al*. (2011), where in their studies based on mitochondrial DNA, they concluded that the genera *Ocyurus* and *Rhomboplites* are considered monotypic, and recommended that they should be included in the genus *Lutjanus*. In the presente study, based on morphometric characterization of otoliths, the species *O. chrysurus* and *R. aurorubens* showed no similarities to the genus *Lutjanus*.

5. Conclusion

In the presente study, a significant difference was observed between species and depths. The combination of otolith and *sulcus acusticus* separated the groups better, both by depth and species. Specimens from shallow depths had larger Sulcus Relative Surface (SRS) medians, with *O. chrysurus* being the main representative. The species *O. chrysurus* and *R. aurorubens* showed similarities among themselves, corroborating with phylogenetic studies that indicate that those are closely related species. Thus, it is plausible to conclude that through the morphometric and morphological characteristics of otoliths obtained for this family it is possible to characterize both ecological and philogenetic patterns, becoming efficient tools for intraspecific studies, expanding the knowledge of species of great ecological and economic importance, such as the Lutjanidae.

6. Contributions

MGB, JEVF, BPF designed the ideas of the researsh. BPF collected data. MGB, JEVF performed the analyses. MGB wrote the most of the text. JEVF, PJDN, JBQS contributed reviewing the text.

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9. Attachment

TABLE 5 Values of *a*, *b*, *p-value*, r^2 and *t* (Student's *t-test* statistics) for the non-linear regressions between otolith weight (OW, g) and otolith length (OL, mm); and, fish weight (BW, g) and fish fork length (FL, cm) for the studied Lutjanidae species. Spaces with "ns" indicate that it was not significant. Blank spaces for grouped species indicate that there was a difference between male and female.

			L. analis	***L. bucanella***	***L. jocu***	***L. purpureus***	***L. synagris***	***L. vivanus***	***O. chrysurus***	***R. aurorubens***
OW (g)	Female	*a*								0,0005098 (-0,000014 – 0,0010)
		b								2.32 (1.91 – 2.73)
		p-value								<0,00
		r^2								0.856
		t								11.03
	Male	*a*								0,00014 (-0,000056 – 0,00033)
		b								2.95 (2.38 – 3.52)
		p-value								<0.00
		r^2								0.84
		t								10.01
	Polled	*a*	0,00023 (0,00012 – 0,00034)	0,000047 (0,000010 – 0,000085)	0,000052 (0,000016 – 0,000088)	0,00014 (0,0000014 – 0,00027)	0,0022 (0,00090 – 0,0034)	0,00021 (0,00012 – 0,00030)	0,00027 (0,000063 – 0,00049)	

BW (g)		*b*	2.78	3.29	3.2	2.94	1.96	2.71	2.57	
			(2.62 – 2.95)	(2.99 – 3.59)	(2.95 – 3.46)	(2.58 – 3.30)	(1.75 – 2.16)	(2.55 – 2.86)	(2.25 – 2.88)	
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	
		r^2	0.84	0.87	0.84	0.93	0.77	0.87	0.7	
		t	32.81	22.15	24.84	16.61	18.6	34.41	16.04	
	Female	*a*							0.034	
									(0.01 – 0.06)	
		b							2.79	
									(2.6 – 2.98)	
		p-value							<0.00	
		r^2							0.687	
		t							27.94	
	Male	*a*							0.05	
									(0 – 0.1)	
		b							2.64	
									(2.39 – 2.89)	
		p-value							<0.00	
		r^2							0.7	
		t							21.03	
	Polled	*a*	0,0077	2.6 e-03	0,010	ns	0.05	0.29		0.12
			(0,00028 – 0,015)	(-0.02 – 0.03)	(0,0063 – 0,014)		(0.03 – 0.06)	(-0.04 – 0.62)		(-0.07 – 0.32)
		b	3.18	3.54	3.11	ns	2.67	2.2		2.38
			(2.94 – 3.42)	(0.55 – 6.53)	(3.01 – 3.22)		(2.58 – 2.76)	(1.90 – 2.51)		(1.94 – 2.82)
		p-value	<0.00	0.0256	<0.00	ns	<0.00	<0.00		<0.00
		r^2	0.95	0.58	0.99	ns	0.98	0.73		0.86
		t	26.28	2.73	63.5	ns	60.97	14.39		11.15

TABLE 6 Values of *a*, *b*, *p-value*, r^2 and *t* (Student's *t-test* statistics) for the linear and non-linear regressions (area) between measurements and indices with fish fork length for the studied Lutjanidae species. Spaces with "ns" indicate that it was not significant. Blank spaces for grouped species indicate that there was a difference between male and female.

			Area (mm²)	**Perimeter (mm)**	**Height (mm)**	**Length (mm)**	**Circularity**	**Rectangularity**	**FormFactor**	**Ellipticity**	**AspectRatio**	**Roundness**
L. analis	polled	*a*	0.61 (0.41 – 0.81)	9.54 (7.13 – 11.95)	2.75 (2.32 – 3.18)	4.32 (3.63 – 5.01)	16.95 (16.13 – 17.77)	0.71 (0.7 – 0.72)	0.71 (0.69 – 0.73)	ns	ns	ns
		b	1,37 (1.29 – 1.45)	0.84 (0.79 – 0.89)	0.16 (0.15 – 0.17)	0.26 (0.25 – 0.27)	0,068 (-0.050 – 0.086)	-0,00025 (-0,00043– -0,000075)	-0,0019 (-0,0025 – -0,0015)	ns	ns	ns
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	ns	ns	ns
		r^2	0.84	0.81	0.83	0.83	0.2	0.03	0.19	ns	ns	ns
		t	32.48	32.35	34.49	34.7	7,.1	-2.78	-7.52	ns	ns	ns
L. bucanella	Female	*a*								ns	ns	
		b								ns	ns	
		p-value								ns	ns	
		r^2								ns	ns	
		t								ns	ns	
	Male	*a*								0.2 (0.15 – 0.25)	0.66 (0.03 – 1.29)	
		b								0,0016 (0,00016- 0,0030)	-0,0020 (-0,0038 – -0,00021)	
		p-value								0.0402	0.0401	
		r^2								0.1776	0.1779	
		t								2.18	-2.18	
	polle	*a*	0.56 (0.20 – 0.93)	6.91 (1.58 – 12.24)	2.93 (2.09 – 3.77)	4.23 (2.92 – 5.54)	13.06 (11.55 – 14.57)	ns	0.84 (0.79 – 0.89)			0.57 (0.53 – 0.61)

		b	1.36	0.86	0.14	0.25	0.18	ns	-0,0057			-0,0011
			(1.18 – 1.53)	(0.74 – 0.98)	(0.12 – 0.16)	(0.21 – 0.29)	(0.14 – 0.22)		(-0,0070 –			(-0,0022 –
									-0,0044)			-0,00010)
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	ns	<0.00			0.0345
		r^2	0.76	0.74	0.67	0.74	0.53	ns	0.53			0.08
		t	15.36	13.83	11.6	13.57	8.52	ns	-8.537			-2.158
		a						0.69				
								(0.68 – 0.7)				
		b						-0.00028				
	Female							(0 - 0)				
		p-value						<0.00				
		r^2						0.062				
		t						-3.34				
		a						ns				
		b						ns				
	Male	*p-value*						ns				
L. jocu		r^2						ns				
		t						ns				
		a	0.93	15.82	3.14	6	20.41	ns	0.61	0.27	0.57	0.5
			(0.71 – 1.15)	(14.13 – 17.51)	(2.9 – 3.38)	(5.53 – 6.47)	(19.41 – 21.41)		(0.58 – 0.64)	(0.26 – 0.28)	(0.55 – 0.59)	(0.47 – 0.53)
		b	1.11	0.47	0.097	0.14	0.027	ns	-0,8	-0,00054	0,00070	0,00037
			(1.05 – 1.17)	(0.44 – 0.5)	(0.09 – 0.1)	(0.13 – 0.15)	(0.01 – 0.05)		(-0,0012 –	(-0,00081 –	(0,00034 –	(0,0000158 -
	polled								-0,00020)	-0,00027)	0,0011)	0,00073)
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	ns	<0.00	<0.00	<0.00	0.0429
		r^2	0.91	0.84	0.91	0.86	0.05	ns	0.05	0.09	0.09	0.03
		t	37.77	29.56	41.27	31.71	2.87	ns	-2.76	-3.9	3.84	2.04
		a	0.38	8.32	1.92	2.7	ns	0.69	ns	0.22	0.63	ns
L.	polle		(0.16 – 0.60)	(5.42 – 11.22)	(1.43 – 2.41)	(1.78 – 3.62)		(0.67 – 0.71)		(0.2 – 0.24)	(0.61 – 0.65)	

		b	1.45 (1.30 – 1.59)	0.8 (0.74 – 0.86)	0.15 (0.14 – 0.16)	0.27 (0.25 – 0.29)	ns	0,00045 (0,00014 – 0,00072)	ns	0,00046 (0,00012 – 0,00081)	-0,00060 (-0,0010 – -0,00016)	ns
		p-value	<0.00	<0.00	<0.00	<0.00	ns	<0.00	ns	<0.00	<0.00	ns
		r²	0.91	0.91	0.93	0.91	ns	0.11	ns	0.1	0.1	ns
		t	20.2	26.01	29.97	27.46	ns	2.83	ns	2.65	-2.66	ns
L. synagris	polled	*a*	0.25 (0.12 – 0.38)	3.58 (0.72 – 6.44)	1.57 (0.98 – 2.16)	1.66 (0.64 – 2.68)	16.46 (15.95 – 16.97)	0.7 (0.69 – 0.71)	0.75 (0.73 – 0.77)	0.23 (0.22 – 0.24)	0.62 (0.61 – 0.63)	0.56 (0.55 – 0.57)
		b	1.67 (1.53 – 1.82)	1.11 (1.02 – 1.2)	0.2 (0.18 – 0.22)	0.39 (0.08 – 0.7)	0.068 (0.05 – 0.08)	0,00051 (0,00025 - 0,00077)	-0,0025 (-0,0030 – -0,0019)	0,0012 (0,00088 - 0,0016)	-0,0015 (-0,0019 – -0,0011)	-0,0010 (-0,0016 – -0,00058)
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00
		r²	0.82	0.81	0.77	0.8	0.33	0.1	0.34	0.26	0.26	0.13
		t	22.8	25.16	22.17	24.37	8.41	3.85	-8.79	7.1	-7.09	-4.59
L. vivanus	polled	*a*	1.07 (0.74 – 1.40)	12.76 (10.94 – 14.58)	3.4 (3.01 – 3.79)	4.96 (4.35 – 5.57)	17.33 (16.72 – 17.94)	ns	0.72 (0.7 – 0.74)	0.21 (0.2 – 0.22)	0.64 (0.62 – 0.66)	ns
		b	1.23 (1.14 – 1.31)	0.79 (0.74 – 0.84)	0.15 (0.14 – 0.16)	0.26 (0.24 – 0.28)	0.061 (0.04 – 0.08)	ns	-0,0021 (-0,0026 - -0,0015)	0,0062 (0,0058 - 0,0066)	-0,00078 (-0,0013 – -0,00024)	ns
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	ns	<0.00	<0.00	<0.00	ns
		r²	0.81	0.82	0.78	0.81	0.2	ns	0.21	0.05	0.04	ns
		t	28.88	29.59	25.84	28.94	6.93	ns	-7.07	2.96	-2.837	ns
O. chrysurus	Female	*a*	0.86 (0.47 – 1.25)		2.81 (2.4 – 3.22)			ns				0.55 (0.53 – 0.57)
		b	1.14 (1.02 – 1.26)		0.1 (0.09 – 0.11)			ns				-0,00074 (-0,0014 – -0,00011)
		p-value	<0.00		<0.00			ns				0.0235
		r²	0.83		0.81			ns				0.03

		t	17.37		17.65			ns				-2.187
	Male	*a*	0.98		2.95			ns				0.58
			(0.59 – 1.37)		(2.6 – 3.3)							(0.55 – 0.61)
		b	1.09		0.097			ns				-0,0018
			(0.99 – 1.19)		(0.09 – 0.11)							(-0,0028 – -0,00085)
		p-value	<0.00		<0.00			ns				<0.00
		r²	0.8		0.83			ns				0.16
		t	19.22		18.36			ns				-3.69
	polled	*a*		9.9		4.39	16.27		0.73	0.23	0.62	
				(8.29 – 11.51)		(3.9 – 4.88)	(15.25 – 17.29)		(0.7 – 0.76)	(0.21 – 0.25)	(0.6 – 0.64)	
		b		0.65		0.18	0.15		-0,0041	0,0061	-0,0076	
				(0.6 – 0.7)		(0.17 – 0.19)	(0.12 – 0.18)		(-0,0049 – -0,0034)	(0,0056 – 0,0066)	(-0,0083 – -0,0070)	
		p-value		<0.00		<0.00	<0.00		<0.00	0.0152	0.0167	
		r²		0.82		0.81	0.39		0.39	0.04	0.04	
		t		27.77		26.36	10.27		-10.32	2.45	-2.41	
R. aurorubens	polled	*a*	1.74	9.9	3.76	4.53	17.01	ns	0.72	0.16	0.7	0.63
			(0.71 – 2.78)	(8.29 – 11.51)	(3.21 – 4.31)	(3.59 – 5.47)	(15.58 – 18.44)		(0.68 – 0.76)	(0.13 – 0.19)	(0.66 – 0.74)	(0.59 – 0.67)
		b	0.96	0.65	0.08	0.19	0.11	ns	-0,0034	0,0024	-0,0031	-0,0030
			(0.79 – 1.12)	(0.6 – 0.7)	(0.06 – 0.1)	(0.16 – 0.22)	(0.07 – 0.15)		(-0,0048 – -0,0020)	(0,0015-0,0033)	(-0,0043 – -0,0019)	(-0,0044– -0,0017)
		p-value	<0.00	<0.00	<0.00	<0.00	<0.00	ns	<0.00	<0.00	<0.00	<0.00
		r²	0.73	0.82	0.64	0.76	0.33	ns	0.31	0.35	0.34	0.27
		t	11.52	27.77	9.71	12.96	5.03	ns	-4.86	5.28	-5.18	-4.39

3 CONCLUSÃO

No presente estudo, foi observada uma diferença significativa entre espécies e profundidades. A combinação do otólito e *sulcus acusticus* separou melhor os grupos, tanto por profundidade como por espécie. Os espécimes de profundidades rasas tiveram medianas de Sulcus Relative Surface (SRS) maiores, sendo *O. chrysurus* o principal representante. As espécies *O. chrysurus* e *R. aurorubens* mostraram semelhanças entre si, corroborando com estudos filogenéticos que indicaram que essas espécies estão estreitamente relacionadas. Assim, é plausível concluir que através das características morfométricas e morfológicas dos otólitos obtidos para esta família é possível caracterizar tanto padrões ecológicos como filogenéticos, tornando-se instrumentos eficientes para estudos intra-específicos, alargando o conhecimento de espécies de grande importância ecológica e econômica, tais como os Lutjanidae.

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