UNIVERSIDADE ESTADUAL DE CAMPINAS
Instituto de Biologia

HELENA SERRANO

# BIODIVERSIDADE DE *OPHIOCOMA* (ECHINODERMATA: OPHIUROIDEA) DO ATLÂNTICO SUL: ESTUDOS MORFOLÓGICOS E MOLECULARES

# BIODIVERSITY OF *OPHIOCOMA* (ECHINODERMATA: OPHIUROIDEA) ALONG SOUTH ATLANTIC: MORPHOLOGICAL AND MOLECULAR STUDIES

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Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em BIOLOGIA ANIMAL, na Área de BIODIVERSIDADE ANIMAL.

Dissertation presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Master, in the area of ANIMAL BIOLOGY.

Supervisor/Orientador: Dra. Michela Borges

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA HELENA SERRANO, E ORIENTADA PELA PROFA. DRA. MICHELA BORGES

CAMPINAS
2021

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Gustavo Lebre de Marco - CRB 8/7977

Se68b

Serrano, Helena, 1992-
Biodiversidade de *Ophiocoma* (Echinodermata: Ophiuroidea) do Atlântico Sul : estudos morfológicos e moleculares / Helena Serrano. – Campinas, SP : [s.n.], 2021.

Orientador: Michela Borges.
Dissertação (mestrado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Bentos. 2. Delimitação de espécies. I. Borges, Michela, 1976-. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Informações para Biblioteca Digital

**Título em outro idioma:** Biodiversity of *Ophiocoma* (Echinodermata: Ophiuroidea) along South Atlantic : morphological and molecular studies
**Palavras-chave em inglês:**
Benthos
Species delimitation
**Área de concentração:** Biodiversidade Animal
**Titulação:** Mestra em Biologia Animal
**Banca examinadora:**
Michela Borges
Fosca Pedini Pereira Leite
Jéssica Prata de Oliveira
**Data de defesa:** 29-11-2021
**Programa de Pós-Graduação:** Biologia Animal

**Identificação e informações acadêmicas do(a) aluno(a)**
- ORCID do autor: https://orcid.org/0000-0003-0656-6526
- Currículo Lattes do autor: http://lattes.cnpq.br/7393475374869861

Campinas, 29 de Novembro de 2021

COMISSÃO EXAMINADORA

Profa. Dra. Michela Borges (Orientadora)

Profa. Dra. Fosca Pedini Pereira Leite

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*Os membros da Comissão Examinadores acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.*

A Ata de defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós Graduação em Biologia Animal do Instituto de Biologia da Universidade Estadual de Campinas.

DEDICATÓRIA

Obstáculos podem diminuir a velocidade da nossa trajetória, mas não nos impedem de alcançar a linha de chegada.

# AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), processo nº 2019/01620-9, e da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. Agradeço também à FAPESP pela bolsa de iniciação científica (processo nº 2019/05997-0), ao Projeto Temático BIOTA-Coleções (processo nº 2018/10313-0), ao suporte financeiro do Projeto ProTrindade (nº 2016/50373-6), ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (processo nº 402186/2016-8 e 443278/2019-9), à Marinha brasileira (1º Distrito) e ao SECIRIM (Comissão Interministerial para os Recursos do Mar) por todo suporte provido na Ilha Trindade e Martin Vaz. O material utilizado referente ao Arquipélago de Trindade e Martin Vaz foi uma contribuição 14 do Projeto de Invertebrados Marinhos do ProTrindade.

Gostaria de agradecer, primeiramente, a Deus, Quem me deu forças e capacidade para realizar meus sonhos, mesmo que muitas vezes parecessem estar longe de serem alcançados. Sonhos que, por mais que tenham sido readaptados, aconteceram, e foram melhores do que eu esperava. Agradeço a Deus por ter colocado pessoas maravilhosas ao meu lado, durante essa jornada.

Agradeço aos meus pais, Claudia e Sérgio, por terem acreditado em mim até agora, e por sempre me incentivarem a correr atrás dos meus sonhos. Agradeço também...

À minha melhor amiga, da faculdade, do mestrado e da vida, Vanessa, que desde 2013 tem feito parte da minha vida, em todas as alegrias, tristezas e processos seletivos. Obrigada pela paciência, pelas caronas, pelas horas estudando na biblioteca comigo, pelas idas ao "bandejão" e na feirinha da UNICAMP. Obrigada por me tornar uma pessoa mais leve e despreocupada.

À Paula, por ter trazido mais alegria durante a faculdade e o mestrado, e por estar sempre guardando lugar nas mesinhas de ardósia do IB, na hora do almoço.

Agradeço a todas as pesquisadoras do EQUI.LAB...

À Michela, minha orientadora, que tem me apoiado desde a iniciação científica. Obrigada pela paciência, pelos ensinamentos da vida e da academia. Obrigada por se tornar a minha referência da mulher no meio acadêmico: Mãe, Mulher e Cientista.

À Renata, minha coorientadora, e minha amiga de laboratório. Obrigada por toda a paciência, desde o primeiro dia de laboratório até hoje. Você me ensinou a dar o meu melhor.

À Cecília, Gabriela e Giovanna, agradeço o empenho e a dedicação de todas vocês em me ajudar e a me ensinar. Cada uma de vocês teve um papel importantíssimo nessa dissertação. Muito obrigada pelos cafezinhos com biscoitos no meio da tarde e as risadas.

Ao Pablo e ao Prof. Walter, por me ajudarem mesmo a distância.

A todos do LabEsc: Karin, Kaleb, Ricardo, Alessandra, Profa. Luciana e Rê Tenório por todo apoio em nossos experimentos moleculares.

À UNICAMP e ao curso de pós-graduação em Biologia Animal do IB/UNICAMP pela formação proporcionada.

Ao Museu de Zoologia da UNICAMP, Museu de Zoologia da USP, Laboratório de Microscopia Eletrônica da UNICAMP e Laboratório de Estudos Cromossômicos da UNICAMP pela infraestrutura para realização deste trabalho.

RESUMO

Os ofiuróides são invertebrados marinhos muito peculiares, com hábitos diversificados, tanto alimentares quanto reprodutivos. A história evolutiva dos Ophiuroidea, ainda é pouco compreendida, devido à falta de apomorfias do grupo, que dificultam a delimitação das espécies. Por isso, com base na taxonomia integrativa (TI), que utiliza distintos caracteres, moleculares, morfológicos, ecológicos e filogeográficos, para a obtenção de resultados mais robustos, é possível delimitar mais facilmente essas espécies. Nesta dissertação utilizamos a TI para testar se os dois morfotipos de *Ophiocoma* encontrados no litoral do Brasil, pertencem ou não a mesma espécie. As ferramentas utilizadas nessa investigação foram: i) morfologia externa; ii) morfologia dos caracteres microestruturais; iii) morfometria das estruturas diagnósticas; iv) dados moleculares (fragmentos do gene mitocondrial 16S). Nossos resultados são apresentados na forma de um artigo científico, com a descrição da nova espécie *Ophiocoma trindadensis* sp. nov, e redescrição de *Ophiocoma echinata* (Lamarck, 1816), até então a única espécie do gênero descrita para o Brasil. Reunimos ferramentas morfológicas e moleculares modernas, importantes para a delimitação das espécies, contribuindo assim para futuros trabalhos taxonômicos do grupo.
Palavras-chave: bentos marinho, DNA *barcoding*, taxonomia integrativa.

ABSTRACT

Ophiuroids (brittle stars) are very peculiar marine invertebrates, with diversified habits, both feeding and reproductive. The evolutionary history of the Ophiuroidea is still poorly understood, due to the lack of apomorphies of the group, which make it difficult to define the species. Therefore, based on integrative taxonomy (IT), which uses different molecular, morphological, ecological and phylogeographic characters, to obtain the most robust results, it is possible to delimit these species more easily. In this dissertation we use IT to test whether the two morphotypes of *Ophiocoma* sampled on the coast of Brazil belong or not to the same species. The tools used in this investigation were: i) external morphology; ii) morphology of microstructural characters; iii) morphometry of diagnostic structures; iv) molecular data (16S mitochondrial gene fragments). Our results are presented in the form of a scientific article, describing the new species *Ophiocoma trindadensis* sp. nov, and redescription of *Ophiocoma echinata* (Lamarck, 1816), until then a single species of the genus described for Brazil. We bring together modern morphological and molecular tools, important for the delimitation of species, thus contributing to future taxonomic work of the group.
Keywords: DNA *barcoding*, benthos, integrative taxonomy.

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# 1. INTRODUÇÃO

O objetivo principal da pesquisa sistemática é construir uma avaliação precisa da diversidade de espécies, a fim de se aplicar uma adequada política de conservação. Espécies crípticas, que por definição são difíceis de se distinguir morfologicamente, representam uma grande dificuldade no cumprimento deste objetivo (Bickford *et al*., 2007), visto que "camuflam" a real diversidade local. Atualmente, cientistas vivem a chamada "crise da taxonomia" onde se perguntam se as análises morfológicas dos caracteres externos são suficientes para se delinear uma espécie, não desconsiderando as morfoespécies, mas tratando-as como hipóteses que devem ser testadas por diferentes abordagens e tipos de dados.

Com o intuito de se testar essas diferenças, foi proposto um novo *framework* para a taxonomia integrativa, que visa delimitar as unidades de biodiversidade a partir de perspectivas múltiplas e complementares, como por exemplo, os caracteres morfológicos, genéticos, ecológicos, comportamentais e filogeográficos (Dayrat, 2005; Padial *et al*., 2010).

A combinação desses fatores aos esforços dos taxonomistas, marcou as últimas décadas como um importante período de novas descobertas de espécies marinhas. Estima-se que a magnitude global dessa diversidade seja de 700 a 1 milhão de espécies, contudo apenas 226 mil foram descritas (Appeltans *et al.*, 2012). Com taxonomistas descobrindo e descrevendo novas espécies, ampliamos o conhecimento sobre os ecossistemas e consequentemente melhoramos as condições para conservação destes, além de proteger as espécies da extinção (Costello *et al*., 2013).

Neste contexto, sobre uma perspectiva taxonômica moderna, escolhemos estudar a classe Ophiuroidea, família Ophiocomidae, visando a delimitação precisa de espécies que compõem a diversa fauna de ofiuróides no Atlântico Sul Ocidental.

A dissertação apresenta os resultados na forma de um artigo científico intitulado "A new species of *Ophiocoma* (Echinodermata: Ophiuroidea) from Archipelago Trindade and Martin Vaz, Southeastern Atlantic", para o qual foram utilizados caracteres morfológicos e moleculares no intuito investigar a atual biodiversidade do gênero *Ophiocoma* no Brasil. Neste estudo comparativo, descrevemos uma nova espécie, *Ophiocoma trindadensis* sp. nov., encontrada no Arquipélago de Trindade e Martin Vaz, e redescrevemos a espécie *Ophiocoma echinata*, encontrada no Nordeste brasileiro. Ambas as espécies foram descritas detalhadamente, a partir de caracteres morfológicos externos e microestruturais, como placas

braquiais, dentais e orais. Dessa forma, nosso estudo contribui para o real conhecimento da biodiversidade do grupo em águas brasileiras.

## 1.1. Características gerais e filogenia dos Echinodermata

Características gerais

Os Echinodermata são um dos grupos mais distintos dos Metazoa, apresentando três características exclusivas como, o endoesqueleto composto por carbonato de cálcio, a presença do sistema hidrovascular aquífero, responsável pela alimentação, locomoção, entre outras funções, e um tecido colágeno mutável (Pawson, 2007; Benavides-Serrato, 2011). Estão divididos em cinco classes viventes, Ophiuroidea (serpentes-do-mar, ofiuróides), com 2087 espécies descritas, Asteroidea (estrelas-do-mar) com 1800 espécies, Holothuroidea (pepinos-do-mar) com 1400 espécies, Echinoidea (ouriços-do-mar e bolachas-da-praia) com 1000 espécies, e Crinoidea (lírios-do-mar) com 700 espécies (Alvarado & Solís-Marín, 2013; Kroh & Mooi, 2018; Mah, 2018; Stöhr *et al*., 2018). Além disso, são descritas aproximadamente 13.000 espécies de equinodermos fósseis que, devido ao alto potencial de fossilização do esqueleto de calcita, têm sido importantes em estudos morfológicos e evolutivos (Thuy & Stohr, 2011; O'Hara *et al*., 2014; Thuy & Stohr, 2016).

No Brasil, os precursores dos estudos dos Echinodermata foram J. Marcgrave (1648), que em seu trabalho, citou quatro espécies (dois asteróides, um equinóide e um ofiuróide) (Marcgrave, 1942), Rathbun (1879) que compôs o primeiro catálogo de Echinodermata da costa brasileira e Ludwig (1882) que apresentou um estudo de 30 espécies. Atualmente são conhecidas pouco mais de 350 espécies no país (Ventura *et al*., 2013).

Nas últimas décadas, os equinodermos têm sido analisados em diversos âmbitos da Ciência, como o tratamento de câncer, pesquisas de bioerosão e modelos robóticos (Amini *et al*., 2017, 2019; Patterson *et* al., 2020; Schneider *et al*., 2011).

Filogenia

Atualmente, o filo Echinodermata e Hemichordata pertencem a um clado monofilético chamado Ambulacraria, com base em padrões de expressões de gene não compartilhados com os Chordata (Brown *et al*., 2008; Winchell *et al*., 2002; Edgecombe *et al*.,

2011). Embora a posição filogenética dos Echinodermata esteja estabelecida dentro dos Metazoa, as relações entre as cinco classes do filo, ainda são contestadas entre os especialistas (Smith, 1984; Janies, 2001; Pisani, 2012; Telford *et al*., 2014).

Segundo registros fósseis, os primeiros Echinodermata surgiram no início do Cambriano, e exibiram vários planos corporais, ainda no final do mesmo período. Sendo assim, as classes passaram por um curto período de irradiação, que ocorreu entre 10 e 15 milhões de anos (Smith *et al*., 2013). Devido a essa rápida e intensa divergência morfológica, entre as linhagens, a reconstrução filogenética ainda é considerada um desafio (Pisani, 2012). Segundo aspectos morfológicos e moleculares, a formação de um grupo monofilético entre os Echinoidea, Asteroidea, Holuthoroidea e Ophiuroidea (Eleutherozoa), é incontestável, porém a posição dos Ophiuroidea em relação as outras classes, é controversa (Telford *et al*., 2014). Historicamente equinodermatólogos discutem duas hipóteses, sobre as relações das classes pertencentes ao Eleutherozoa. A primeira é a hipótese Asterozoa – Echinozoa, e a segunda, é a hipótese Cryptosyringida, e ambas discutem a relação dos Ophiuroidea com os demais grupos (Linchangco *et al*., 2017).

Proposta por Bather (1900), a hipótese Asterozoa – Echinozoa (Fig. 1A), define como grupo irmão de Ophiuroidea, os Asteroidea, formando o clado Asterozoa, e uma relação similar entre Echinoidea e Holothuroidea, formando o clado Echinozoa. A monofilia dos Asterozoa está baseada na sinapomorfia do plano corporal do adulto com a forma do corpo estrelado e a construção do quadro oral e ossículos ambulacrais (Mooi & David, 2000). Nos Echinozoa, a monofilia é suportada pela forma globosa do corpo (Smith, 1984).

Descrita por Smith (1984), a hipótese Cryptosyringida (do grego, Kryptos, escondido; Syringos, tubo) (Fig. 1B), reúne as classes Echinoidea, Holothuroidea e Ophiuroidea, no clado Cryptosyringida, grupo irmão dos Asteroidea. A monofilia dos Cryptosyringida, neste caso, está baseada na sinapomorfia morfológica dos componentes ectoneurais (sensoriais) do anel nervoso e nervos radiais aprofundados na derme. Diferente dos Cryptosyringida, nos Asteroidea esses componentes estão localizados externamente (Smith, 1984; Barnes *et al*., 2005).

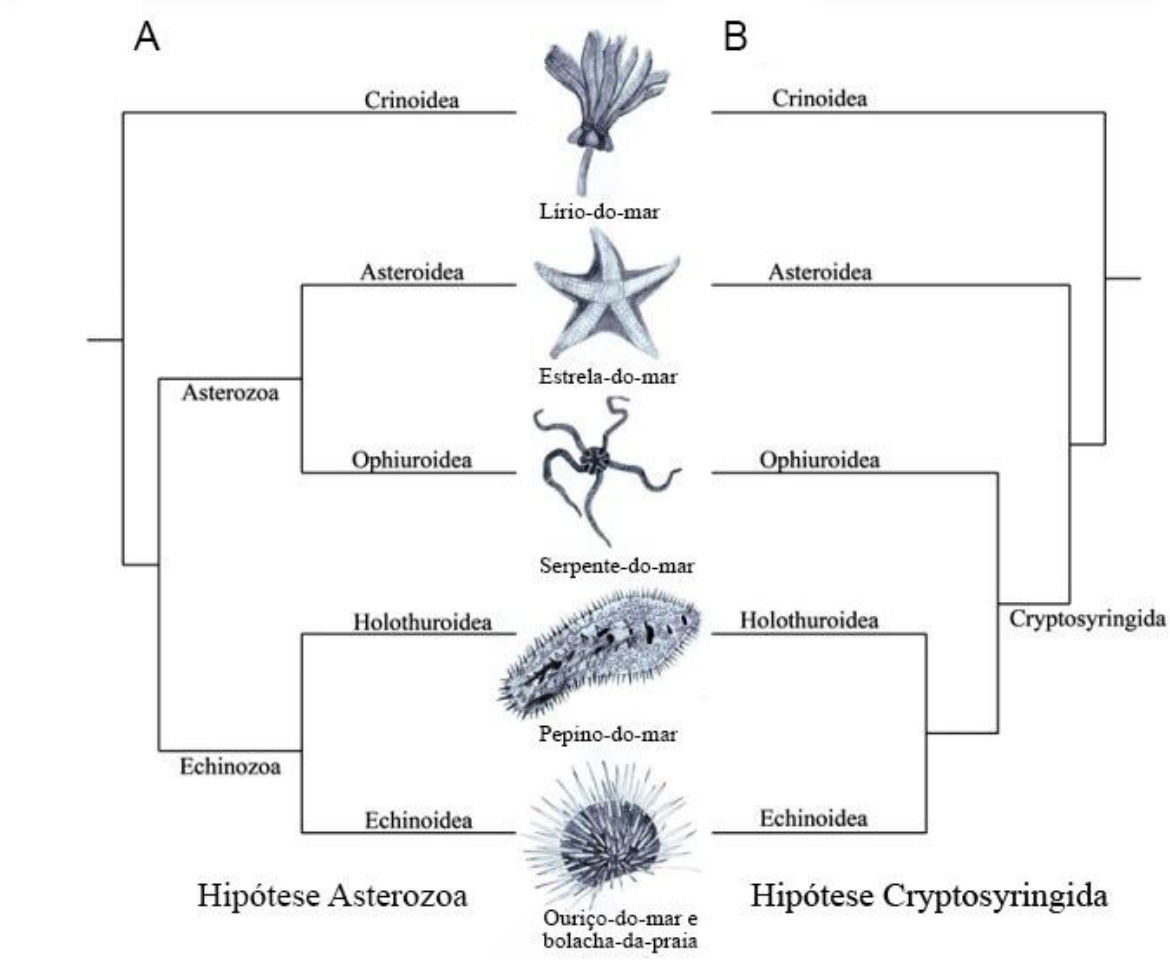


**Figura 1.** Hipóteses das relações filogenéticas dentro do grupo dos Echinodermata viventes. A) Hipótese Asterozoa. B) Hipótese Cryptosyringida. Modificado de Reich *et al.* (2015).

Estudos filogenômicos recentes, suportaram a hipótese Asterozoa (Telford *et al*., 2014; O'Hara *et al*., 2014; Linchangco *et al*., 2017), assim como desvendaram a incógnita de Xyloplax, uma rara espécie de águas profundas, que foi incluída na classe Asteroidea, a partir de análises do transcriptoma.

Ao que parece, as relações filogenéticas dentro dos Deuterostomia ainda são discutidas. Com a descoberta de fósseis do período Ordoviciano inferior, os Estilóforos seriam membros ancestrais dos equinodermos e, morfologicamente extremamente próximos ao último ancestral em comum de Echinodermata e Hemichordata (Lefebvre *et al*., 2019).

Dessa forma, os estudos filogenéticos atuais, não podem ignorar a importância dos caracteres morfológicos na evolução dos equinodermos, principalmente na comparação entre espécies viventes e fósseis. Resolver essas relações é fundamental para inferir estados

plesiomórficos dos caracteres dos Echinodermata e Hemichordata, assim como entender as relações entre Ambulacraria e Deuterostomia (Cannon *et al*., 2014).

Dentre os Echinodermata, a classe Ophiuroidea é considerada a mais intrigante. Com uma grande capacidade de mobilidade, devido ao seu tamanho pequeno, os ofiuróides, exploram diferentes habitats, inalcançáveis por outros equinodermos. Exibem hábitos alimentares diversos, além de uma variedade de estratégias de dispersão.

A reprodução ocorre assexuadamente (fissão) ou sexualmente, com indivíduos hermafroditas sequenciais ou simultâneos. Habitam desde a plataforma continental até profundidade hadal, do equador até regiões mais polares (O'Hara, 2017; Benavides-Serrato *et al*., 2011; Stöhr *et al*., 2012).

Além disso, atualmente, a sistemática dos ofiuroides passa por oscilações, onde análises moleculares recentes entram em conflito com as classificações tradicionais baseadas na morfologia (Thuy & Stöhr, 2016).

## 1.2. Morfologia e taxonomia dos Ophiuroidea

Morfologia

Os Ophiuroidea podem ser confundidos com os Asteroidea, porém algumas características exclusivas os diferenciam. Nos ofiuróides o sulco ambulacral, encontrado na região ventral dos braços, é completamente fechado pelas placas braquiais laterais e ventrais, enquanto nos asteróides esse sulco é totalmente aberto (Stöhr *et al*., 2012).

Os ofiuróides possuem um disco central, redondo ou pentagonal, nitidamente destacado dos braços, geralmente finos e delgados. Dorsalmente sobre o disco, estão localizados os escudos radiais que, assim como o disco, podem ser nus ou estarem parcialmente ou completamente cobertos por escamas, espinhos, grânulos ou tegumento (Fig. 2A). Na região ventral, estão localizadas as bursas, que se conectam com o meio externo por meio das fendas bursais (Fig. 2C).

Na região ventral, também está localizada a boca, a qual é rodeada por um conjunto de estruturas que formam as mandíbulas. Estas, nada mais são do que ossículos dos braços que sofreram modificações ao longo do desenvolvimento do indivíduo, por isso, o número de mandíbulas corresponde ao número de braços (Fig. 2C) (Hendler, 2018).

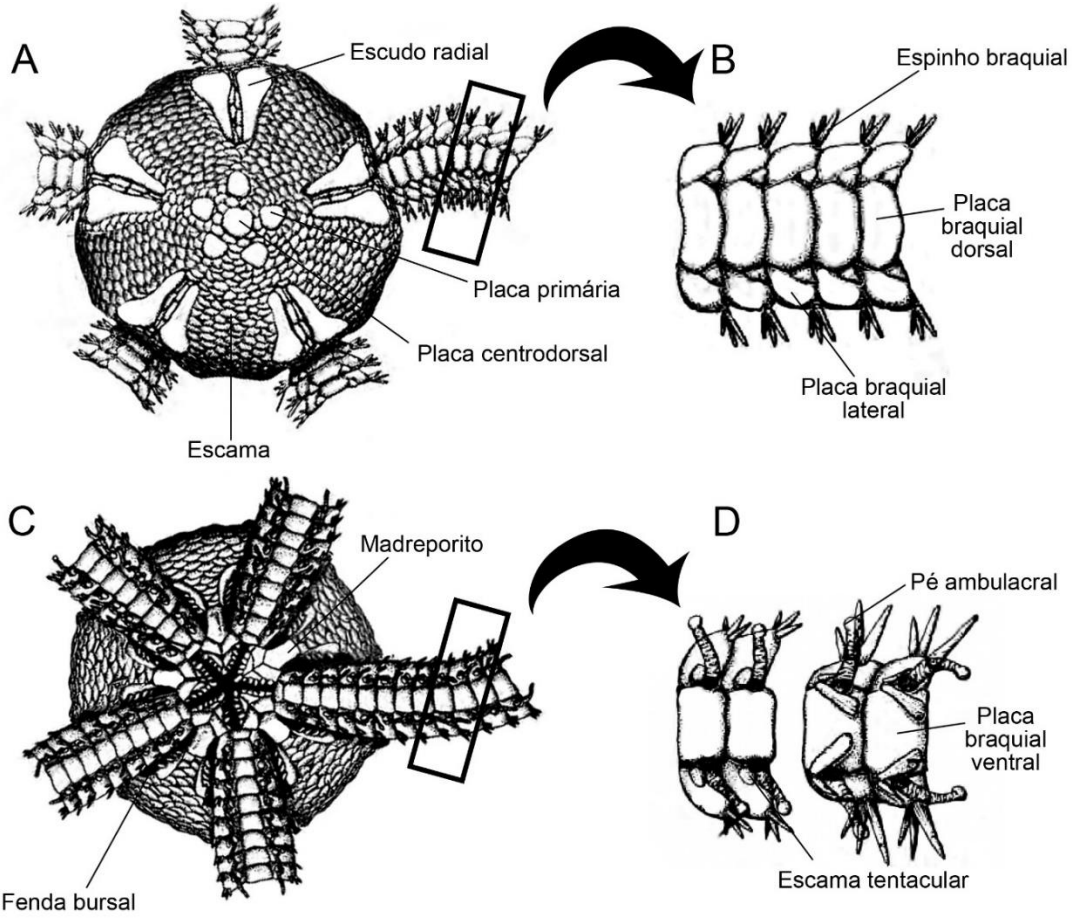


**Figura 2.** Morfologia externa dos Ophiuroidea. A) Disco - vista dorsal. B) Braço - vista dorsal. C) Disco - vista ventral. D) Braço - vista ventral. A, B, C e D, modificado de Hendler *et al*. (1995).

Cada mandíbula é formada por um escudo oral (EO), dois escudos adorais (EAd), dentes (De) localizados na parte interna, e um conjunto de ossículos denominados, papilas orais (lato sensu) (Fig. 3A). Até então, o termo utilizado para "papilas orais", englobava todos os ossículos dispostos na mandíbula, no qual eram apenas nomeados em números.

Recentemente, um estudo baseado na ontogenia dos ofiuróides, mostrou que as papilas orais dos indivíduos adultos, são homólogas a uma diversidade de ossículos. Dessa forma, o termo "papila oral" passou a ser usado para designar os seguintes ossículos: ossículo de Lyman (OsLy), derivado do esqueleto peribucal, associado ao compartimento do tentáculo oral ventral (CTOV), que está disposto entre a primeira placa braquial ventral (1º PBV) e o escudo adoral (EAd); espinho do escudo adoral (EsEAd), derivado do escudo adoral; segundo espinho do escudo adoral (2ºEsEAd), derivado da placa oral; papila infradental (PaI), derivada da placa oral; papilas dentais (PaD) e dentes (De), ambas derivadas da placa dental (Figs. 3A e

B). Além disso, algumas famílias podem apresentar outras papilas, como a papila infradental secundária, papila oral lateral e papilas orais acessórias (Hendler, 2018).

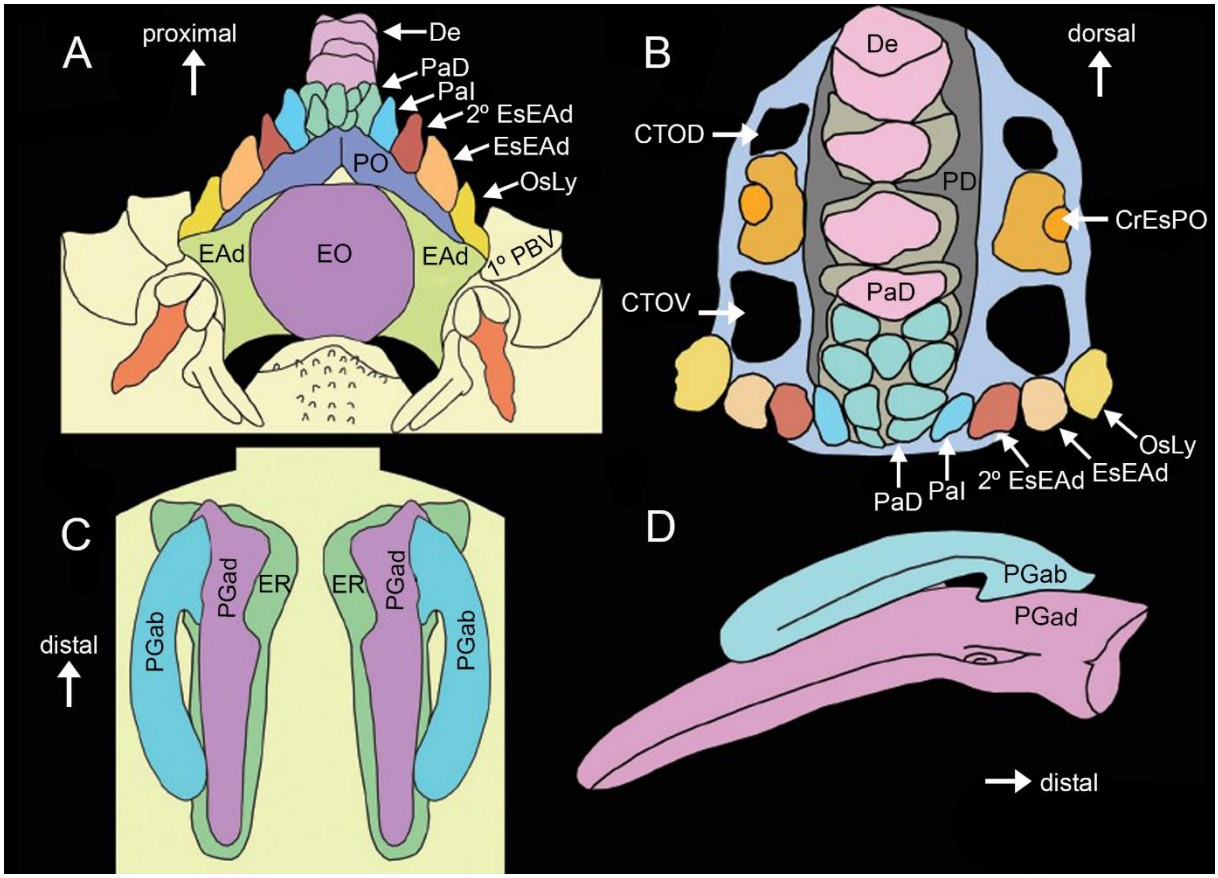


**Figura 3.** Morfologia das estruturas internas e externas de *Ophiocomella alexandri* (Ophiocomidae). A) Detalhe da mandíbula - vista ventral. B) Detalhe da mandíbula - vista transversal. C) Placas genitais articuladas com o escudo radial. D) Placas genitais. Abreviações: CrEsPO: Crista do espinho da placa oral; CTOD: Compartimento do tentáculo oral dorsal; CTOV: Compartimento do tentáculo oral ventral; De: Dentes; EO: Escudo oral; EAd: Escudo adoral; EsEAd: Espinho do escudo adoral; 2º EsEAd: Segundo espinho do escudo adoral; OsLy: Ossículo de Lyman; PaD: Papilas dentais; PaI: Papila infradental; PD: Placa dental; PGab: Placa genital abradial; PGad: Placa genital adradial; PO: Placa oral; 1º PBV: Primeira placa braquial ventral. A, B, C e D, modificado de Rosales-Contreras *et al*. (2021).

Em geral, os ofiuróides possuem de cinco a seis braços, bem articulados, onde cada segmento do braço é constituído externamente por: duas placas braquiais laterais, das quais suportam os espinhos braquiais, uma placa braquial dorsal (Fig. 2B) e uma ventral (Fig. 4C)

(Benavides-Serrato *et al*., 2011). Em cada segmento, observa-se também, escamas tentaculares, de onde emergem os pés ambulacrais (Fig. 2D).

As estruturas braquiais externas, envolvem ossículos internos, denominadas vértebras (Fig. 4C). Cada vértebra possui seis faces: proximal (Fig. 4D), distal (Fig. 4E), dorsal (Fig. 4F), ventral (Fig. 4G), e duas laterais.

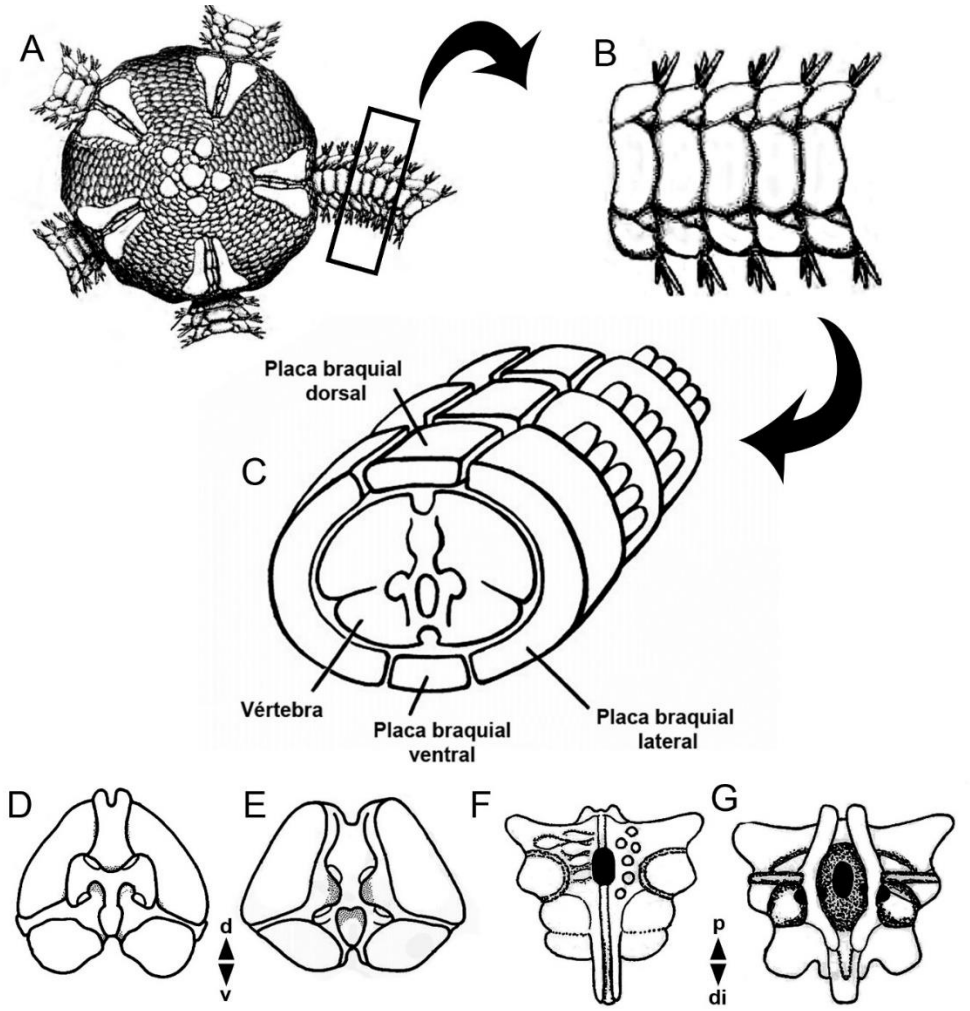


**Figura 4.** Ossículos braquiais de Ophiuroidea. A) Disco dorsal. B) Braço - vista dorsal. C) Esquema do corte transversal do braço. Vértebra: D) vista proximal, E) vista distal, F) vista dorsal e G) vista ventral. Abreviações: d: dorsal; di: distal; p: proximal; v: ventral. A e B, modificado de Hendler *et al*. (1995). C, D e E, modificado de LeClair & LaBarbera (1997). F e G, modificado de Irimura & Fujita (2003).

As faces se encaixam entre si, devido as superfícies articulares, classificadas da seguinte maneira: i) articulação com botões – provavelmente mais primitiva, flexível em todas

as direções, porém os botões de articulação são ocos e por isso os movimentos mais bruscos não são estáveis; ii) articulação em forma de sela – permite maior flexibilidade ao braço, com movimentos em todas as direções, inclusive espiral; iii) articulação universal – flexível mas sem movimento espiral; iv) articulação retangular – mais alongada no sentido dorso ventral e movimentação mais restrita com curva para baixo e v) articulação aberrante – flexível verticalmente e restrito horizontalmente (Litvinova, 1994).

Taxonomia e filogenia

Após a grande extinção em massa no período do Permiano, os ofiuróides passaram por uma rápida irradiação, que os possibilitou a ocupação em larga escala dos ambientes bentônicos. Devido a essa rápida evolução do grupo, a reconstrução filogenética dos Ophiuroidea é dificultada, pela falta de apomorfias (Perseke *et al*., 2010) que resultam na sobreposição de caracteres diagnósticos entre as muitas famílias (Stöhr *et al*., 2012), ao invés de melhor delimitá-las.

No final do século XIX, as tentativas de classificação do grupo, que deram início aos estudos sistemáticos dos Ophiuroidea, não passavam de observações anatômicas de novos gêneros e espécies. Posteriormente, Matsumoto em 1915 e 1917, deu início as primeiras tentativas de reconstrução filogenética e evolutiva dos ofiuróides (Thuy & Stöhr, 2016), com base nas placas braquiais dorsais e o tipo de articulação entre as placas genitais.

Porém, até então, a única tentativa de se compreender a história evolutiva destes animais, compilando análises moleculares (RNA ribossomal) e caracteres morfológicos, havia sido realizada por Smith *et al*., (1995), a qual resultou na subdivisão dos ofiuróides modernos em Euryalida e Ophiurida (Thuy & Stöhr, 2016). Smith *et al*. (1995) demonstrou a importância da integração de análises como ferramentas para entender a história evolutiva, e ainda é referenciado em diversos estudos filogenéticos dos Ophiuroidea (Martynov, 2010; Thuy & Stöhr 2016; O'Hara *et al*., 2017).

Entretanto, ao longo das décadas, novas técnicas moleculares e caracteres morfológicos foram implementados, aumentando a robustez das análises filogenéticas. Um exemplo, foi a descoberta da eficácia das placas braquiais laterais (PBL) e da articulação dos espinhos braquiais, na delimitação de famílias, gêneros e espécies (Thuy & Stöhr, 2011). Tal classificação das PBL coincidiu com os resultados filogenômicos, envolvendo análises de

sequências do transcriptoma. A partir desse trabalho, todos os esquemas de classificações históricas dos Ophiuroidea, baseado na morfologia, foram refutados (O'Hara *et al*., 2014).

Em resposta a esses últimos trabalhos, Thuy & Stöhr (2016), demonstraram a importância da integração dos dados moleculares e morfológicos para a classificação do grupo, contribuindo para novas perspectivas de trabalhos futuros.

A família Ophiocomidae

Desde o último século, a família Ophiocomidae sofreu algumas revisões taxonômicas, com o intuito de melhor delimitar os táxons, os quais apresentavam caracteres morfológicos diagnósticos sobrepostos. A primeira revisão resultou na subdivisão de Ophiocomidae em três grupos: brevipes, pumila e scolopendrina, baseado na granulação do disco, no número de escamas tentaculares, na coloração e na distribuição geográfica das espécies (Clark, 1921). Devaney (1970), além de incluir um novo gênero *Clarkcoma*, delimitou mais um grupo, denominado pica, com base na sequência de espinhos do braço, coloração e formato da placa dental.

A última revisão taxonômica, envolvendo sequenciamento de última geração (genes nucleares e mitocondriais obtidos a partir do método exon-capture system) e caracteres morfológicos, resultou na divisão da família Ophiocomidae em quatro gêneros: *Breviturma* (8 espécies), *Ophiocoma* (7 espécies), *Ophiocomella* (6 espécies) e *Ophiomastix* (22 espécies) (O'Hara *et al*., 2019). Atualmente, as espécies dos grupos não formais de Devaney (1970), brevipes e pica, representam o gênero *Breviturma*, as espécies do grupo pumila, pertencem a *Ophiocomella*, e algumas espécies do grupo scolopendrina, pertencem a *Ophiocoma* (O'Hara *et al*., 2019).

Embora a nova divisão de gêneros tenha sido aceita entre os taxonomistas, existem algumas lacunas que ainda necessitam ser preenchidas. Segundo a nova filogenia, *Ophiocoma* passou a ser grupo irmão de *Ophiocomella*, gênero formado por espécies fissíparas de seis braços, porém compartilha o mesmo padrão de alternância do número de espinhos braquiais, que *Ophiomastix* (O'Hara *et al*., 2019).

Atualmente, *Ophiocoma* é composto por: *Ophiocoma aethiops*, *O. anaglyptica*, *O. cynthiae*, *O. echinata*, *O. erinaceus*, *O. schoenleinii* e *O. scolopendrina*. Além disso, é considerado um grupo monofilético, devido a sinapomorfia morfológica do envelope

ornamentado (espinhoso) no ovo, e sinapomorfias moleculares (Cisternas *et al*., 2013; O'Hara *et al*., 2019).

No Brasil, *Ophiocoma* é representado apenas por *O. echinata*, uma vez que *O. pumila* foi transferida para o gênero *Ophiocomella* (*Ophiocomella pumila*) e *O. wendtii* para *Ophiomastix* (*Ophiomastix wendtii*) (Tommasi & Aron, 1988; O'Hara *et al*., 2019).

A distribuição de *Ophiocoma echinata* ao longo da costa brasileira, inclui os estados do Ceará, Paraíba, Pernambuco, Alagoas e Bahia (Tommasi, 1970; Albuquerque, 1986; Rathbun, 1879; Tommasi, 1970; Lima & Fernandes, 2009; Miranda *et al*., 2012; Manso *et al*., 2008). Ainda, pouco se sabe sobre a biodiversidade de *Ophiocoma* nas ilhas oceânicas do Brasil, uma vez que grande parte dos trabalhos de diversidade está restrita a região costeira. Por isso, estudos conduzidos em áreas remotas ao continente, são fundamentais para o real conhecimento da biodiversidade e conservação das espécies, especialmente as endêmicas.

## 2. RESULTADOS

Este trabalho estudou dois morfotipos do gênero *Ophiocoma* encontrados no Atlântico Sul do Brasil, por meio da morfologia dos caracteres externos, morfologia dos caracteres microestruturais, morfometria e dados moleculares (fragmentos do gene 16S). Todas as análises tiveram o mesmo peso nos resultados finais.

Visando uma melhor organização dos resultados, o conteúdo deste trabalho encontra-se na forma de um artigo científico, aqui já formatado conforme normas da revista científica.

# A new species of *Ophiocoma* (Echinodermata: Ophiuroidea) from Archipelago Trindade and Martin Vaz, Southeastern Atlantic.

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The brittle stars of the family Ophiocomidae inhabit shallow waters and coral reefs of tropical regions. The species of *Ophiocoma* are difficult to identify, due the morphological variations, especially when different stages of development are observed. Here, based on morphological analyses, two Brazilian morphotypes of *Ophiocoma* were studied and characterized, *Ophiocoma* sp. NB (Northeastern Brazil) and *Ophiocoma* sp. TMV (Trindade and Martin Vaz Archipelago), and then they were thoroughly analyzed to confirm whether they were the same species or not. After all the morphological studies, including external morphology, morphometry and microstructural characters, and molecular data, involving the 16S gene, was concluded that *Ophiocoma* sp. NB and *Ophiocoma* sp. TMV do not correspond to the same species, and the latter is new species. This new species is characterized by two tentacle scales on the first arm segment and then one (posterior segments), and dorsalmost arm spines robust and rounded as wide as long or almost as wide as long.

Keywords: brittle stars, DNA barcoding, dorsalmost arm spines, integrative taxonomy, morphometry, new species, Ophiocomidae, South Atlantic, taxonomy, tentacle scale.

## INTRODUCTION

Ophiocomids are abundantly found in shallow waters and coral reefs of all tropical regions (O'Hara *et al*., 2019). Their fascinating camouflage skills, which in addition to be used as visual protection against predators, facilitates negative phototaxis under a range of lights intensities (Hendler, 1984; Hendler & Byrne, 1987; O'Hara *et al*., 2004, 2019).

The taxonomy of *Ophiocoma* L. Agassiz, 1836, has been shrouded in uncertainty due to morphological characters not easily observable, and also because of changes related to growth, such as the number of arm spines, size of the disc and dorsal arm plates (Sumida *et al*., 1998; Stöhr, 2005; Stöhr *et al*., 2008). The genus is currently considered monophyletic, and the presence of an ornate egg envelope has been regarded as a synapomorphy of the *Ophiocoma* clade (O'Hara *et al*., 2019).

Before the review by O'Hara *et al*., (2019), in Brazilian waters, *Ophiocoma* was represented by four species (Tommasi, 1970; Tommasi & Aron, 1988). Currently, *O. pumilla* was transferred to *Ophiocomella*, *O. wendtii* (synonymous with *O. riisei*) to *Ophiomastix* and *O. echinata* (Lamark, 1816), is the only *Ophiocoma* species recorded from the Brazilian coast so far, whose distribution ranges from Florida (~20°N) to Bahia (~12°S) (Tommasi, 1970; Manso, 1993). Additional localities along the Brazilian coast include the states of Ceará (Albuquerque, 1986), Paraíba (Rathbun, 1879), Pernambuco (Tommasi, 1970; Lima & Fernandes, 2009), Alagoas (Miranda *et al*., 2012), and Bahia (Tommasi, 1970; Manso, *et al*., 2008).

During a long-term survey of the remote oceanic archipelago of Trindade and Martin Vaz located off the coast of southeastern Brazil (Tavares *et al*., 2017) a wealth of specimens of *Ophiocoma* were obtained. A taxonomic study based on morphological and molecular analyses from both insular and coastal specimens revealed that the Trindade and Martin Vaz samples actually belongs to a new species, whereas the coastal specimens belong to *O. echinata*. The new species is herein described and illustrated.

## MATERIALS AND METHODS

*Abbreviations:* BA, Bahia; CBS, Caribbean Sea; ES, Espírito Santo; NB, Northeastern Brazil; RI, Reunion Island; SPSPA = São Pedro and São Paulo Archipelago; TMV, Trindade and Martin Vaz Archipelago; LDA, linear discriminant analysis; SEM, scanning electron microscope; UNICAMP, University of Campinas; USP, University of São Paulo; ZUEC, Zoology Museum of UNICAMP; MZUSP, Zoology Museum of USP.

Initially, morphological comparisons were made among *Ophiocoma* sp. TMV and seven other *Ophiocoma* species, recently reviewed by O'Hara *et al*., 2019: *Ophiocoma aethiops* Lütken, 1859; *Ophiocoma anaglyptica* Ely, 1944; *Ophiocoma cynthiae* Benavides-Serrato & O'Hara, 2008; *Ophiocoma echinata* (Lamark, 1816); *Ophiocoma erinaceus* Müller & Troschel, 1842; *Ophiocoma schoenleinii* Müller & Troschel, 1842; and *Ophiocoma scolopendrina*

(Lamark, 1816). These comparisons were made according to the original descriptions and most recent publications (Say, 1825; Müller & Troschel, 1842; Matsumoto, 1917; Ely, 1944; Devaney, 1970; Tommasi, 1970; O'Hara *et al*., 2018, 2019).

The studied specimens were collected by the project ProTrindade/CNPq – TMV and belong to the scientific collections of the ZUEC and MZUSP. However, additional samples were collected in the NB, specifically in Salvador – BA.

The TMV is located in the southwestern Atlantic, approximately 1200 km east of Vitoria, ES. The Trindade Island (20°30'S, 29°20'W) is formed by a single large island of 13.5 km², while Martin Vaz Archipelago (20°30'S, 28°51'W) is located 47 km east of Trindade Island and formed by several smaller islands (Figure 1) (Marques *et al*., 1999; Almeida, 2007; Anker *et al*., 2016; Alitto *et al*., 2019). The expeditions executed by the ProTrindade/CNPq project, occurred between the years 2012-2019, and the material was collected from scuba diving between depths of 0.5-30 m, which resulted in 397 lots of Ophiuroidea.

Salvador is located in the state of Bahia, on the northeast coast of Brazil. The Itapuã beach (12°57'S, 38°21'W), where the specimens were obtained, consists of a wide area of exposed reefs, which receive few energy from the shore (Alves & Cerqueira, 2000). All the collections were made manually between the rock fragments at a depth of approximately 10 cm.

Some additional specimens were examined and were important for our delimitation: Saint Thomas Danish West Indies, U.S. Virgin Islands, type locality of *Ophiocoma echinata*, sampled in 1963 (Voucher ZUEC OPH 1953) and Carrie Bow Cay Belize (Voucher ZUEC OPH 1893), sampled in 1989. Both vouchers contain one specimen and are in ethanol 70%.

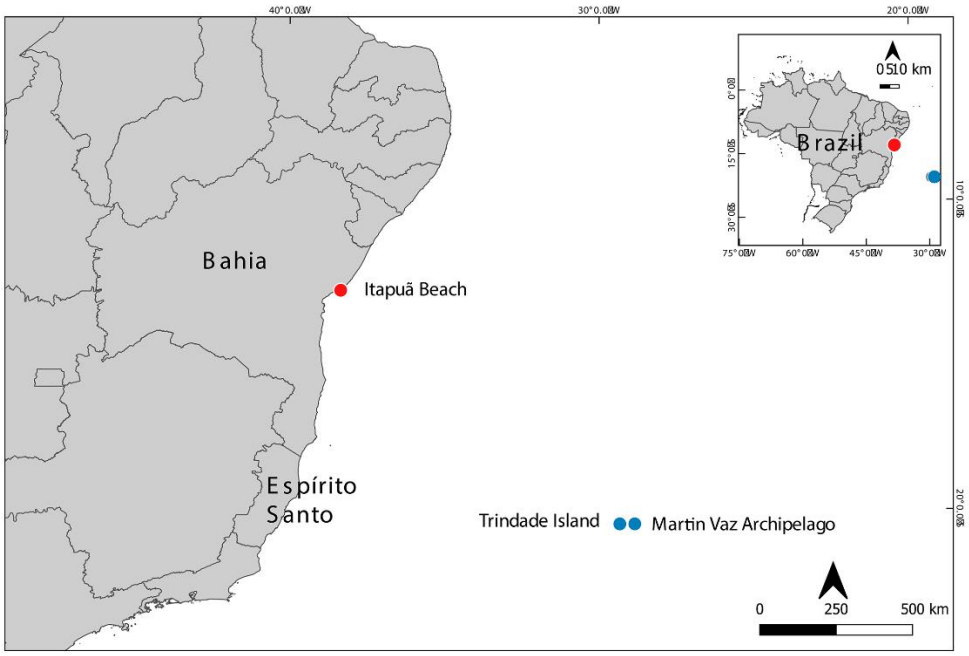


**Fig. 1.** Study areas: Trindade Island and Martin Vaz Archipelago, Espírito Santo and Itapuã Beach, Bahia.

The specimens were initially separated based on the external morphological characters, traditionally used, such as the type of disc cover, number, size and characteristic of spines, tentacle scales, among others. The arm spines were counted from the first to the 21st follow-up on both sides of each arm (Benavides-Serrato & O'Hara, 2008) and for granulometry, granules were counted in three frames measuring 0.2 mm², in the central area of the disc. The average of these three frames was then multiplied by five, creating an estimate of the number of disc granules in an area of 1 mm² (Stöhr *et al*., 2013). Classical morphological taxonomy was adopted for external and internal morphological studies with the support of a specialized bibliography (Borges *et al*., 2002; Borges & Amaral, 2005; Stöhr *et al*., 2012; Stöhr *et al*., 2013; Thuy & Stöhr, 2016; O'Hara *et al*., 2018; Hendler, 2018).

The microstructures of the arm ossicles, such as dorsal, ventral and lateral arm plates, as well as vertebrae, were taken from a fragment of the arm (between the fifth and tenth segments) of an adult from both coastal and insular specimens each morphotype. For other ossicles, such as oral, dental and genital plates, the entire disc or part thereof were used. Each arm or disc fragment was immersed in regular household bleach (NaClO) until the tissues were removed (Stöhr *et al*., 2008). The ossicles were then washed in distilled water and ethanol, air-dried, and arranged on aluminum stubs, metallized for observation under a scanning electron microscope (SEM) at the Electron Microscopy Laboratory of the Institute of Biology at the University of Campinas (UNICAMP).

The terminology used in the description of the ossicles essentially followed Thuy & Stöhr (2011, 2016), Alitto *et al*., (2018), O'Hara *et al*., (2018) and Hendler (2018).

Morphometry was used to refine and improve the morphological studies of external characters. Measurements were taken using the program AxioVision VS. 40.4.8.20 (Carl Zeiss Microscopy, Germany) attached to a stereomicroscope ZEISS Discovery V20 for specimens and structures smaller than 10 mm and/or with a digital caliper for those larger than 10 mm. A detailed list of the measured morphological characters is shown in Table 1.

**Table 1.** Abbreviations and definitions of morphological characters measured for morphometric analysis.

| Abbreviation | Character | Definition |
|---|---|---|
| dd | Disc diameter | Length from the distal edge of the radial shield to the inter-radial edge of the disc |
| disc_granule | Granules of disc | Granules present on 1 $mm^2$ of disc |
| as_l | Length of arm spine | Length of the largest arm spine |
| as_w | Width of arm spine | Width of the largest arm spine |
| as | Arm spine of first segment | Number of arm spines on the first segment |
| as2 | Arm spine of second segment | Number of arm spines on the second segment |
| as3 | Arm spine of third segment | Number of arm spines on the third segment |
| as10 | Arm spine of tenth segment | Number of arm spines on the tenth segment |
| od | Oral diameter | Length from the proximal edge of the oral shield to the proximal edge of the first ventral arm plate |
| os_l | Length of oral shield | Length of one oral shield |
| os_w | Width of oral shield | Width of one oral shield |
| ads_l | Length of adoral shield | Length of one adoral shield |
| ads_w | Width of adoral shield | Width of one adoral shield |
| dap_l | Length of dorsal arm plate | Length of the dorsal arm plate of the third arm segment |
| dap_w | Width of dorsal arm plate | Width of the dorsal arm plate of the third arm segment |
| vap1_l | Length of first ventral arm plate | Length of first ventral arm plate |
| vap1_w | Width of first ventral arm plate | Width of first ventral arm plate |
| vap2_l | Length of second ventral arm plate | Length of second ventral arm plate |
| vap2_w | Width of second ventral arm plate | Width of second ventral arm plate |
| ts_1 | Tentacle scale of first arm segment | Number of tentacle scales of the first arm segment |
| ts_2 | Tentacle scale of second arm segment | Number of tentacle scale of the second arm segment |
| ts_3 | Tentacle scale of third arm segment | Number of tentacle scale of the third arm segment |
| ts_4 | Tentacle scale of fourth arm segment | Number of tentacle scale of the fourth arm segment |
| pp | Oral papillae | Number of oral papillae in each half jaw |

The linear discriminant analysis (LDA) was applied in R version 4.0.4 (2021-02-15) following a script. A correlation matrix was constructed and the variables that were significantly correlated were removed with a threshold value of 0.9, aiming to avoid multicollinearity among morphological characters. Besides, the variables near-zero variance was removed (Kuhn & Johnson, 2013). The LDA was performed using R software packages, such as: MASS (Venables & Ripley, 2002; R Development Core Team, 2016), ggord (Beck, 2017), vegan (Oksanen *et al*., 2019), tidyverse (Wickham *et al*., 2019) and cowplot (Wilke, 2019).

The total genomic DNA was extracted according to the protocol previously established and tested by Alitto *et al*., (2019), where tube feet from the arm and/or gonads were soaked in two changes of Tris-EDTA (TE) buffer for 30 minutes. The sample was then macerated in 50µl of TE buffer with a pistil. More 450µl of TE buffer and 300µl of Chelex 100 10% (Walsh *et al*., 1991) were added, and then the samples were incubated for 60 minutes at 55°C, boiled in a water bath for eight minutes and, after reaching room temperature, vortexed and centrifuged for one minute. The supernatant (300µl) containing the genomic DNA was removed and incubated at -20 °C.

Fragments of the mitochondrial gene 16S were amplified by Polimerase Chain Reaction (PCR) through the primers 16S Sofi F and Sofi R (Pérez-Portela *et al*., 2013). PCR reactions were performed with a final volume of 25 µl, with 2.5 µl of reaction buffer (Taq Buffer), 2.5 µl of dNTP, 4 µL of MgCl2 (1.5 mM, Invitrogen), 1 µl of each of the primers, 1µl of Taq DNA polymerase, the amount of DNA and water are variable, aiming to complete the total volume (25µl).

The thermal cycling consisted of a single step at 94˚C for 7 minutes, which was followed by 39 cycles (denaturation at 94˚C for 60 seconds, annealing at 50˚C to 56˚C for 70 seconds, and extension at 72˚C for 80 seconds) and a final extension at 72˚C for 5 minutes on a thermal cycler (Eppendorf Mastercycler1). For purification, the Promega Kit was used, and subsequently, the samples were sent to the sequencing service of Myleus Biotechnology.

For the phylogenetic analysis of *Ophiocoma*, the 16S fragment of the species *Ophiocomella ophiactoides*, also from the Ophiocomidae family, was used as outgroup. All samples used from GenBank are listed below (Table 2).

**Table 2.** GenBank access number for DNA sequences used for phylogenetic analyses. Abbreviations: NMRJ = National Museum of Rio de Janeiro, Brazil; URUN = University of Reunion Island, Reunion.

| Museum | Species | Locality | GenBank | References |
|---|---|---|---|---|
| Absent | *Ophiocoma echinata* | Caribbean | KR090275 | Rojas & Schizas |
| Absent | *Ophiocoma echinata* | Caribbean | KR090224 | Rojas & Schizas |
| Absent | *Ophiocoma echinata* | Caribbean | KR090225 | Rojas & Schizas |
| URUN | *Ophiocoma erinaceus* | Reunion Island | KF662942 | Stöhr *et al*., |
| URUN | *Ophiocoma scolopendrina* | Reunion Island | KF662941 | (2013) |
| | | | | Stöhr *et al*., |
| | | | | (2013) |
| NMRJ | *Ophiocomella ophiactoides* | São Pedro and São Paulo Archipelago | KM234227 | Barboza *et al*., (2015) |

In phylogenetic analyzes two approaches were used: Maximum Parsimony (MP) and Bayesian Inference (BI). The cladogram obtained by the MP, had its nodes tested by the Bootstrap sampling test (Felsenstein, 1985), based on 1000 pseudoreplicates using the New Technology.

Two simultaneous analyzes were performed, with four chains in each one (three heated and one cold). There were execute 3.000.000 generations in each run, with one tree sampled every 100 generations. A consensus topology with the subsequent probability for each node was produced after the disposal of the first trees generated (initial 25%). The value of Average Standard Deviation of Split Frequencies was less than 0.01. Phylogenetic trees were visualized and edited using the FIGTREE v.1.4.3 program (http://tree.bio.ed.ac.uk/software/figtree/).

The genetic distances between and within the different groups were estimated by p-distance using the program MEGA v. X (Kumar *et al*., 2018).

## RESULTS

Here we present the results of the multiple comparative analysis, including data from external and microstructural morphology, and morphometry, in order to define whether the two morphotypes of the genus *Ophiocoma* (*Ophiocoma* sp. TMV and *Ophiocoma* sp. NB) found in Brazil belong or not to the same species.

**Morphotypes**

A total of 107 specimens were analyzed from two regions: (i) TMV (91 specimens) and (ii) NB (16 specimens). They were separated into two morphotypes: *Ophiocoma* sp. TMV and *Ophiocoma* sp. NB. In the following, each morphotype is presented with a brief description (Table 3, Figure 2).

**Table 3.** Characters identified as diagnostics in the separation of the two morphotypes of *Ophiocoma*.

| **Characters** | ***Ophiocoma* sp. TMV Figures 2A, B and C** | ***Ophiocoma* sp. NB Figures 2D, E and F** |
|---|---|---|
| Dorsal arm plates (DAP) | Contiguous, overlapping proximally | Not contiguous |
| Dorsalmost arm spines (DASp) | Robust and rounded, as wide as long or almost as wide as long (with 3.4 mm of length and 1.5 mm of width) | Robust, longer than wide (with 2.2 mm of length and 1.5 mm of width) |
| Number of tentacle scales (TSc) | Two ovals TSc on each pore of the first arm segment and only one in the rest | Two ovals TSc on each pore, except in the final third of the arm |

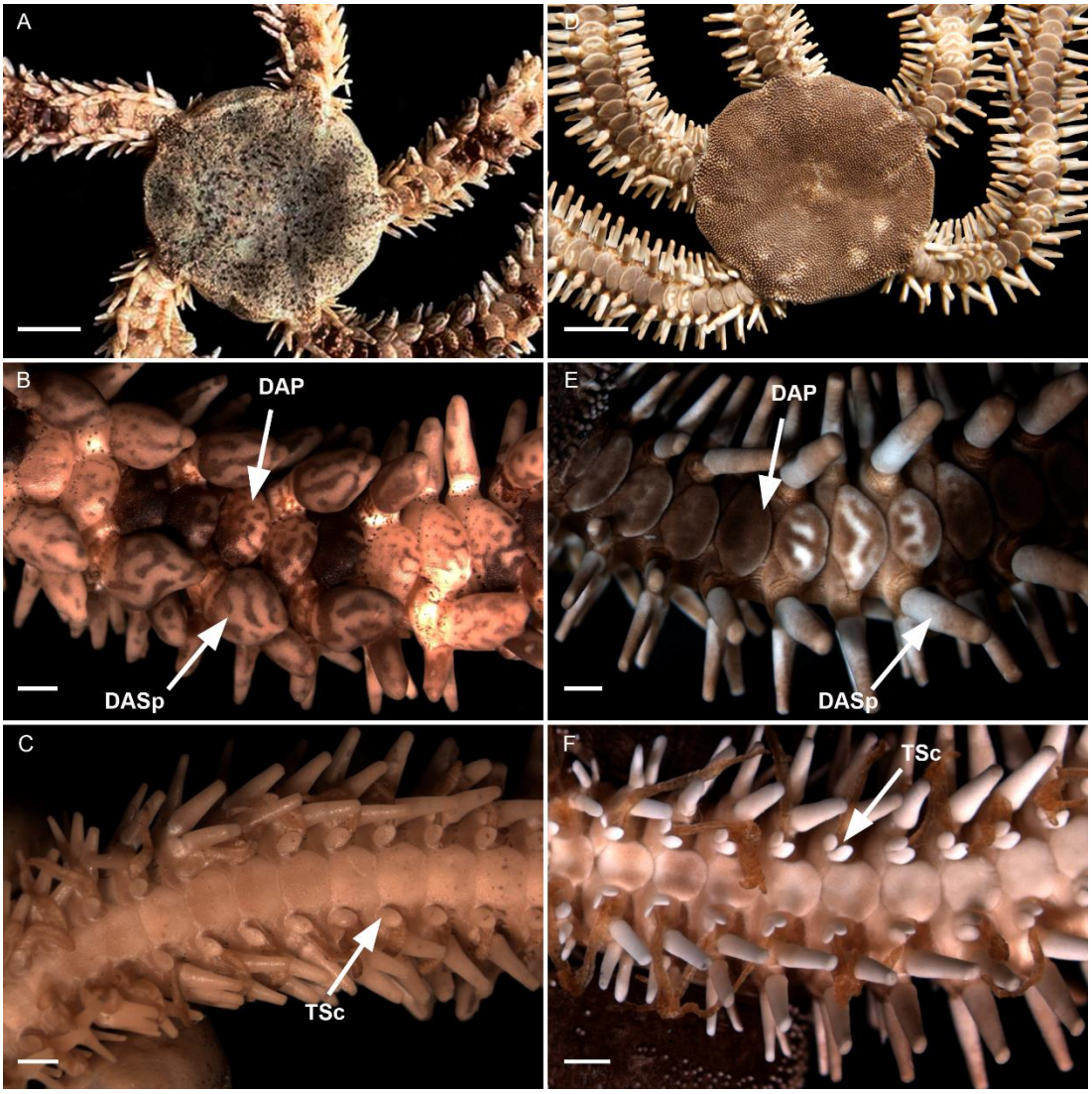


**Fig. 2.** Morphotypes of *Ophiocoma*: (A-C) *Ophiocoma* TMV – sample ZUEC OPH 3095 (19.3 mm dd). (D-F) *Ophiocoma* NB – sample ZUEC OPH 1538 (19.3 mm dd). Abbreviations: DAP: dorsal arm plate; DASp: dorsalmost arm spine; TSc: tentacle scale. Scale bars: A and D, 5 mm; B, C, E and F, 1 mm.

**Morphological characters**

STUDY OF THE MICROSTRUCTURAL CHARACTERS (OSSICLES)

We studied eight specimens of which: five were from TMV and three from NB. We described the dorsal and ventral arm plates, dorsalmost arm spines and dental plate of the two morphotypes. In the following is presented the description according to each morphotype previously stated.

*Ophiocoma* sp. TMV

Material examined. Samples ZUEC OPH 3093, ZUEC OPH 3094, ZUEC OPH 3095, ZUEC OPH 3103 and ZUEC OPH 3104.

Dental plate (Figure 3A): General outline: long with dorsal and ventral portion of the same width. Outer side: dorsalmost portion with two tooth sockets divided by a thin septum into two halves, and in the central portion one tooth sockets divided into two small halves. The holes of the tooth sockets are all longer than wide and are surrounded by protruding knobs. In the median portion there are horizontal ridges and in the ventralmost there is a cluster of circular knobs with central depression. Inner side: dorsalmost portion with two tooth sockets divided by a thin septum into two halves longer than wide. From the median to the ventralmost portion there are a series of horizontal ridges.
Dorsal arm plates (Figure 3B): Flabeliform, 1.8 times as wide as long; lateral edges tapered.
Ventral arm plates (Figure 3C): Heptagonal (in dissociated plate), as wide as long, distal portion 1.25 times wider than the proximal one; distal edge straight, proximal edge incised and lateral edges of proximalmost with incisions for the tentacles openings.
Dorsalmost arm spines (Figure 3D): robust and rounded, 2.3 times as long as wide.

*Ophiocoma* sp. NB
Material examined. Samples ZUEC OPH 1538, ZUEC OPH 3085 and ZUEC OPH 3077.
Dental plate (Figure 3E): General outline: long with dorsal portion wider than ventral portion. Outer side: dorsalmost portion with two tooth sockets divided by a thick septum into two halves, and in the central portion one tooth sockets divided into two small halves. The holes of the tooth sockets are all longer than wider and are surrounded by protruding knobs. In the median portion there a horizontal ridge and in the ventralmost there is a cluster of circular knobs with central depression. Inner side: dorsalmost portion with two tooth sockets divided by a thick septum into two halves longer than wide. From the median to the ventralmost portion there are a series of horizontal ridges.
Dorsal arm plates (Figure 3F): Flabeliform, 1.4 times as wide as long; lateral edges rounded.
Ventral arm plates (Figure 3G): Heptagonal (in dissociated plate), as wide as long, distal portion as wider as the proximal one; distal edge rounded, proximal edge incised and lateral edges of proximalmost with incisions for the tentacles openings.
Dorsalmost arm spines (Figure 3H): robust, 3.7 times as long as wide.

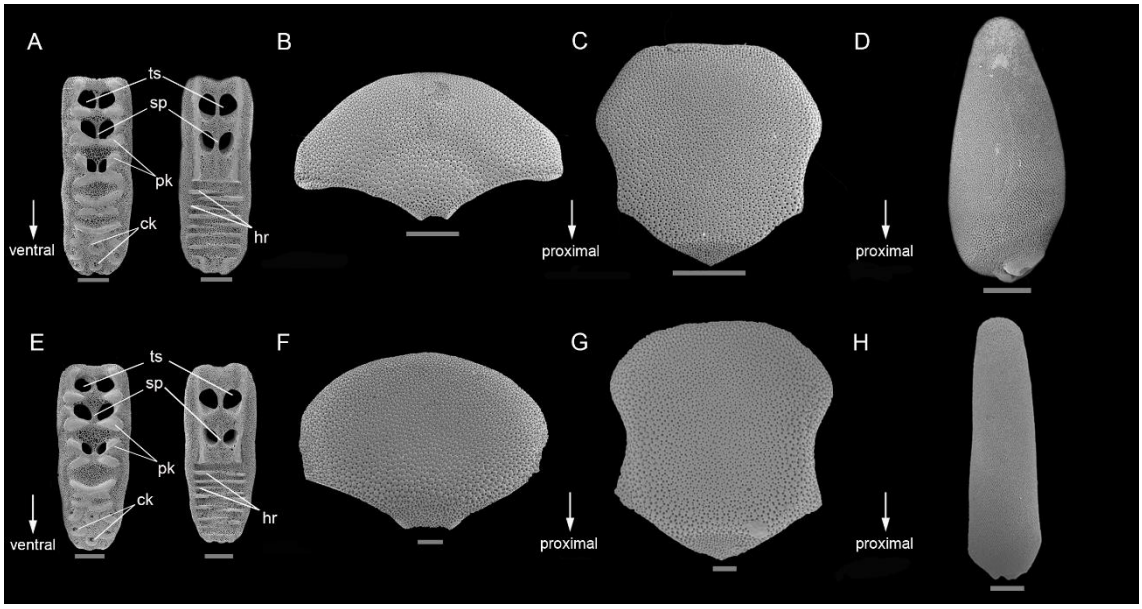


**Fig. 3.** Microstructural characters: dental plate (DP) outer and inner side; dorsal (DAP), and ventral arm plates (VAP); dorsalmost arm spine (DASp). *Ophiocoma* sp. TMV (sample ZUEC OPH 3095, dd = 19.3 mm): (A) DP (outer and inner side); (B) DAP; (C) VAP; (D) DASp. *Ophiocoma* sp. NB (sample ZUEC OPH 1538, dd = 19.3 mm): **(**E) DP (outer and inner side); (F) DAP; (G) VAP; (H) DASp. Abbreviations: ck: circular knobs; hr: horizontal ridge; pk: protruding knobs; sp: septum; ts: tooth sockets. Scale bars: A-E and H, 500 µm; F-G, 200 µm.

In the following, a comparative table of the principal differences of the microstructural characters, between the morphotypes, is presented (Table 4).

**Table 4.** Comparative table of the microstructural differences found between the morphotypes (dorsal and ventral arm plates, dorsalmost arm spines and dental plate).

| **Character** | ***Ophiocoma* sp. TMV** | ***Ophiocoma* sp. NB** |
|---|---|---|
| Dental plate | With dorsal and ventral portion of the same width<br>Thin septum | With dorsal portion wider than ventral portion<br>Thick septum |
| Dorsal arm plates | Almost two times as wide as long<br>With lateral edges tapered | Almost one and a half times as wide as long<br>With lateral edges rounded |
| Ventral arm plates | Distal portion 1.25 times as wide as long the proximal one<br>Distal edge straight | Distal portion as wide as the proximal one<br>Distal edge rounded<br>Pronounced lateral concavities |
| Dorsalmost arm spines | Robust and rounded two times as long as wide | Robust, almost four times as long as wide |

**Morphometry**

A total of 79 specimens of *Ophiocoma* were measured, 63 of TMV and 16 of NB. In order to perform the LDA analysis, a total of 24 morphological characters were used, and such analysis were shown to be effective in discriminating between the two species of *Ophiocoma*. The number of tentacle scales from the first to the fourth arm segment (ts_1, ts_2, ts_3 e ts_4) support the separation of the species, and for that reason it was excluded from the analysis. Only one axis (LD1) was necessary to separate the two morphotypes (Figure 4), and the characters with the highest positive coefficients were the width of second ventral arm plate (vap2_w) and the length of first ventral arm plate (vap1_l), and those with the lowest negative coefficients were the length of oral shield (os_l) and the oral diameter (od).

Morphometrically, *Ophiocoma* sp. NB differs from *Ophiocoma* sp. TMV in having the width of second ventral arm plate (Figure 5A), the length of first ventral arm plate (Figure 5B), the oral diameter (Figure 5C) and the length of oral shield (Figure 5D) greater than *Ophiocoma* sp. TMV.

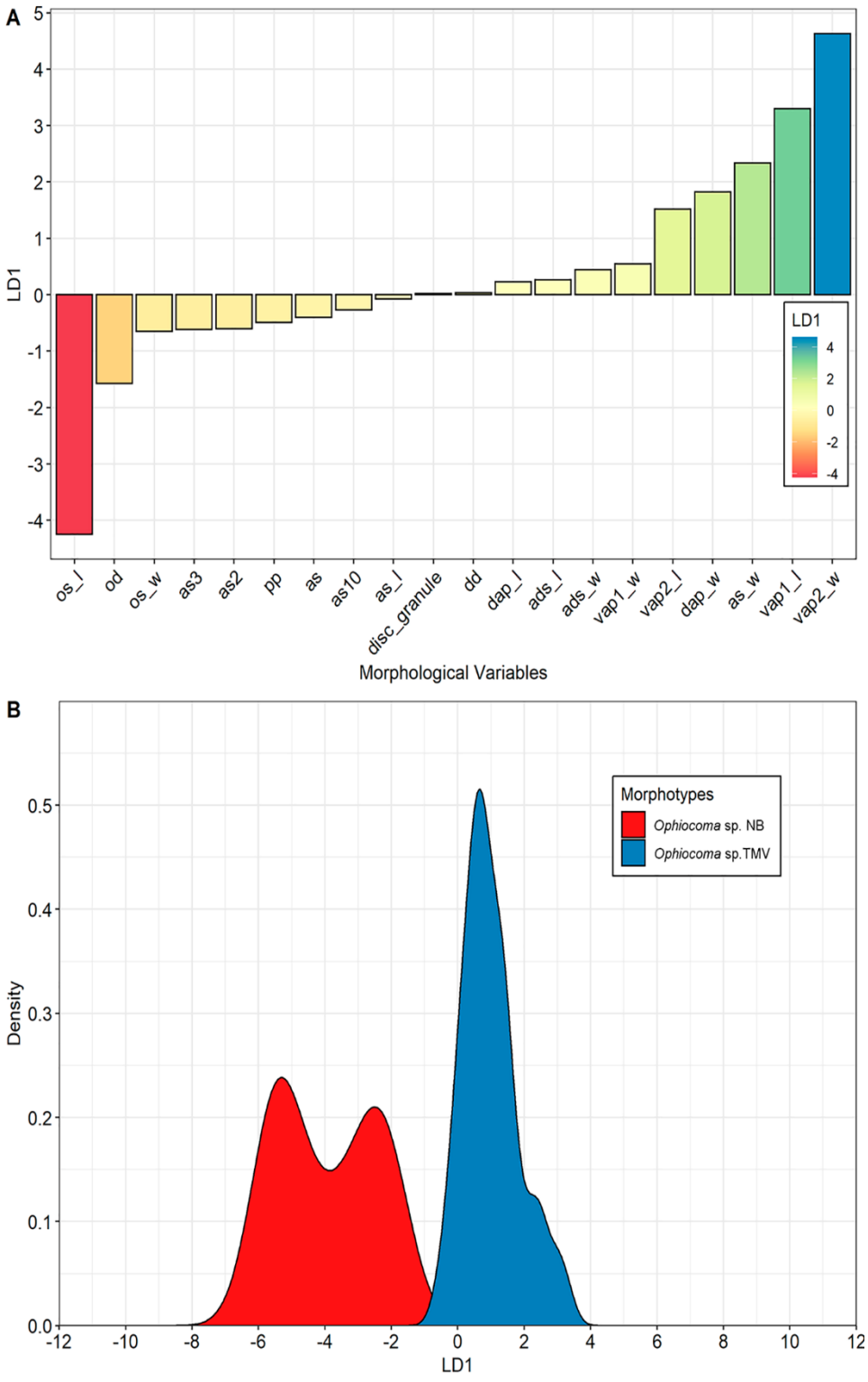


**Fig. 4.** LDA analysis (*Linear Discriminant Analysis*) based on 24 morphological variables (described in Table 1) from two morphotypes: *Ophiocoma* sp. TMV and *Ophiocoma* sp. NB. (A) Histogram of the linear discriminant analysis scores; (B) density plot of the values of the discriminant function for the samples from different morphotypes. Abbreviations: dd: disc diameter; disc_granule: granules of disc; as_l: length of arm spine; as_w: width of arm spine; as: arm spine of first segment; as2: arm spine of second segment; as3: arm spine of third segment; as10: arm spine of tenth segment; od: oral diameter; os_l: length of oral shield; os_w: width of oral shield; ads_l: length of adoral shield; ads_w: width of adoral shield; dap_l: length

of dorsal arm plate; dap_w: width of dorsal arm plate; vap1_l: length of first ventral arm plate; vap1_w: width of first ventral arm plate; vap2_l: length of second ventral arm plate; vap2_w: width of second ventral arm plate; pp: oral papillae.

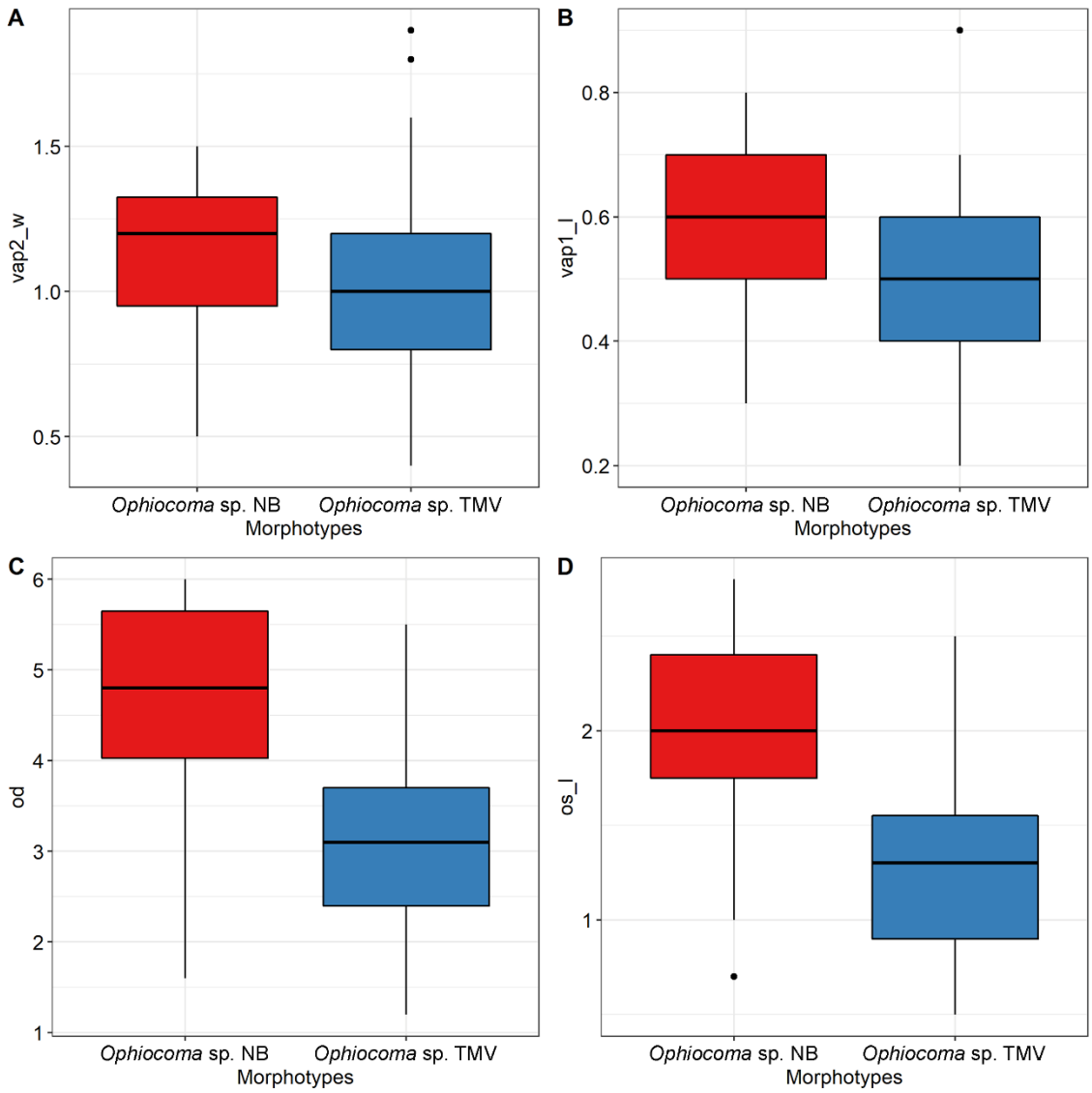


**Fig. 5.** *Boxplots* generated by the LDA analysis of two morphotypes: *Ophiocom*a sp. TMV and *Ophiocoma* sp. NB. *Boxplot* of positive morphological variables: (A) vap2_w e (B) vap1_l; and negative (C) od and (D) os_l. Abbreviations: od: oral diameter; os_l: length of oral shield; vap1_l: length of first ventral arm plate; vap2_w: width of second ventral arm plate.

## Molecular characters

A 361 bp from three individuals were obtained for 16S, two from TMV and one from NB. The phylogenetic reconstruction, obtained from Bayesian Inference by MrBayes analysis, shows two clades, A and B (Figure 6), a similar result to the analysis of Maximum Parsimony (MP).

Clade A (in orange), which contains *Ophiocoma echinata* from the Caribbean Sea and *Ophiocoma* sp. NB, presented a high support value for both the BI and the MP (0.99 and 99%,

respectively). Clade B (in blue), which contains specimens of *Ophiocoma* sp. from the TMV, also showed a high support value for the two analyzes, BI and MP (1 and 99%, respectively).

In BI, the standard deviation of the split frequencies between the two runs reached a value lower than c. 0.005 at two million generations. The analysis of four executions resulted in similar likelihood scores, with ESS> 200, as verified using TRACER. The most suitable model for molecular evolution was SYM + G, chosen based on the Akaike information criterion and Hierarchical Likelihood Ratio Tests.

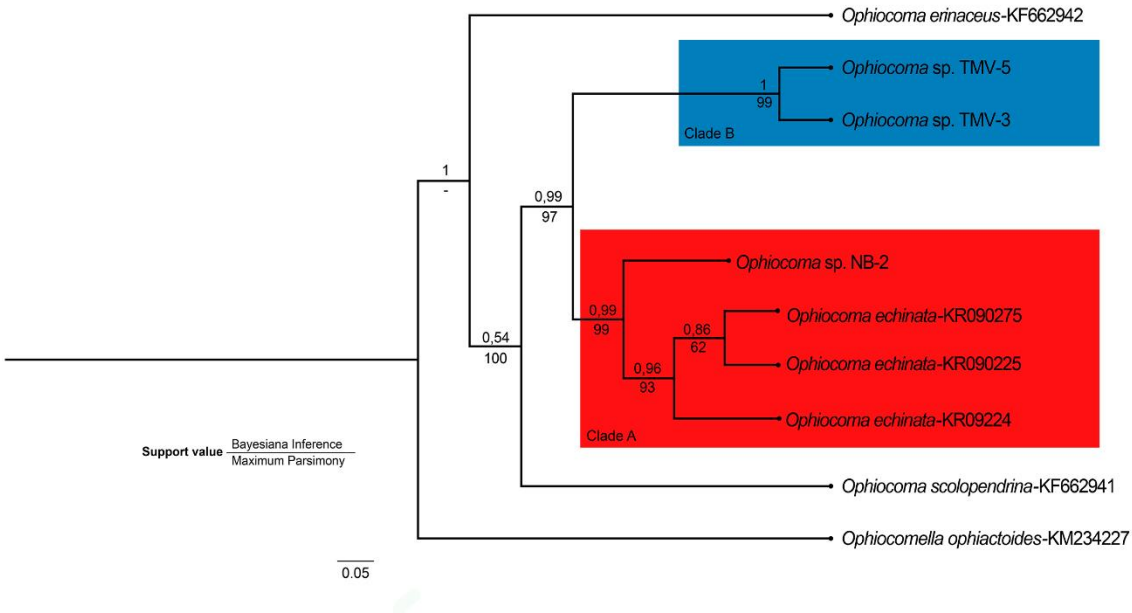


**Fig. 6.** Phylogenetic reconstruction of the 16S gene obtained with Bayesian Inference in the MrBayes analysis. The numbers above the branches represent later probabilities. For clades also inferred in the MP analysis, bootstrap values (%) are provided (below the branches).

The genetic distances within clade A (TMV) were low, 0.77%, while the genetic distance between clades A and B was 12.9-13.1%. These results corroborate those morphological and morphometric analysis in the separation of these two morphotypes. The genetic distances of the 16S sequences are shown in Table 5.

**Table 5.** Genetic distance (%) between and within groups of 16S. Abbreviations: # = value of the genetic distance within the group not calculated because only one sequence was considered in the analysis.

| | **Between groups** | | | | | | **Within groups** |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| 1 *Ophiocoma* sp. TMV | | | | | | | 0.77 |
| 2 *Ophiocoma* sp. NB | 12.9 | | | | | | # |
| 3 *Ophiocoma echinata* - CBS | 13.1 | 3.7 | | | | | 1 |
| 4 *Ophiocoma erinaceus* - RI | 17.6 | 15.7 | 14.9 | | | | # |
| 5 *Ophiocoma scolopendrina* - RI | 18 | 16.6 | 15.9 | 14.5 | | | # |
| 6 *Ophiocomella ophiactoides* - SPSPA | 27.1 | 26.6 | 26.9 | 24.2 | 23.6 | | # |

**Species delimitation**

All studies previously analyzed indicated the separation of the two morphotypes of *Ophiocoma* sp. TMV and *Ophiocoma* sp. NB. Divergences were found between external morphology (number of tentacles scales), microstructural characters (dorsal and ventral arm plates, dental plates and especially dorsalmost arm spine), morphometry and molecular data (16S and genetic distances). Such results obtained corroborate the initial hypothesis of the existence of two species of *Ophiocoma* in the Brazilian coast, being *O*. sp. TMV a new species for science and *O*. sp. NB corresponding to *Ophiocoma echinata*.

In the following, we present the detailed descriptions of the new species, named as *Ophiocoma trindadensis*, and a redescription of *Ophiocoma echinata*.

SYSTEMATICS

Order OPHIACANTHIDA O'Hara, Hugall, Thuy, Stöhr & Martynov, 2017

Family OPHIOCOMIDAE Ljungman, 1867

Genus *Ophiocoma* L. Agassiz, 1836

*Ophiocoma trindadensis* Serrano; Damiano; Alitto; Borges sp. nov.

(Figures 7, 8 and 9)

*Type locality.* Trindade and Martin Vaz Archipelago, ES, BRAZIL.

*Size range.* dd: 1.6 – 19.3 mm.

*Material examined.* 91 specimens (dd: 1.6 – 19.4 mm). See Table S1.

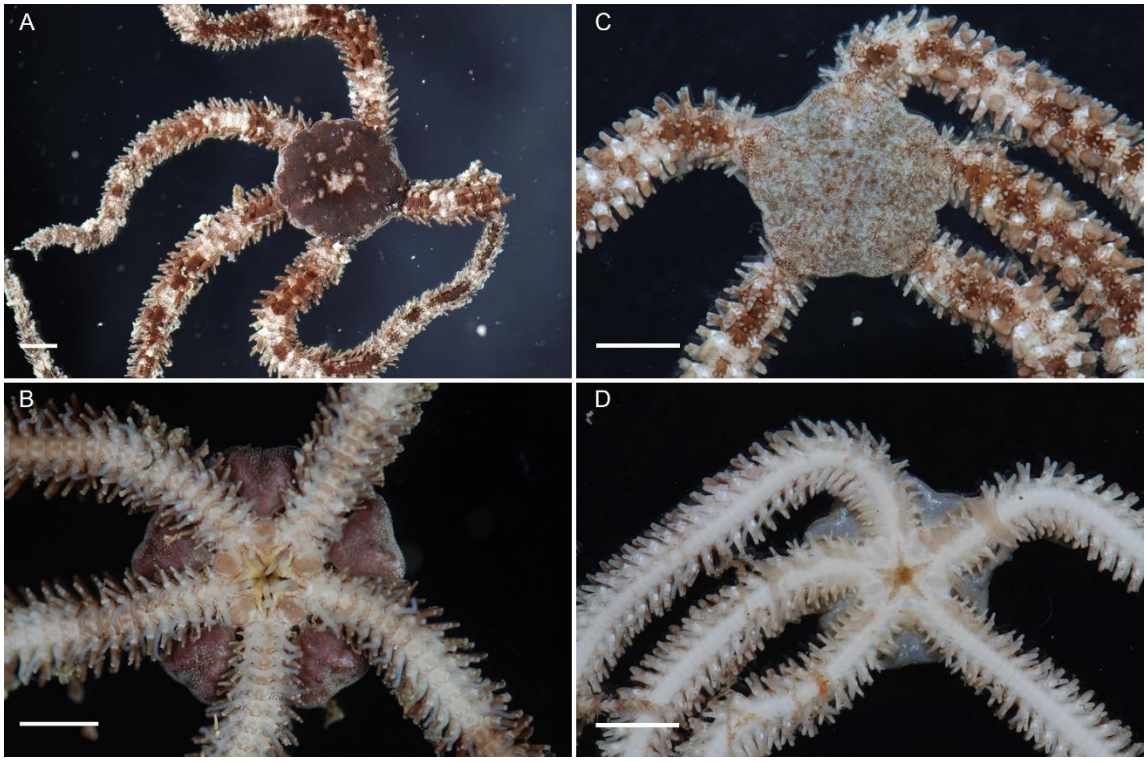


**Fig. 7.** *Ophiocoma trindadensis* sp. nov. in vivo. (A and B) paratype sample MZUSP 1909 (17.7 mm dd). (C and D) paratype sample MZUSP 1916 (12.1 mm dd). (A) and (C) Dorsal view; (B) and (D) Ventral view. Scale bars: A - D, 5 mm. All preserved in ethanol.

*Holotype*: adult 19.4 mm dd; preserved in 70% ethanol. (Trindade Island, Ponta da Calheta, Espírito Santo, Brazil; coordinates: 20º30'18.72"S 29º18'31.67"W; water depth: 17.7 m) (MZUSP 1973); coll. Joel B. Mendonça, 03 August 2015.

*Paratypes*: 8.5 mm dd; preserved in 70% ethanol. (Trindade Island, Parcel Tartarugas, Espírito Santo, Brazil; coordinates: 20º31'01.3"S 29º17'56.9"W; water depth: 9.8 m) (ZUEC OPH 3066); coll. Joel B. Mendonça, 30 April 2014. 9.7 mm dd; preserved in 70% ethanol. (Trindade Island, Parcel Tartarugas, Espírito Santo, Brazil; coordinates: 20º31'01.3"S 29º17'56.9"W; water depth: 9.8 m) (ZUEC OPH 3066); coll. Joel B. Mendonça, 30 April 2014. 9.1 mm; preserved in 70% ethanol. (Trindade Island, Parcel Tartarugas, Espírito Santo, Brazil; coordinates: 20º31'01.3"S 29º17'56.9"W; water depth: 9.8 m) (ZUEC OPH 3066); coll. Joel B. Mendonça, 30 April 2014. 12.6 mm dd; preserved in 70% ethanol. (Trindade Island, Parcel Tartarugas, Espírito Santo, Brazil; coordinates: 20º31'01.3"S 29º17'56.9"W; water depth: 9.8 m) (ZUEC OPH 3066); coll. Joel B. Mendonça, 30 April 2014. 12.1 mm dd; preserved in 70% ethanol. (Trindade Island, Ponta da Calheta, Espírito Santos, Brazil; coordinates: 20º30'18.72"S 29º18'31.67"W; water depth: 9.9 m) (MZUSP 1916); coll. Joel B. Mendonça, 26 October 2014. 10.9 mm dd; preserved in 70% ethanol. (Trindade Island, Enseada da Cachoeira, Espírito Santos, Brazil; coordinates: 20º30'55.6"S 29º20'1.7"W; water depth: 12 m)

(MZUSP 2747); coll. Joel B. Mendonça, 12 July 2012. 17.7 mm dd; preserved in 70% ethanol. (Trindade Island, Farrilhões, Espírito Santo, Brazil; coordinates: 20º31’22.4”S 29º19’52.0”W; water depth: 12.2 m) (MZUSP 1909); coll. Joel B. Mendonça, 4 April 2014. 8.7 mm dd; preserved in 70% ethanol. (Trindade Island, Farrilhões, Espírito Santo, Brazil; coordinates: 20º31’22.4”S 29º19’52.0”W; water depth: 12.2 m) (MZUSP 2757); coll. Joel B. Mendonça, 4 April 2014. 3.7 mm dd; preserved in 70% ethanol. (Trindade Island, Ponta da Calheta – Atrator de Fauna, Espírito Santo Brazil; coordinates: 20º30’18.72”S 29º18’31.6”W; water depth: 17 m) (MZUSP 2534); coll. Joel B. Mendonça, 24 November 2019. 8 mm dd; tissues removed and ossicles arranged on SEM stubs. (Trindade Island, Ponta da Calheta – Atrator de Fauna, Espírito Santo Brazil; coordinates: 20º30’18.72”S 29º18’31.6”W; water depth: 17 m) (MZUSP 2772); coll. Joel B. Mendonça, 24 November 2019. 1.6 mm dd; tissues removed and ossicles arranged on SEM stubs. (Trindade Island, Ponta da Calheta – Atrator de Fauna, Espírito Santo Brazil; coordinates: 20º30’18.72”S 29º18’31.6”W; water depth: 17 m) (MZUSP 2773); coll. Joel B. Mendonça, 24 November 2019. 3.9 mm dd; preserved in 70% ethanol. (Trindade Island, Praia do Noroeste, Espírito Santo, Brazil; coordinate: 20º29’46.4”S 29º20’35.4”W; water depth: 9.4 m) (MZUSP 2526); coll. Joel B. Mendonça, 28 November 2017. 5.1 mm dd; preserved in 70% ethanol. (Trindade Island, Praia do Noroeste, Espírito Santo, Brazil; coordinate: 20º29’46.4”S 29º20’35.4”W; water depth: 9.4 m) (MZUSP 2774); coll. Joel B. Mendonça, 28 November 2017. 2.9 mm dd; preserved in 70% ethanol. (Trindade Island, Praia do Noroeste, Espírito Santo, Brazil; coordinate: 20º29’46.4”S 29º20’35.4”W; water depth: 9.4 m) (MZUSP 2775); coll. Joel B. Mendonça, 28 November 2017. 3.1 mm dd; preserved in 70% ethanol. (Trindade Island, Praia do Noroeste, Espírito Santo, Brazil; coordinate: 20º29’46.4”S 29º20’35.4”W; water depth: 9.4 m) (MZUSP 2776); coll. Joel B. Mendonça, 28 November 2017. 11 mm dd; preserved in 70% ethanol. (Trindade Island, Praia do Noroeste, Espírito Santo, Brazil; coordinate: 20º29’46.4”S 29º20’35.4”W; water depth: 9.4 m) (MZUSP 2777); coll. Joel B. Mendonça, 28 November 2017. 9.3 mm dd; preserved in 70% ethanol. (Trindade Island, Enseada da Cachoeira, Espírito Santo, Brazil; coordinate: 20º30’53.8”S 29º20’19.2”W; water depth: 15 m) (ZUEC OPH 3098); coll. Joel B. Mendonça, 08 July 2013. 10 mm dd; preserved in 70% ethanol. (Martin Vaz Archipelago, Martin Vaz Island, Espírito Santo, Brazil; coordinate: 20º30’45.7”S 29º18’21.9”W; water depth: 13 m) (ZUEC OPH 3101); coll. Joel B. Mendonça, 23 July 2013. 5.1mm dd; preserved in 70% ethanol. (Trindade Island, Enseada Portuguesa, Espírito Santo, Brazil; coordinate: 20º30’20”S 29º18’43.7”W; water depth: 12.2 m) (MZUSP 2746); coll. Joel B. Mendonça, 18 July 2013. 11.3mm dd; preserved in 70%

ethanol. (Trindade Island, Enseada Portuguesa, Espírito Santo, Brazil; coordinate: 20º30’20”S 29º18’43.7”W; water depth: 12.2 m) (MZUSP 2745); coll. Joel B. Mendonça, 18 July 2013.

*Diagnosis*

Disc and radial shields completely covered by sparse and rounded granules. Dorsalmost arm spines as wide as long or almost as wide as long, rounded and robust. Dorsal arm plates contiguous, overlapping proximally. Two ovals tentacle scales on each pore of the first arm segment, then one.

*Description*

Disc. (dd: 19.3 mm): circular, completely covered dorsally by sparse and rounded granules, extending ventrally. About 13 granules per mm² (Figure 8A).

Mouth. Oral shields (OSh) rectangular with rounded edges, 1.2 times as long as wide. Adoral shield (AdSh) triangular, twice as wide as long, separated proximally (Figure 8C, D). In each half jaw a line of five papillae: Lyman’s ossicle (LyOs) large, partially covered by a triangular adoral shield spine (AdShSp), 1.3 times as long as wide; secondary adoral shield spine (2º AdShSp) semi triangular, 1.2 times as long as wide; infradental papilla (IPa) oval, 1.7 times as long as wide; at oral plate edge, tooth papilla (TPa), oval and shorter than the other papillae.

Arms. Dorsal arm plates (DAP) contiguous, overlapping proximally. Ventral arm plates (VAP) hexagonal. First ventral arm plate (VAP-1) with the proximal edge covered by LyOs (Figure 8C). VAP-2, 1.5 times as wide as long, distally concave with an angular lateral edge on both sides. Lateral edge of other VAPs with concavity and a convex/rounded distal edge (Figure 8F). The number of arm spines on each side, from the first arm segment until 21st, are arranged in the following sequence:

4 4 4 3 4 3 3 4 3 4 3 4 3 4 3 3 3 3 4 3 3
4 4 3 4 4 3 4 3 4 3 4 3 4 3 4 4 3 4 3 3 4 } Arm 1

4 3 4 3 4 3 4 3 4 3 3 4 3 4 3 3 4 3 3 3 3
4 4 3 4 3 4 3 4 3 4 3 3 4 3 3 4 3 4 3 4 3 } Arm 2

4 3 4 3 3 4 3 3 4 3 4 3 4 4 3 4 3 4 3 4 3
4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 3 4 } Arm 3

4 4 3 4 3 4 3 4 3 4 3 4 3 4 3 3 4 3 4 3 3
4 4 4 3 4 3 4 3 4 3 4 3 4 3 3 4 3 4 3 4 3 } Arm 4

4 4 5 3 4 3 4 3 4 3 4 3 3 3 3 3 4 3 3 4 3
4 4 3 4 3 4 3 4 4 3 4 3 4 3 3 4 3 4 4 3 3 } Arm 5

Three to four arm spines. The number of spines alternates in the same arm segment (on one side with three, on the other side with four). Among 3rd and 21st arm segments the dorsalmost arm spines (DASp) are more robust and rounded (Figure 8E), as wide as long or almost as wide as long (with 2.2 mm of length and 1.5 mm of width) in segments with four spines. Two ovals tentacle scales (TSc) on each pore of the first arm segment and only one in the rest (Figure 8F). Morphological variations. Granules: the larger specimens (19.2 and 19.3 mm dd) can present 4-13 granules per mm² in the center of the disc, while the smallest specimens (1.6 to 4.8 mm dd) can present 61-105 granules per mm². Arm spines: some specimens have dorsalmost spines more rounded and bigger than others, and ventralmost spines bifid and flattened. Tentacle scales (TSc): the number can vary on the same individual and between specimens, in approximately 35% it can present one and two TSc on the first arm segment, and 65% only two. Approximately 5 % have two TSc on the second arm segment.

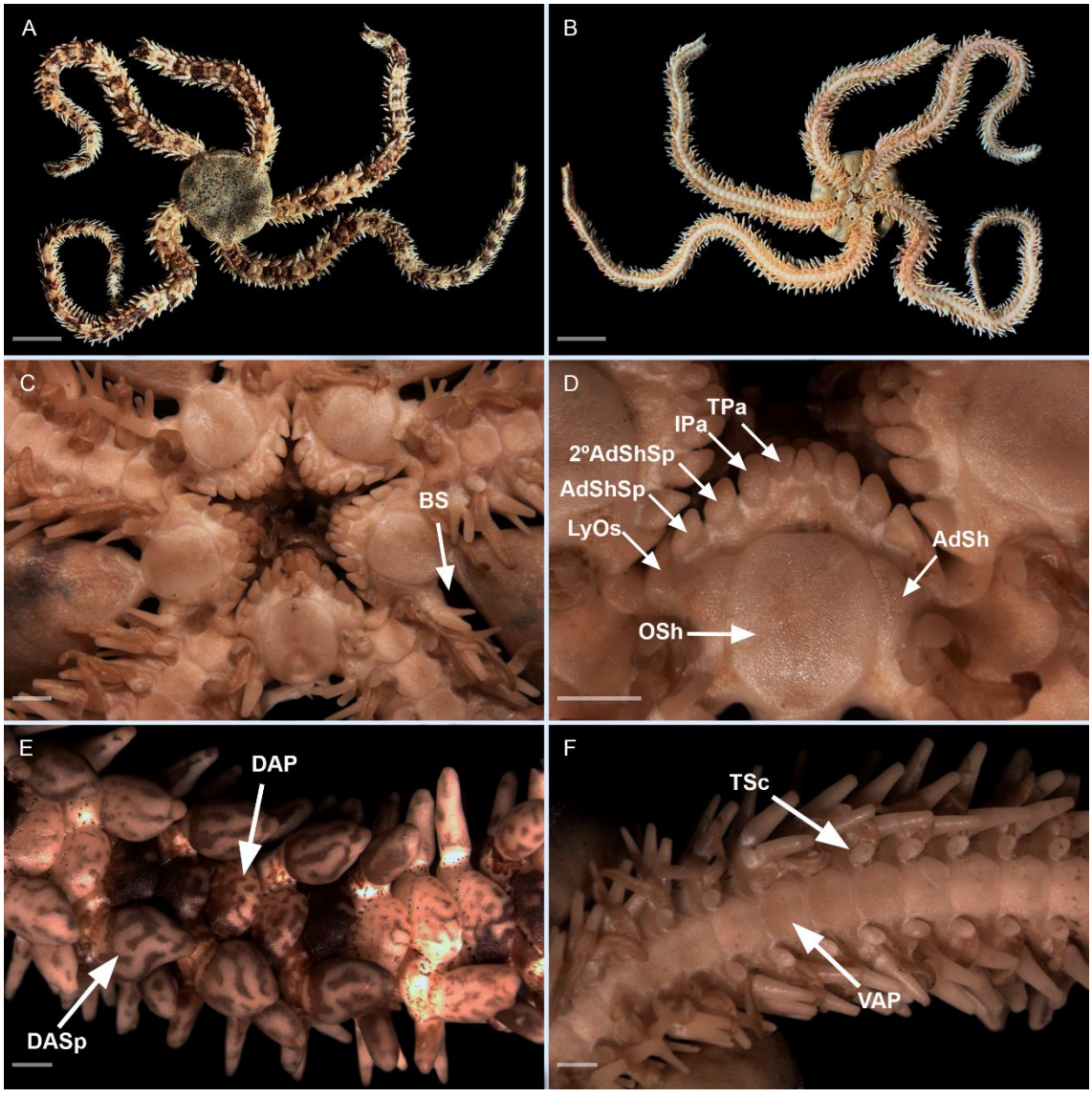

**Fig. 8.** *Ophiocoma trindadensis* sp. nov. Sample ZUEC OPH 3095 (19.3 mm dd). (A) Dorsal view; (B) Ventral view; (C) Detail of the oral view; (D) Detail of the jaw; (E) Detail of dorsal arm; (F) Detail of ventral arm. Abbreviations: 2º AdShSp: secondary adoral shield spine; AdSh: adoral shield; AdShSp: adoral shield spine; BS: bursal slits; DAP: dorsal arm plate; DASp: dorsalmost arm spine; IPa: infradental papilla; LyOs: Lyman's ossicle; Osh: oral shield; TPa: tooth papilla; TSc: tentacle scale; VAP: ventral arm plate. Scale bar: A and B, 10 mm; C-F, 1 mm.

*Microstructural ossicles* (Figure 9)

Material examined. Sample ZUEC OPH 3095 (dd: 19.3 mm).

Radial shield. Abradial distal edge incise (Figure 9A).

Genital plates. Adradial plate: long, thin and with a distal spacing (Figure 9B). Abradial plate: longer than half the length of the adradial plate; with the shape of a longitudinally curved blade (Figure 9C).

Dental plate. General outline: long with dorsal and ventral portion of the same width. Outer side: dorsalmost portion with two tooth sockets divided by a thin septum into two halves, and in the central portion one tooth sockets divided into two small halves. The holes of the tooth sockets are all longer than wide and are surrounded by protruding knobs. In the median portion there are horizontal ridges and in the ventralmost there is a cluster of circular knobs with central depression. Inner side: dorsalmost portion with two tooth sockets divided by a thin septum into two halves longer than wide. From the median to the ventralmost portion there are a series of horizontal ridges (Figure 9D).

Oral plate. General outline: 1.2 times as long as wide. Abradial: presents a large and well-defined muscle flange with horizontal and diagonal striations. Adradial: muscle attachment area is arranged vertically in the distal region of the oral plate, forming a set of strong folds (Figure 9E).

Dorsal arm plates. Flabeliform, 1.8 times as wide as long; lateral edges tapered (Figure 9F).

Ventral arm plates. Heptagonal (in dissociated plate), as wide as long, distal portion 1.25 times as wide as long the proximal one; distal edge straight, proximal edge incised and lateral edges of proximalmost with incisions for the tentacles openings (Figure 9G).

Dorsal arm spine. Almost two times as wide as long, with lateral edges tapered (Figure 9H).

Vertebrae. Zygospondylous vertebrae. Proximal side with two articular knobs and massive stereom, positioned in the middle of dorsal portion (Figure 9I). Distal side with zygosphene fused with pair of parallel zygocondyles (Figure 9J). Dorsal side with a groove not projecting beyond the zygocondyles (Figure 9K). Ventral side with a strong depression in the groove upper

proximal half, a "V" cavity in the proximal edge, and zygosphene not projecting beyond ventral edge of zygocondyles (Figure 9L).

Lateral arm plates. General outline: arched (wrapped around the arm). Spine articulations: four, all on same level as the outer side; dorsalmost knobs larger than others, because supports the more robust spine (Figure 9M). Shape: "c" like.

Morphological variations. Dorsal arm plate: some DAP can be irregular, with one of edges longer than the other. Dental plate: can present two or three tooth sockets divided by a septum into two halves.

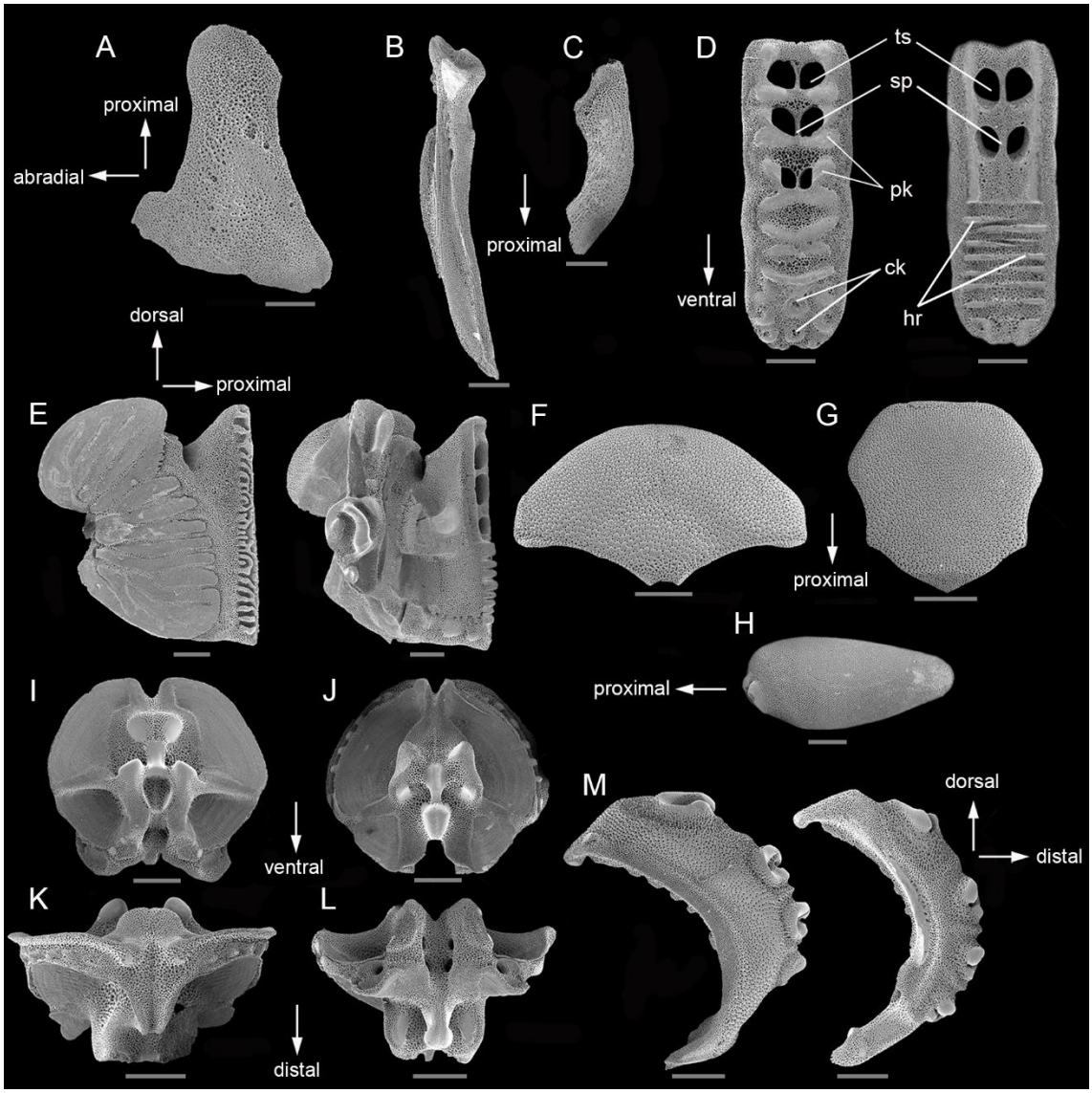


**Fig. 9.** Microstructural characters of *Ophiocoma trindadensis* sp. nov. Sample ZUEC OPH 3095 (19.3 mm dd): (A) Radial shield – outer side; (B) Genital plate – adradial; (C) Genital plate – abradial; (D) Dental plate – outer and inner side; (E) Oral plate – abradial and adradial; (F) Dorsal arm plate – outer side; (G) Ventral arm plate – outer side; (H) Dorsal arm spine; (I) Vertebrae ossicle – proximal surface; (J) Vertebrae ossicle – distal surface; (K) Vertebrae ossicle – dorsal surface; (L) Vertebrae ossicle – ventral surface; (M) Lateral arm plate – outer

and inner side. Abbreviations: ck: circular knobs; hr: horizontal ridge; pk: protruding knobs; sp: septum; ts: tooth sockets. Scale bars: A–M, 500 µm.

*Etymology*

The species name '*trindadensis*' corresponds to the location where the species was collected, and it also corresponds to the name given by Tommasi (1970) for the species of *Ophiothrix trindadensis* from Trindade and Martin Vaz Archipelago.

*Ecology and distribution*

*Ophiocoma trindadensis* sp. nov. was collected from rubble bottom. It has also been sampled in corals and associated with calcareous. The present study samples occurred at depths ranging from 8 to 23.5 m, only at the Trindade and Martin Vaz Archipelago.

*Ophiocoma echinata* (Lamark, 1816)

(Figures 10 and 11)

*Type locality.* Antilles

*Size range.* dd: 4.9 – 22.7 mm.

*Material examined.* 16 specimens (dd: 4.9 – 22.7 mm). See Table S1.

*Diagnosis*

Disc and radial shields completely covered by sparse and rounded granules. Dorsalmost arm spines rounded, longer than wide. Dorsal arm plates not contiguous. Two ovals tentacle scales on each pore, except in the final third of the arm.

*Description*

Disc. (dd: 19.3 mm): circular, completely covered dorsally by sparse and rounded granules, extending ventrally. About 15 granules per mm² (Figure 10A).

Mouth. Oral shields (OSh) rectangular with angular margins, 1.2 times as long as wide. Adoral shield triangular, 1.8 times as wide as long, separated proximally (Figure 10C, D). In each half jaw a line of five papillae: Lyman's ossicle (LyOs) large, 1.5-1.8 times as long as wide; adoral shield spine (AdShSp) triangular 1.4 times as long as wide; secondary adoral shield spine (2º AdShSp) elongate 2.2 times as long as wide; infradental papilla (IPa) elongate 1.6 times as long as wide; at oral plate edge, a tooth papilla (TPa), oval and shorter than the other papillae.

Arms. Dorsal arm plates (DAP) not contiguous. Ventral arm plates (VAP) hexagonal. First ventral arm plate (VAP-1) with the proximal edge covered by LyOs (Figure 10C). VAP-2, almost as long as wide, distally concave with an angular lateral edge on both sides. Lateral edge of other VAPs with concavity and a convex/rounded distal edge (Figure 10F). The number of arm spines on each side, from the first arm segment until 21st, are arranged in the following sequence:

4 4 3 4 3 4 3 3 4 3 3 4 4 3 4 3 4 3 4 3 3
4 4 4 3 4 3 4 3 4 3 4 3 4 3 3 4 3 3 4 3 4 } Arm 1

4 5 3 4 3 4 3 4 4 3 4 3 3 4 3 3 4 3 4 3 3
5 4 4 3 4 3 4 3 4 3 4 3 4 3 3 4 3 4 3 3 4 } Arm 2

5 4 4 3 4 3 4 4 3 4 3 3 4 3 4 3 3 3 4 3 3
4 4 3 4 3 4 4 3 4 3 3 4 3 4 3 4 3 3 4 3 4 } Arm 3

4 5 3 4 3 4 3 4 3 3 4 3 4 3 3 4 3 4 3 3 4
5 4 4 3 4 3 4 3 3 4 3 3 4 3 4 3 3 4 3 4 3 } Arm 4

For counting the number of dorsal arm spines, another specimen of similar size (19.5 mm dd) was used, which had the most conserved arms. The specimen used has only four entire arms. Three to five cylindrical arm spines. The number of spines alternates in the same arm segment (on one side with three, on the other side with four). Among 5º and 14º arm segments the dorsalmost arm spines (DASp) are more robust (Figure 10E), longer than wide (with 3.4 mm of length and 1.5 mm of width) in segments with four spines. Two and three ovals tentacle scales (TSc) on each pore, except in the final third of the arm (Figure 10F)

Morphological variations. Granules: the larger specimens (22.6 and 22.7 mm dd) can present 10-13 granules per mm² in the center of the disc, while the smallest specimens (4.9 and 8.2 mm dd) can present 27-38 granules per mm². Tentacle scales (TSc): in 88% of specimens there are two ovals TSc on the first arm segment, 6% present three and in approximately 6% present four TSc on the first arm segment.

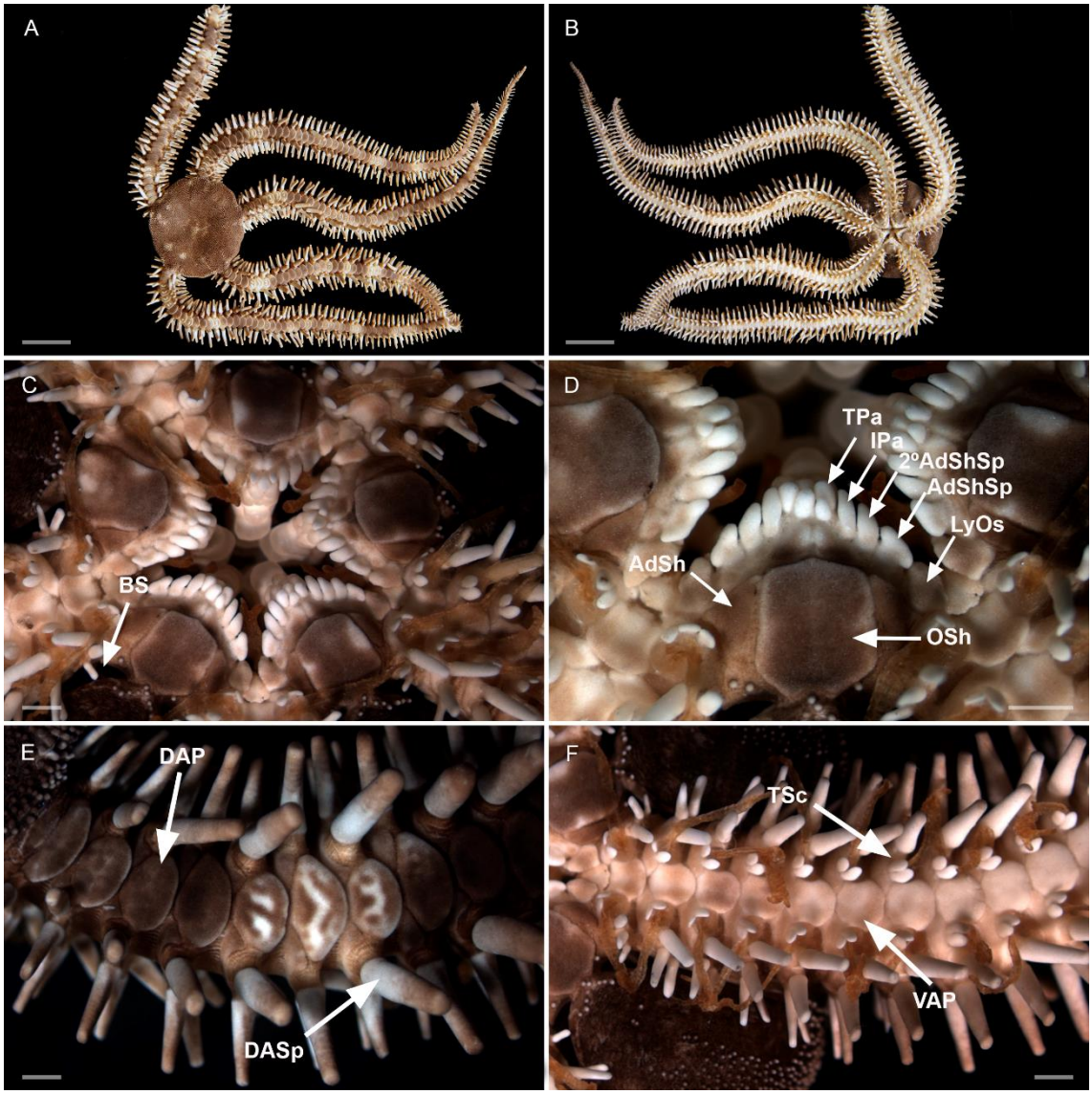


**Fig. 10.** *Ophiocoma echinata*. Sample ZUEC OPH 1538 (19.3 mm dd). (A) Dorsal view; (B) Ventral view; (C) Detail of the oral view; (D) Detail of the jaw; (E) Detail of dorsal arm; (F) Detail of ventral arm. Abbreviations: 2º AdShSp: secondary adoral shield spine; AdSh: adoral shield; AdShSp: adoral shield spine; BS: bursal slits; DAP: dorsal arm plate; DASp: dorsalmost arm spine; IPa: infradental papilla; LyOs: Lyman's ossicle; Osh: oral shield; TPa: tooth papilla; TSc: tentacle scale; VAP: ventral arm plate. Scale bars: A and B, 10 mm; C-F 1 mm.

*Microstructural ossicles* (Figure 11)

Material examined. Sample ZUEC OPH 1538 (dd: 19.3 mm)

Radial shield. Abradial distal edge incise (Figure 11A).

Genital plates. Adradial plate: long, thin and with a distal spacing (Figure 11B). Abradial plate: longer than half the length of the adradial plate; with the shape of a longitudinally curved blade (Figure 11C).

Dental plate. General outline: long with dorsal portion wider than ventral portion. Outer side: dorsalmost portion with two tooth sockets divided by a thick septum into two halves, and in the central portion one tooth sockets divided into two small halves. The holes of the tooth sockets are all longer than wide and are surrounded by protruding knobs. In the median portion there is a horizontal ridge and in the ventralmost there is a cluster of circular knobs with central depression. Inner side: dorsalmost portion with two tooth sockets divided by a thick septum into two halves longer than wide. From the median to the ventralmost portion there are a series of horizontal ridges (Figure 11D).

Oral plate. General outline: 1.3 times as long as wide. Abradial: presents a large and well-defined muscle flange with horizontal and diagonal striations. Adradial: muscle attachment area is arranged vertically in the distal region of the oral plate, forming a set of strong folds (Figure 11E).

Dorsal arm plates. Flabeliform, 1.4 times as wide as long; lateral edges rounded (Figure 11F).

Ventral arm plates. Heptagonal (in dissociated plate), as wide as long, distal portion as wider as the proximal one; distal edge rounded, proximal edge incised and lateral edges of proximalmost with incisions for the tentacles openings (Figure 11G).

Dorsal arm spine. Robust, almost four times as long as wide (Figure 11H).

Vertebrae. Zygospondylous vertebrae. Proximal side with two articular knobs and massive stereoma, positioned in the middle of dorsal portion (Figure 11I). Distal side with zygosphene fused with a pair of parallel zygocondyles (Figure 11J). Dorsal side with a groove not projecting beyond the zygocondyles (Figure 11K). Ventral side with a strong depression in the groove upper proximal half, a "V" cavity in the proximal edge, and zygosphene not projecting beyond ventral edge of zygocondyles (Figure 11L).

Lateral arm plates. General outline: Arched (wrapped around the arm). Spine articulations: four, all on same level as the outer side; dorsalmost knobs larger than others, because supports the more robust spine (Figure 11M). Shape: “c” like.

Morphological variations. Dorsal arm plate: some DAP have lateral edges more elongated than rounded. Dental plate: with two or three tooth sockets divided by a septum into two halves.

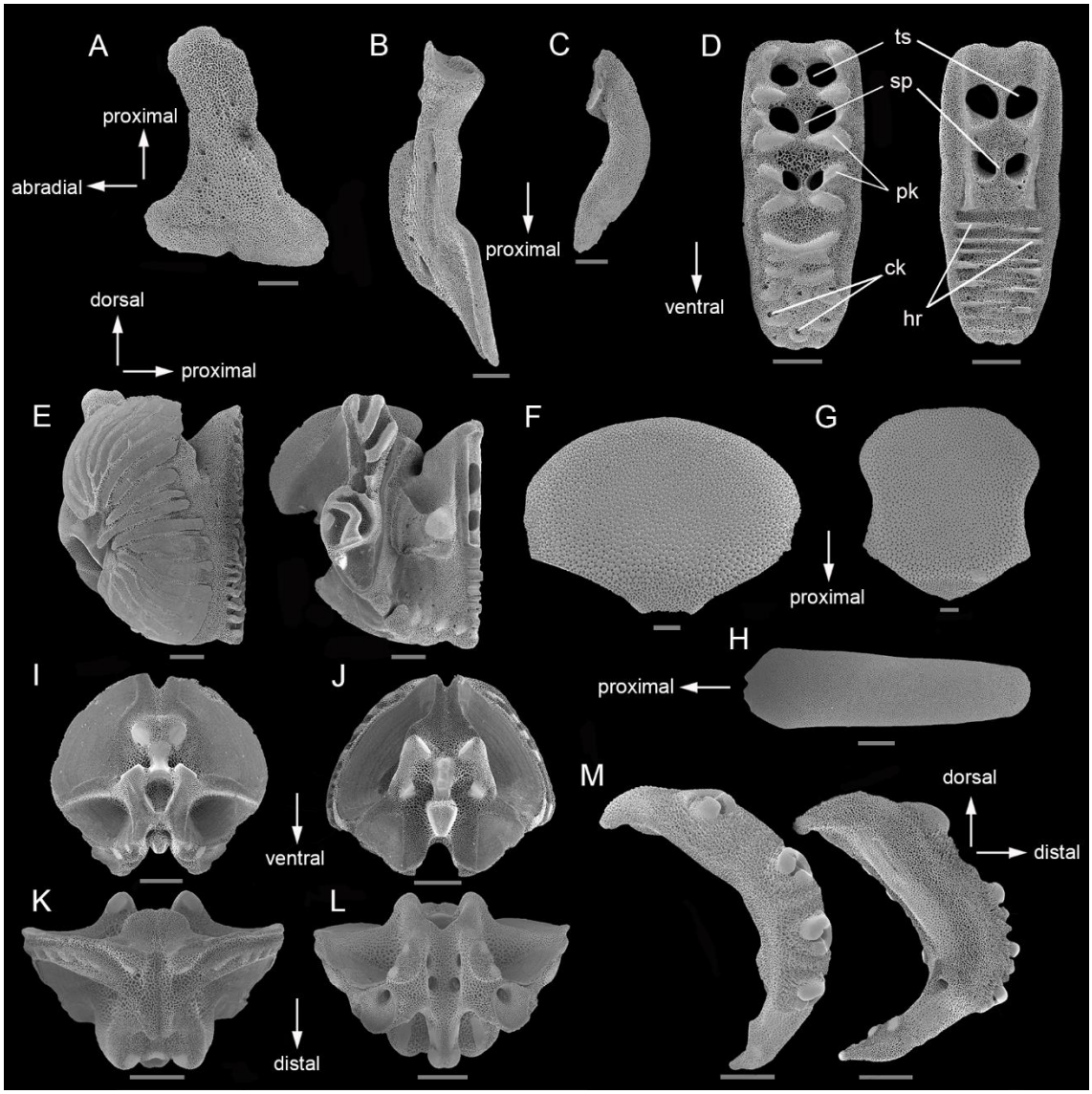


**Fig. 11.** Microstructural characters of *Ophiocoma echinata*. Sample ZUEC OPH 1538 (19.3mm dd): (A) Radial shield – outer side; (B) Genital plate – adradial; (C) Genital plate – abradial; (D) Dental plate – outer and inner side; (E) Oral plate – abradial and Oral plate – adradial; (F) Dorsal arm plate – outer side; (G) Ventral arm plate – outer side; (H) Dorsal arm spine; (I) Vertebrae ossicle – proximal surface; (J) Vertebrae ossicle – distal surface; (K) Vertebrae ossicle – dorsal surface; (L) Vertebrae ossicle – ventral surface; (M) Lateral arm plate – outer and inner side. Abbreviations: ck: circular knobs; hr: horizontal ridge; pk: protruding knobs; sp: septum; ts: tooth sockets. Scale bar: A-E and I-M, 500 µm; F and G, 200µm.

*Ecology and distribution*

The present study samples were collected in Itapuã beach, from exposed reefs at depths of 10 cm. Species from other localities occurred at depths from 0.1 to 49 m, occurring on substrates such as coral and sandstone reefs. In Brazil: Bahia (Tommasi, 1970; Manso *et al*., 2008), Ceará (Albuquerque, 1986), Pernambuco (Tommasi, 1970; Lima & Fernandes, 2009), Alagoas (Miranda *et al*., 2012) and Paraíba (Rathbun, 1879). Atlantic Ocean: From the Florida to Brazil (Tommasi, 1970).

DISCUSSION

As a starting point, two morphotypes of *Ophiocoma* were characterized from initial morphological analyzes, occurring in Brazil: *Ophiocoma* sp. NB (Northeastern Brazil) and *Ophiocoma* sp. TMV (Trindade and Martin Vaz Archipelago). To investigate whether the two morphotypes corresponded to one or two species, an integrative analysis of morphological (external morphology, morphometry and microstructural characters) and molecular characters (16S) was performed. All data sets suggest that the analysed specimens belong to two distinct species: *Ophiocoma echinata* from NB and *Ophiocoma trindadensis* sp. nov. from TMV. All the specimens of *Ophiocoma echinata* from NB were compared with *O. echinata* from the type locality, belonging to the collection of Ophiuroidea of the ZUEC.

Ecologically, the species *Ophiocoma echinata* and *O. trindadensis*, were found in the similar types of substrates and also in the same bathymetric distribution. However, the geographic distribution of the species is distinct, since *O. echinata* is a restricted species from the continental coastal region, in which it is widely distributed (Ceará to Bahia), while *O. trindadensis* is exclusive to the remote oceanic archipelago of Trindade and Martin Vaz.

Although *Ophiocoma echinata* is the only species of the genus that occurs on the Brazilian coast, the analyzed specimens of *Ophiocoma trindadensis* sp. nov. do not correspond morphologically with the species in question. However, much these species share, spherical granular cover and thick and evident spines (Lamark, 1816), they differ in number of tentacle scales, size and shape of the dorsalmost arm spines. According to Say, T. (1825) *Ophiocoma echinata* has two oval and equal tentacles scales along the entire arm, dilated dorsalmost arm spines, which are almost oval. Unlike *Ophiocoma echinata*, *Ophiocoma trindadensis* sp. nov. has two tentacles scales in the first arm segment and only one in the others, besides very robust dorsalmost arm spines, which are more rounded than *Ophiocoma echinata*.

As much as the dorsalmost arm spines of *Ophiocoma anaglyptica* be described as swollen and enlarged, they are characterized as bottleshaped, expanded in the middle but slighty compressed, narrowing abruptly to form a short neck (Ely, 1944). In addition, this species from the Pacific Ocean has flattened disc granules, and two tentacle scales on all, except for the first segments, which can support up to three tentacle scales (Ely, 1944; Devaney, 1970). Lyman, T. (1861), describes *Ophiocoma molaris* (synonymized as *Ophiocoma scolopendrina*), with two tentacle scales in the first arm segment and then one, but dorsalmost arm spines slender, longest and tapering, being three to three and a half times as long as dorsal arm plates. The description of *Ophiocoma erinaceus* does not indicate similarities with *O. trindadensis*, since it has two

tentacle scales and bright red tubular feet in live, and the same is true for *Ophiocoma cynthiae*, which has two tentacle scales (Müller & Troschel, 1842; Benavides-Serrato & O'Hara, 2008). However, *Ophiocoma schoenleinii* and *Ophiocoma aethiops* have a single tentacle scale, except in the first segments of the arm (Muller & Troschel, 1842; Matsumoto, 1917; Devaney, 1970; Kœhler, 1922) both do not have dilated dorsalmost arm spines as *O. trindadensis* (Fatemi *et al*., 2010; Kœhler, 1922). In addition, *O. scheonleinii* occurs in the Indian Ocean and the Pacific while *O. aethiops* occurs only in the Pacific Ocean.

The study of the ossicles allowed to observe differences between the morphotypes analyzed. The dental plates of *Ophiocoma trindadensis* has thin septum, the ventral arm plates are wider distally than proximally, and the dorsal arm plates have more prominent edges, creating a curvature towards the proximal portion. While *Ophiocoma echinata* from NB, differs by presenting more pronounced concavity in the ventral arm plates, due to the fact that it has two tentacle scales, and dorsal arm plates are more oval.

The LDA analysis also corroborated the separation of specimens into two groups. *Ophiocoma echinata* from NB in addition to presenting the first ventral arm plates longer, and the second ventral arm plates wider, they present oral shield longer and the oral diameter larger than in *Ophicoma trindadensis*.

Regarding molecular analysis, from 16S sequences, differences were also found between the morphotypes. In both phylogenetic inferences, Maximum Parsimony and Bayesian Inference, two clades were observed, one for the TMV species and the other for *Ophiocoma* sp. NB and specimens from Antilles. The genetic distances between these clades were considered high (12.9% - 13.1%).

Until the present moment *Ophiocoma echinata* and *Ophiocoma trindadensis* sp. nov. are the only two species of *Ophiocoma* described for Brazil, the latter being only described to the Trindade and Martin Vaz Archipelago.

ACKNOWLEDGMENTS

We are grateful for all professors and researchers involved in this work: especially Luciana and Karin (LABESC), and Gabriela Granadier and Giovanna Aranha (EQUILAB), and Vanessa Silva Vicente. We also thank to MZUSP for specimens donated.

FINANCIAL SUPPORT

This work was supported by the São Paulo Research Foundation (FAPESP, Process nº 2019/01620-9), and CAPES, (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil) – Finance Code 001. I also thank FAPESP for the scientific initiation scholarship (Process nº 2019/05997-0), to the BIOTA-Collections Project (Process nº 2018/10313-0), to the financial support of the ProTrindade Project (Process nº 2016/50373-6), to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Process nº 402186/2016-8 and 443278/2019-9), the Brazilian Navy (1st District) and SECIRIM (Interministerial Secretariat for Marine Resources) for all support provided in Trindade and Martin Vaz. The material used referring to the Trindade and Martin Vaz Archipelago was a contribution 14 of the ProTrindade Marine Invertebrates Project.

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# 3. CONCLUSÕES

Na presente dissertação, a partir da taxonomia integrativa, foi possível reconhecer e descrever uma nova espécie de Ophiocomidae (*Ophiocoma trindadensis* sp. nov.) e redescrever mais detalhadamente *Ophiocoma echinata*, espécie já conhecida na costa brasileira.

Foram analisados 107 espécimes do gênero *Ophiocoma*, pertencentes às coleções científicas do Museu de Zoologia da UNICAMP (ZUEC) e do Museu de Zoologia da USP (MZUSP), coletadas no Arquipélago de Trindade e Martin Vaz e Salvador (Bahia).

Este estudo mostrou a importância de distintas ferramentas da taxonomia integrativa, na separação e identificação de espécies, como por exemplo: análise dos caracteres diagnósticos externos, caracteres microestruturais internos e externos (ossículos), morfometria e análises moleculares.

Os principais caracteres morfológicos que contribuíram na separação das espécies de *Ophiocoma* foram: tamanho e formato dos espinhos braquiais dorsais, placas dentais, placas braquiais dorsais e ventrais e número de escamas tentaculares.

A análise de LDA também corroborou na separação das espécies, indicando diferenças morfológicas só percebidas por meio da morfometria, tais como: o comprimento da primeira placa braquial ventral, a largura da segunda placa braquial ventral, o comprimento do escudo oral, e o diâmetro oral. Além disso, tanto as inferências filogenéticas, de Máxima Parcimônia e Bayesiana, quanto a distância genética, obtidas pelas análises moleculares do gene 16S, separaram as espécies em dois clados.

A redescrição de *Ophiocoma echinata* fez-se necessária, não desvalorizando descrições anteriores, mas sim complementando-as com o uso de novas ferramentas que possibilitaram a identificação de mais caracteres diagnósticos e um maior nível de detalhamento das espécies.

A descrição da nova espécie *Ophiocoma trindadensis* sp. nov. mostra que muito ainda se tem para conhecer acerca da biodiversidade dos Ophiuroidea em ilhas oceânicas, como é o caso do Arquipélago de Trindade e Martin Vaz. Assim, como em trabalhos anteriores realizados na região, os dados apresentados nessa dissertação, mostram a importância da utilização da taxonomia integrativa em estudos de espécies endêmicas, e enfatizam a

importância do conhecimento e monitoramento de áreas remotas, como o TMV, a fim proteger a biodiversidade marinha brasileira.

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# ANEXO 1

**Ministério do Meio Ambiente**
**CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO**

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

**Comprovante de Cadastro de Acesso**

**Cadastro nº A3014E0**

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

| | |
|---|---|
| Número do cadastro: | **A3014E0** |
| Usuário: | **UNICAMP** |
| CPF/CNPJ: | **46.068.425/0001-33** |
| Objeto do Acesso: | **Patrimônio Genético** |
| Finalidade do Acesso: | **Pesquisa** |

**Espécie**

**Ophiocoma sp.**

**Ophiocomella sp.**

| | |
|---|---|
| Título da Atividade: | **Taxonomia Integrativa Ophiocomidae** |

**Equipe**

| | |
|---|---|
| **Michela Borges** | **UNICAMP** |
| **Helena Serrano** | **Unicamp** |

| | |
|---|---|
| Data do Cadastro: | **28/08/2019 16:14:26** |
| Situação do Cadastro: | **Concluído** |

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **16:14** de **28/08/2019**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

ANEXO 2

COORDENADORIA DE PÓS-GRADUAÇÃO
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## DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***BIODIVERSIDADE DE OPHIOCOMA (ECHINODERMATA: OPHIUROIDEA) DO ATLÂNTICO SUL: ESTUDOS MORFOLÓGICOS E MOLECULARES***", desenvolvida no Programa de Pós-Graduação em Biociências e Tecnologia de Produtos Bioativos do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Helena Serrano

Assinatura: _____
Nome do(a) aluno(a): Helena Serrano

Michela Borges

Assinatura: _____
Nome do(a) orientador(a): Michela Borges

Data: 28 de Janeiro de 2022.

# ANEXO 3

**Declaração**

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **BIODIVERSIDADE DE OPHIOCOMA (ECHINODERMATA: OPHIUROIDEA) DO ATLÂNTICO SUL: ESTUDOS MORFOLÓGICOS E MOLECULARES** , não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 28 de Janeiro de 2022

Helena Serrano

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