UNIVERSIDAD DE CIENCIAS Y ARTES DE CHIAPAS

INSTITUTO DE CIENCIAS BIOLÓGICAS

ELABORACIÓN DE TEXTO

Morphological and molecular insights into *Beroe forskalii* Milne Edwards, 1841: a truly circumglobal species?

QUE PARA OBTENER EL TÍTULO DE

LICENCIADO EN BIOLOGÍA

PRESENTA

MONTSERRAT AREVALO NUCAMENDI

Tuxtla Gutiérrez, Chiapas Septiembre de 2025

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Resumen

La taxonomía de los ctenóforos sigue siendo un desafío debido a las similitudes morfológicas entre especies estrechamente relacionadas, el frágil cuerpo gelatinoso que complica la preservación y los datos moleculares limitados disponibles para muchos taxones. *Beroe forskalii* Milne Edwards, 1841 se considera una especie global, pero no se han evaluado las variaciones morfológicas y genéticas regionales. Este estudio integra análisis moleculares y morfológicos para investigar B. forskalii del Pacífico Central Mexicano (CMP) y compararlo con poblaciones del Atlántico, Mediterráneo, Pacífico y Golfo de California. Las muestras se caracterizaron utilizando marcadores mitocondriales, nucleares y ribosómicos junto con descripciones morfológicas detalladas, donde se disponía de datos. Las comparaciones morfológicas revelaron diferencias en los patrones macrociliares y la estructura de las papilas aborales entre los especímenes CMP y Atlántico-Mediterráneo. El análisis filogenético del gen ITS reveló una clara distinción en la secuencia del organismo mediterráneo de los clados bien respaldados de las poblaciones del Pacífico. No fue posible realizar un análisis entre cuencas para COI, pero las filogenias basadas en COI indicaron una diferenciación genética entre el Pacífico y el Golfo de California. Estos hallazgos apoyan la hipótesis de que *B. forskalii* comprende al menos dos especies distintas en las regiones del Pacífico y Atlántico-Mediterráneo, con diversidad adicional dentro del Pacífico. Se requieren más estudios moleculares y morfológicos, incluido un muestreo más amplio, para refinar la resolución taxonómica de *B. forskalii* y determinar su verdadera distribución.

ABSTRACT

Ctenophore taxonomy remains challenging due to morphological similarities among closely related species, the fragile gelatinous body that complicates preservation, and the limited molecular data available for many taxa. *Beroe forskalii* Milne Edwards, 1841 is considered a global species, but regional morphological and genetic variations have not been assessed. This study integrantes molecular and morphological analyses to investigate *B. forskalii* from the Central Mexican Pacific (CMP), and compare it with populations from the Atlantic, Mediterranean, Pacific and Gulf of California. Specimens were characterized using mitochondrial, nuclear and ribosomal marks along with detailed morphological descriptions, where data was available. Morphological comparisons revealed differences in macrociliary patterns and aboral papillae structure between the CMP and Atlantic-Mediterranean specimens. Phylogenetic analysis of the ITS gene revealed a clear distinction in the sequence of the Mediterranean organism from the well-supported clades of the Pacific populations. A cross-basin analysis was not possible for COI, but COI-based phylogenies indicated genetic differentiation between the Pacific and Gulf of California. These findings support the hypothesis that B. forskalii comprises at least two distinct species across the Pacific and Atlantic-Mediterranean regions, with additional diversity within the Pacific. Further molecular and morphological studies, including broader sampling, are required to refine the taxonomic resolution of *B. forskalii* and determine its true distribution.

I. INTRODUCCIÓN

Los ctenóforos o medusas peine, son animales transparentes que pertenecen a un pequeño filo de invertebrados exclusivamente marinos, comprendiendo alrededor de 100-150 especies, muchas de las cuales aún no han sido descritas. Tienen como característica principal ocho hileras de peines ciliares (ctenos) o paletas natatorias (Mills y Haddock, 2007; Cruz-Gonzáles *et al*., 2018). Este grupo se alimenta de una amplia variedad de zooplancton, incluyendo especies de importancia ecológica y comercial, teniendo un impacto significativo en la red trófica (Purcell, 2009). Entre los ctenóforos se encuentran los miembros del orden Beroida, los cuales presentan cuerpo aplanado lateralmente y carecen de tentáculos, por lo tanto dependen de una gran boca para engullir a sus presas. Son depredadores especializados de otros ctenóforos zooplanctónicos desempeñando un papel ecológico en la regulación de sus poblaciones (Shiganova y Abyzova, 2022).

Una de las especies dentro de este grupo es *Beroe forskalii* (Milne Edwards, 1841), especie de aguas cálidas reportada tanto en los océanos Pacífico como Atlántico (Shiganova y Abyzova, 2022). *Beroe forskalii* ha sido documentada en México en el Golfo de México (Puente-Tapia et al., 2021) y a lo largo de la costa del Pacífico sur frente a Oaxaca (Cruz-González *et al.,* 2018; Enríquez-García *et al.,* 2013; Ruíz-Escobar et al., 2015). Aún no existen registros publicados para el Pacífico central mexicano, aunque ha sido registrada en informes técnicos de la Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT, 2009) y en plataformas como *!naturalist* que reportan avistamientos en la península de Baja California y cerca de Puerto Vallarta (Puentes-Tapia *et al*., 2021). La mayoría de los estudios previos en México se basan en la identificación morfológica y el único análisis molecular de *B. forskalii* se basa en secuencias del citocromo C oxidasa I (COI) del Golfo de California (Christianson *et al.*, 2022). La escasez de datos moleculares no es exclusiva de México, ya que estudios globales sobre la diversidad genética de la especie siguen siendo limitados.

Dadas las limitadas características morfológicas diagnósticas de los ctenóforos, marcadores moleculares como ITS, COI y 18S han demostrado un potencial para resolver incertidumbres taxonómicas y aclarar la distribución de las especies (Christianson *et al*., 2022; Podar *et al.,* 2001; Shiganova y Abyzova, 2022). Debido a los escasos estudios de *B. forskalii* se plantean preguntas sobre la distribución global que actualmente se conoce y se sugiere que podría no ser una especie única y ampliamente distribuida, sino un complejo de especies que se extienden por diferentes cuencas oceánicas.

Con la integración de enfoques moleculares y morfológicos, este estudio proporciona nuevas perspectivas sobre la distribución taxonómica y geográfica de *B. forskalii*. Por que, en este estudio se presenta el primer reporte documentado de *B. forskalii* en el pacífico central Mexicano (CMP) y la primera caracterización molecular de los genes ITS y 18S rADN de especímenes mexicanos, junto con nuevas secuencias COI que complementan registros previos. El análisis incorpora registros anteriores y tiene como objetivo aclarar la diferenciación de linajes en las cuencas oceánicas, así como revisar la morfología de los ejemplares para compararlos con ejemplares previos e identificar posibles variaciones regionales a nivel morfológico. Estos hallazgos contribuyen a tratar de resolver la incertidumbre respecto a la distribución global de *B. forskalii* y respaldan los esfuerzos actuales para perfeccionar la sistemática y biogeografía de los ctenóforos.

II. MARCO TEÓRICO

1.1 CTENÓFOROS

Los ctenóforos son un filo de organismos marinos que se encuentran en hábitats epipelágicos a abisopelágicos en formas planctónicas y bentónicas, como el género Platyctenea que ha adaptado su vida a la vida bentónica en la etapa adulta (Hernandez-Nicaise, 1984; Pang y Martindale, 2008; Cruz-Gonzáles *et al*., 2018; Puente-Tapía *et al.*, 2020). Tienen una amplia distribución geográfica estando en la mayoría de los hábitats marinos, desde aguas polares hasta tropicales y se pueden encontrar en zonas neríticas así como en lagunas costeras, habitando en diferentes estratos de profundidad en los océanos (someros y profundos), muchas especies están presentes en la superficie del océano, así como existen formas que habitan en aguas más profundas incluyendo zonas abisales hasta al menos 7000 metros (Mianzan *et al.,* 2009; Moss, 2009; Dunn *et al*., 2015). Los ctenóforos más conocidos se encuentran cerca de la costa y son típicamente planctónicas, siendo estacionalmente más abundantes en primavera y principios del verano (Millis, 2010).

Forman un grupo de zooplancton gelatinoso, en las que se encuentran alrededor de 150-200 especies descritas, aunque el número varía dependiendo la fuente. Los ctenóforos se distinguen de otras especies por sus hileras de crestas que son su medio de locomoción, cada una de ocho hileras de crestas que lo recorren longitudinalmente que dividen su cuerpo en secciones iguales, cada una de prolonga alrededor de cuatro quintas partes entre el polo aboral y el extremo oral de su cuerpo (Pang y Martindale, 2008; Cruz-Gonzáles *et al*., 2018).

La mayoría de las especies presentan bioluminiscencia, expulsando sustancias que crean una nube que probablemente utilicen como medio para evitar la depredación (Enríquez-García *et al.*, 2013). Todos los ctenóforos son carnívoros, alimentándose de zooplancton, algunos como el género Beroida se alimentan de plancton gelatinoso, como otros ctenóforos, medusas o salpas (Mianzan et al., 2009). Gran parte de las especies del filo son hermafroditas simultáneas y muy pocas especies pueden reproducirse asexualmente (Cruz-Gonzáles *et al*., 2018).

Los primeros registros sobre el filo en el pacífico sur de México fueron por Bigelow (1912), quien reportó tres especies en la región de Acapulco. Estudios posteriores se centran a nivel de filo mencionado como un componente regular del zooplancton (Pantelón-López *et al*., 2005). Mientras que, existen registros recientes de ctenóforos que fueron realizados por fueron realizados por Enríquez-García *et al.* (2015), Gamero-Mora *et al*. (2015), Ruiz-Escobar *et al.* (2015) y Puente-Tapia *et al*. (2021).

2.1 *Beroe forskalii* Milne Edwards, 1841

La especie *B. forskalii* tiene un cuerpo en forma de saco o cono comprimido, con coloración rosácea. La anchura de su cuerpo se va estrechando desde la mitad de su cuerpo hacia la extremidad aboral. Tiene ocho canales meridionales que se extienden desde la región aboral hacia su boca y está conectado a un canal circular alrededor de la boca por una malla de canales anastomosados; las hileras de peines se extienden dos terceras partes de su tamaño surgiendo debajo del estatocisto (Olivera y Migotto, 2014; Cruz-Gonzáles *et al*., 2018). Presenta filas de ctenos subtentaculares y submodeales con una longitud similar que se van extendiendo desde el extremo aboral hasta un séptimo de la distancia del cuerpo a la boca (Ruiz-Escobar *et al*., 2015).

Beroe forskalii presenta una boca ancha, semicircular que se extiende en toda la región oral y se va abriendo hacia una gran faringe que ocupa gran parte de la zona central interna del organismo (Olivera y Migotto, 2014).

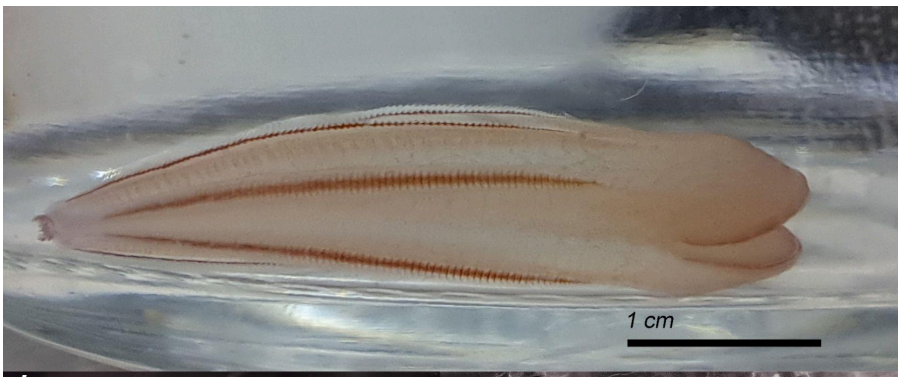

Figura 1. Vista lateral del espécimen vivo de *Beroe forskalii*.

2.1.1 *Beroe forskalii* en México

En México el filo Ctenóforos es uno de los filos menos conocidos y aunque se han realizado estudios específicos acerca de este filo, su diversidad en México es poco conocida y marcada por una discontinuidad temporal. Los estudios sobre *B. forskalii* en México, son principalmente de registros de especies de ctenóforos pero no en la especie misma, centrándose en áreas específicas o áreas geográficas restringidas y a escalas de tiempo cortas (Martínez-Meyer et al., 2014; Puentes-Tapia et al., 2021).

La distribución *B. forskalii* en México se encuentra en el Pacífico nororiental: en la Isla de Guadalupe (SEMARNAT, 2009), en el Golfo de California, en las cuencas de Farallón y Mazatlán (Puente-Tapia *et al.*, 2021), El Quelele, La Paz, Yelapa, Jalisco (naturalista.mx); en el pacífico Tropical oriental se distribuye en el Carrizalillo, Estacahuite, La boquilla, La Mina, Mazunte, Panteón, Puerto Ángel y Zipolite, Oaxaca (Enríquez-García *et al.*, 2013; Ruíz-Escobar *et al.*, 2015; Cruz-Gonzáles *et al*., 2018). Datos que se han obtenido principalmente de estudios acerca sobre registros de especies de ctenóforos o registros de biodiversidad en zonas específicas.

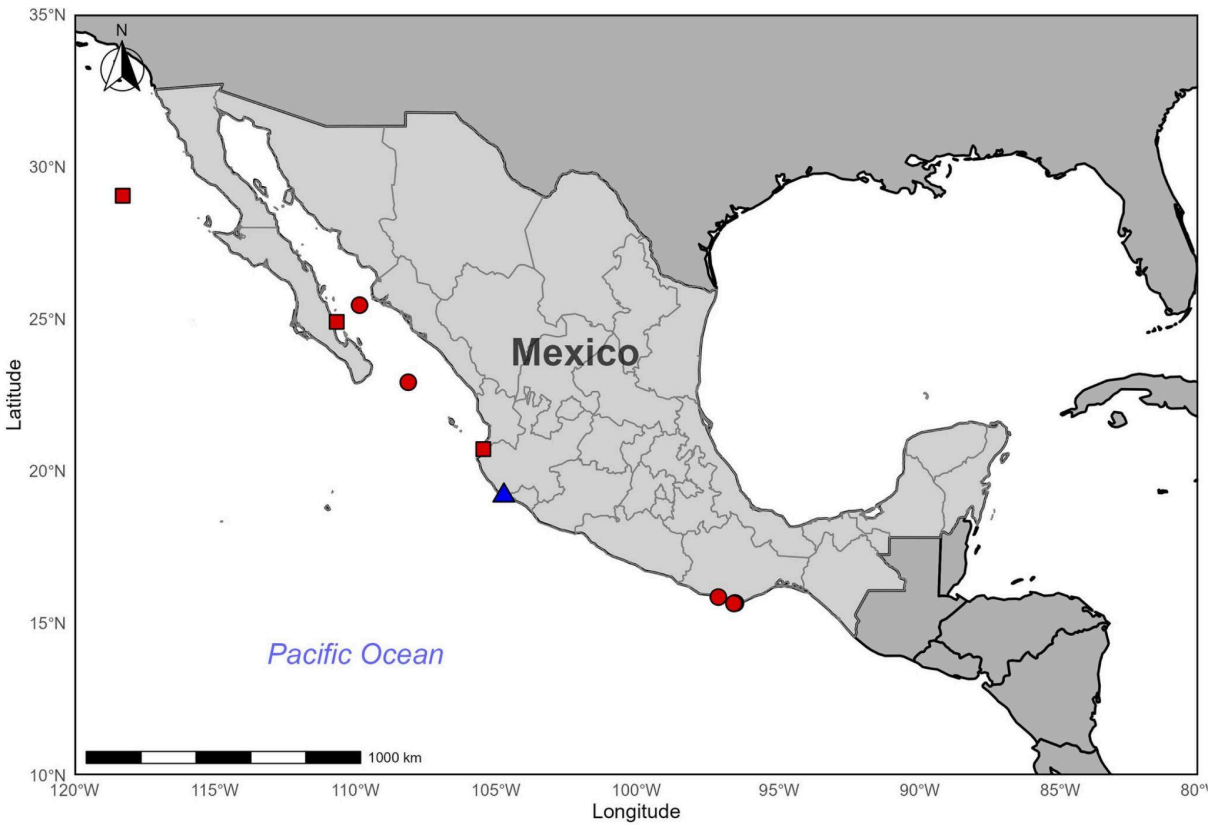

Figura 2. Mapa de ubicaciones de muestreo de *Beroe forskalii* a lo largo del Pacífico mexicano. Los círculos rojos representan registros publicados anteriormente, mientras que los cuadrados rojos indican informes anteriores de la literatura gris. El triángulo azul marca el nuevo récord documentado en este estudio, de Bahía de Navidad.

2.1.2 Estudios moleculares

El estudio de marcadores moleculares mitocondriales ha tenido gran relevancia dentro de la biología evolutiva por permitir la comparación entre linajes antiguos de metazoos. Los ctenóforos aunque son abundantes en la columna de agua, las características morfológicas son difíciles de detectar o fotografiar debido a su transparencia y movimiento constante y son difíciles de recolectar y mantener en vida para realizar estudios moleculares, por ello se sabe poco sobre su biología y las comparaciones hechas anteriormente con otros filos han confirmado que este filo comparte muy pocas cualidades con otros filos. Por lo tanto, el estudio de su genoma mitocondrial permite conocer sobre su origen y la diversificación temprana del filo (Harbinson, 1985; Johansson *et al*., 2018; Schultz *et al*., 2020).

Uno de los primeros estudios filogenéticos moleculares del filo Ctenophora es el de Podar *et al.* (2021) en el que mediante el uso de secuencias de ARN ribosomal 18S de 26 especies de ctenóforos compararon la filogenia ya descrita de este filo con la filogenia derivada de la secuencia obtenida en el estudio.

Por otro lado, en un estudio realizado por Schultz *et al*., (2020), observan que solo se habían secuenciado los genomas mitocondriales de cinco especies de ctenóforos y cada uno de ellos contenía marcos de lectura abiertos (ORF) que si se traducen, no cuentan con ortólogos identificables, sin ellos se denominan marcos de lectura no identificados (URF). Si realmente codifican proteínas, los URF mitocondriales de los ctenóforos representan una ruta limitada en la evolución y metabolismo mitocondrial de los metazoos de divergencia temprana. Para ello se secuenció y anotó el genoma mitocondrial de *Beroe forskalii,* encontrando así, que presentan en su genoma mitocondrial dos URF a diferencia de las otras especies secuenciadas. Por lo que, este estudio proporcionó evidencia de que el genoma mitocondrial de los ctenóforos secuenciados contienen URF que parecen codificar proteínas de transporte transmembrana, mientras que, los URF del genoma

mitocondrial de B. forskalii se encuentra bajo selección negativa, por lo tanto, se traducen y son funcionales dentro de las mitocondrias.

Shiganova y Abyzova, (2022), investigaron especies de Ctenóforos en los mares de Europa junto con otras especies relevantes del orden *Beroe* de otros océanos, realizando estudios taxonómicos, análisis de ADN con secuencias ITS y estudios morfológicos con el objetivo de revisar las posiciones sistemáticas de las especies de *Beroe*, así redefinir el estatus taxonómico de las especies válidas y con ello refinar su morfología. Como resultado, el estudio sugiere cambios sistemáticos, entre los que destaca que *B. Forskalii* podría estar representada por dos especies en el Mediterráneo y en la Antártida, sin embargo, sugieren un análisis genético adicional para definir adecuadamente la especie.

Mientras que, en un estudio de Johansson *et al.* (2018), se analizaron muestras de ADN, así como métodos morfológicos de 109 posibles especies del género Beroe de Europa utilizando citocromo oxidasa 1 (COI) y espaciador transcrito interno 1 (ITS1), junto con secuencias publicadas en GenBank para conocer las relaciones filogenéticas y conocer si las muestras analizadas pertenecían al género. Los árboles filogenéticos tanto de ITS como de COI fueron consistentes en gran medida con la identificación morfológica y se identificaron cinco clados bien soportados que posiblemente corresponden a cinco especies, de los cuales tres podrían asignarse a especies conocidas, y otros dos linajes no coinciden con ninguna especie conocida, por lo tanto, podría reflejar nuevas especies, pero se requieren estudios adicionales para confirmar si se trata de nuevas especies.

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Morphological and molecular insights into *Beroe forskalii* Milne Edwards, 1841: A truly circumglobal species?

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ABSTRACT

Ctenophore taxonomy remains challenging due to morphological similarities among closely related species, the fragile gelatinous body that complicates preservation, and the limited molecular data available for many taxa. *Beroe forskalii* Milne Edwards, 1841 is considered a global species, but regional morphological and genetic variations have not been assessed. This study integrates molecular and morphological analyses to investigate *B. forskalii* from the Central Mexican Pacific (CMP), and compare it with populations from the Atlantic, Mediterranean, Pacific, and Gulf of California. Specimens were characterized using mitochondrial, nuclear, and ribosomal markers along with detailed morphological descriptions, where data was available. Morphological comparisons revealed differences in macrociliary patterns and aboral papillae structure between the CMP and Atlantic-Mediterranean specimens. Phylogenetic analysis of the ITS gene revealed a clear distinction in the sequence of the Mediterranean organism from the well-supported clades of the Pacific populations. A cross-basin analysis was not possible for COI, but COI-based phylogenies indicated genetic differentiation between the Pacific and Gulf of California. These findings support the hypothesis that *B. forskalii* comprises at least two distinct species across the Pacific and Atlantic-Mediterranean regions, with additional diversity within the Pacific. Further molecular and morphological studies, including broader sampling, are required to refine the taxonomic resolution of *B. forskalii* and determine its true distribution.

1. Introduction

Ctenophores, commonly known as comb jellies, are exclusively marine, gelatinous zooplankton. They are characterized by biradial symmetry, eight rows of ciliary combs (ctenes), and, in most species, a pair of tentacles used for prey capture (Podar et al., 2001). Species within this group prey on a wide variety of zooplankton, including ecologically and commercially important species, and can have important impacts on the marine food web (Purcell, 2009). Members of the order Beroida are specialized predators on other zooplanktonic ctenophores and play an ecological role in regulating their populations (Shiganova & Abyzova, 2022). Unlike other pelagic ctenophores, beroids are laterally flattened and lack tentacles, relying instead on a large mouth to engulf prey. One widely distributed species within this group is *Beroe forskalii* Milne Edwards (1841) a warm-water species reported both in the Pacific and Atlantic oceans (Shiganova and Abyzova, 2022).

In Mexico, *Beroe forskalii* has been documented in the Gulf of California (Puente-Tapia et al., 2021) and along the southern Pacific coast of Oaxaca (Cruz-González et al., 2018; Enríquez-García et al., 2013; Ruíz-Escobar et al., 2015). No published records exist for the Central Mexican Pacific (CMP) although there is some gray literature that reports sightings off the Baja California Peninsula and near Puerto Vallarta, including records from the iNaturalist platform (Puentes-Tapia et al., 2021) and a technical report from a Mexican natural resource agency (SEMARNAT, 2013) (Fig. 1). Most prior studies in Mexico have relied on morphological identification, with the only molecular analysis one sequence of Cytochrome C Oxidase subunit 1 (COI) from a specimen sampled in the Gulf of California (Christianson et al., 2022). This scarcity of molecular data is not unique to Mexico, as global studies on the genetic diversity of this species remain limited.

Given the limited diagnostic morphological characteristics in ctenophores, molecular markers such as Internal Transcribed Spacer

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(ITS), and COI have shown great potential for resolving taxonomic uncertainties and clarifying species distributions (Christianson et al., 2022; Podar et al., 2001; Shiganova and Abyzova, 2022). The few studies conducted on *B. forskalii* raise questions about its true global distribution, suggesting that it may not be a single widespread species, but rather a species complex across different ocean basins. Johansson et al. (2018) used two ITS sequences (one from France and one from California) as outgroups in their analysis of *Beroe* species in the Atlantic, and found that they only shared 84.6 % sequence identity, therefore proposing that they might be cryptic or misidentified species. However, those findings were preliminary, and subsequent studies have yet to expand upon or confirm this hypothesis. Nevertheless, in their broader analysis, interspecific comparisons among *Beroe* species typically showed ITS sequence identities ranging from 82.5 % to 95.4 %, while intraspecific variation was consistently above 98.8 %.

By integrating molecular and morphological approaches, this study provides the first species-focused assessment of *B. forskalii* combining internal and external morphology with multi-locus genetic data. We present the first documented occurrence of *B. forskalii* in the CMP and the first molecular characterization of ITS and 18S rDNA genes from Mexican specimens, along with new COI sequences to supplement previous records. We estimated a phylogeny with published data and newly generated sequences to clarify lineage differentiation across ocean basins, while comparative morphology highlighted potential regional variations. These findings contribute to resolving uncertainties regarding the global distribution of *B. forskalii* and support ongoing efforts to refine ctenophore systematics and biogeography.

2. Materials and methods

2.1. Specimen collection

Zooplankton were sampled on July 3, 2024, off Bahía de Navidad, Central Mexican Pacific (19°09′00″N, 104°44′50″W; ~100 m depth; Fig. 1). Near-surface samples (<10 m) were collected between 04:00 and 05:00 am, approximately one hour before sunrise, using a 1 m diameter plankton net (250 µm mesh) equipped with a hard cod-end. The sample was gently transferred into a cooler filled with in situ seawater and transported to the laboratory within one hour. In the lab, four actively swimming *B. forskalii* were isolated and maintained in 3 L aerated tanks with filtered seawater at 25°C for further analysis, and two were preserved in 96 % ethanol for molecular analysis.

2.2. Morphological characterization

Specimens were examined macroscopically, with photographs and videos of swimming organisms taken using a digital camera against a light background. Morphological structures were observed in finer detail using a ZEISS-STEMI 305 stereomicroscope with a black background, and images were captured with an Axiocam 208c. Species identification was based on morphological keys and descriptions from Mills and Haddock (2007), Ruíz-Escobar et al. (2015), and Shiganova and Abyzova (2022). Four days after the sampling, the organisms died and disintegrated overnight, therefore it was not possible to preserve a specimen for future morphological reference.

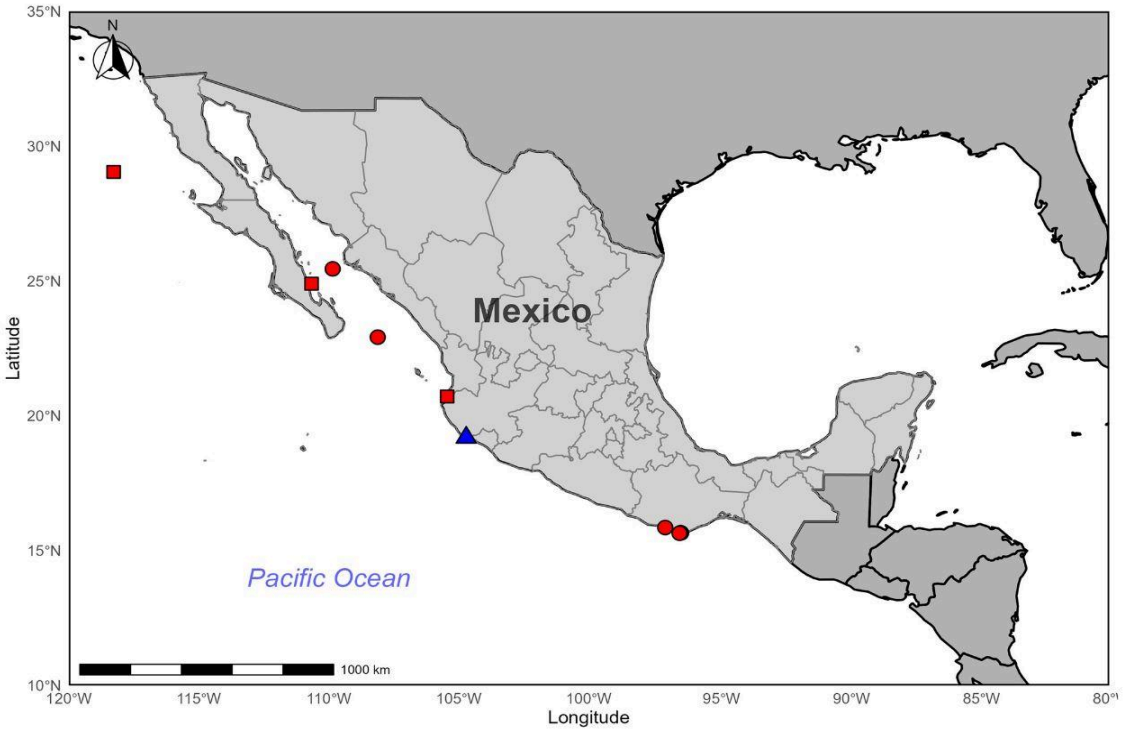

Fig. 1. Map of sampling locations of *Beroe forskalii* along the Mexican Pacific. Red circles represent previously published records, while red squares indicate previous reports from gray literature. The blue triangle marks the new record documented in this study, from Bahía de Navidad.

2.3. Molecular identification

We extracted genomic DNA from two specimens using PROMEGA Wizard® Genomic DNA Purification Kit, following the manufacturer's instructions. Three genes were amplified by polymerase chain reaction (PCR). The ribosomal 18S rDNA (~1800 bp) was amplified with the primers 18SA (5´-AACCTGGTTGATCCTGCCAGT-3´) and 18SB (5´-TGATCCTTCYGCAGGTTCACCTAC-3´; Medlin et al., 1988). Partial sequences of the internal transcribed spacer (ITS, ITS1–5.8-ITS2; ~600 bp) were amplified using the primers ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) and ITS5 (5´-GGAAGTAAAAGTCGTAACAAGG-3´; White et al., 1990). Furthermore, a partial sequence of the mitochondrial cytochrome C oxidase subunit 1 gene (COI, ~600 bp) was amplified using the degenerate primers dgLCOI490 (5´-GGTCAACAAATCATAAAGAYATYGG-3´) and dgHCOI21908 (5´-TAAACTTCAGGGTGACCAAARAAYCA-3´; Meyer, 2003). PCR conditions for all genes were as follows: one cycle at 94 °C for 2 min of denaturing; 35 cycles at 94 °C for 1 min; 48 °C (COI), or 55 °C (18S rDNA), or 52 °C (ITS) for 1 min, annealing, 72 °C 1 min, and a final extending of one cycle at 72 °C 5 min. The PCR products were visualized by electrophoresis on 2 % Tris-acetate-EDTA agarose gels. The PCR fragments were purified using the Wizard SV Gel and PCR Clean-Up System (Promega®) and sequenced at Macrogen® Inc (Seoul, Korea).

The forward and reverse sequences were manually edited to obtain a consensus using Geneious Basic (ver. 4.8.5) and then analyzed using the Basic Local Alignment Search Tool (BLAST; blast.ncbi.nlm.nih.gov) to confirm the taxonomic identification. The gene sequences were deposited in the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/nuccore) with accession numbers 18S rDNA: PV233723 – PV233724; ITS: PV232944 – PV232945 and COI: PV233714 – PV233715.

We determined the systematic position of the examined organisms relative to other ctenophore species, including representatives of both the Nuda and Tentaculata classes, using 18S rDNA, ITS and COI. All sequences were first aligned using ClustalW. We then selected the best-fit evolutionary substitution model for each marker using JModelTest 2.0. Phylogenetic relationships were inferred through Maximum Likelihood (ML) with 1000 bootstrap replicates, using MEGA v.X1 and The Bayesian inference (BI) analysis was conducted using Mr. Bayes 3.2.1 with two Markov four Chain Monte Carlo simulations of over 100,000 generations carried out with sampling every 1000 generations. The appropriate burnin value was determined by examining the standard deviation of split frequencies (< 0.001). A 50% majority rule consensus tree was constructed from all generations sampled after the burnin and

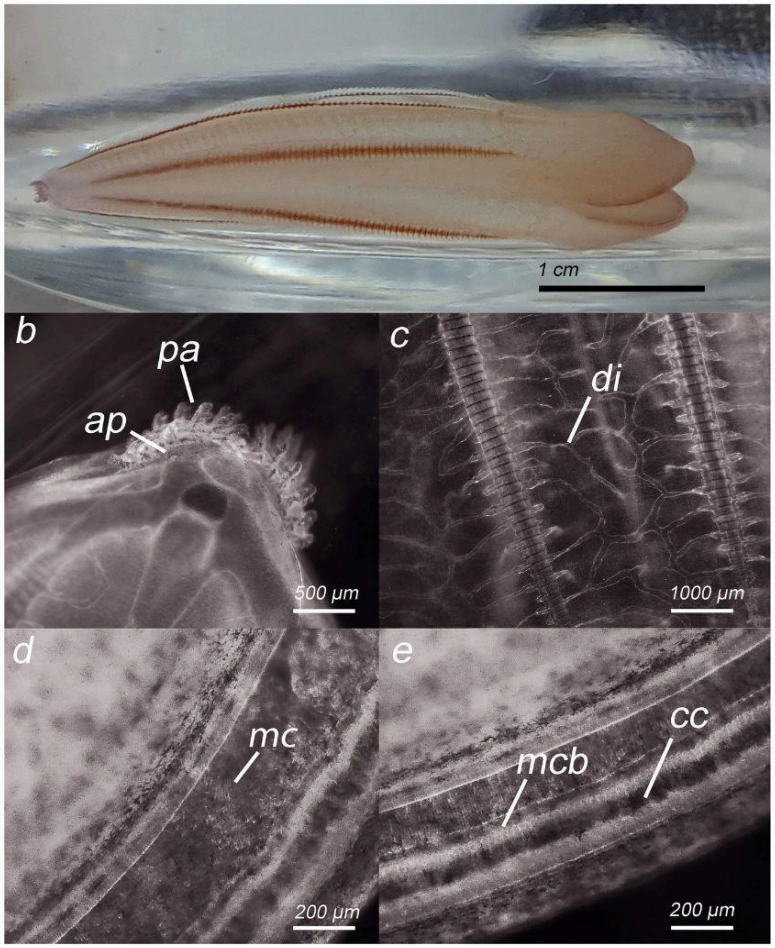

Fig. 2. a) Lateral view of the living specimen of *Beroe forskalii*, b) detailed view the aboral pole, surrounded by papillae, c) internal view showing the meridional canals forming a network through anastomosed diverticula, d) close-up of the mouth revealing macrocilia, e) close up of narrow band of macrocilia along the inner lip. Abbreviations: ap, anal pore; cc, circular canal; di, divirticula; mc, macrocilia; mcb, macrociliary band; pa, papillae.

after discarding 25% of the original samples. Additionally, we calculated pairwise genetic distances among *Beroe* species using Kimura's two-parameter model in MEGA v.XI.

3. Results

3.1. Morphological and behavioral description

The body is elongated and laterally compressed and tapers towards the aboral pole, measuring approximately 4–5 cm. The aboral pole is conical, the while the oral pole is oval, and features a wide mouth that spans the entire oral region. The coloration is a transparent pinkish brown, with a distinctly darker tinge along the ciliary comb rows. Eight rows of ciliary combs extend up to three-quarters of the body length, from the aboral pole towards the mouth (Fig. 2a). The apical organ at the aboral pole is surrounded by short, moderately branched pink colored papillae (Fig. 2b). Internally, the meridional canals form a network through anastomosed diverticula (Fig. 2c). Macrocilia can be observed inside the mouth (Fig. 2d). Additionally, there is a stable, narrow band of macrocilia along the inner lip (Fig. 2e), each with a length of 40–60 μm.

Beroe forskalii moves through the water by a coordinated beating of its eight rows of ctenes. When not actively swimming, it typically remains immobile, with an open or closed mouth, with intermittent beating of the ctenes. It usually swims in a straight line with a closed mouth. However, when initiating feeding behavior, it transitions into a characteristic spiral swimming pattern before suddenly opening its mouth wide (Video S1). No bioluminescence was observed in the specimens; however, the organisms were not dark-adapted and were only observed under dark conditions for a few minutes. Color on the ctenes were briefly mistaken for bioluminescence but were confirmed to be light reflections.

3.2. Molecular phylogenetic analyses and pairwise genetics distance inferences

The phylogenetic reconstruction employing maximum likelihood (ML) and Bayesian methods (BI) with ribosomal (18S rDNA), mitochondrial (COI) and nuclear (ITS) genes demonstrates moderate to high bootstrap values (BV) and posterior probability (PP) within the Phylum Ctenophora. The analyses placed the organisms collected in this study in the Nuda class, forming a well-supported subclade (BV > 90; PP > 0.74) alongside *B. forskalii* specimens collected from other locations (Figs. 3–5; see Table 1).

We conducted analyses of 18S rDNA using 16 sequences (1801 selected sites; 1745 constant, 56 variable and 41 parsimony- informative). The resulting tree (Fig. 3) grouped all sequences of *B. forskalii* into a well-supported clade (BV = 95; PP = 1.00, only two variable sites in all sequences).

We then reconstructed the phylogeny using the ITS with 28 sequences (698 selected sites; 361 constant, 334 variable, and 199 parsimony-informative). The resulting topology (Fig. 4) placed the sequences of *B. forskalii* into a well-supported clade, with maximum values. The Mediterranean sequence was separated from the well-supported grouping of the Pacific Ocean sequences.

Finally, we analyzed the COI gene using 27 sequences (524 selected sites; 294 constant, 230 variable, and 208 parsimony-informative). The resulting phylogeny grouped *B. forskalii* sequences into a well-supported clade (BV = 90; PP = 0.74). The Pacific sequences formed a subclade (BV = 66; PP = 0.99), while the Gulf of California sequence was separated (Fig. 5).

The genetic distance (K2P) calculated from ribosomal (18S), mitochondrial (COI), and nuclear (ITS) genes places the organisms collected in the CMP (this study) within congeneric species of *B. forskalii*. Among the analyzed genes, 18S rDNA exhibited the least intraspecific genetic divergence. The organisms from the CMP and other specimens collected in the Pacific Ocean were genetically identical but diverged slightly from organisms from the Mediterranean and Atlantic regions (0.001 ± 0.000), which also exhibited minimal distances (0.001 ± 0.001) within populations (Table S1). The intraspecies genetic distances values calculated for ITS sequences revealed differences between the sequences of this study and those of other organisms collected on the California coast (PP905524: 0.004 ± 0.04; AF293698: 0.009 ± 0.005; Table S2). Furthermore, the *B. forskalii* sequences from the CMP exhibited substantial genetic divergence from the Mediterranean sequence (0.044 + 0.012). Intraspecies genetic distances based on COI sequences indicated variability among Pacific Ocean specimens. CMP sequences were genetically identical to the two organisms collected in Monterey Bay

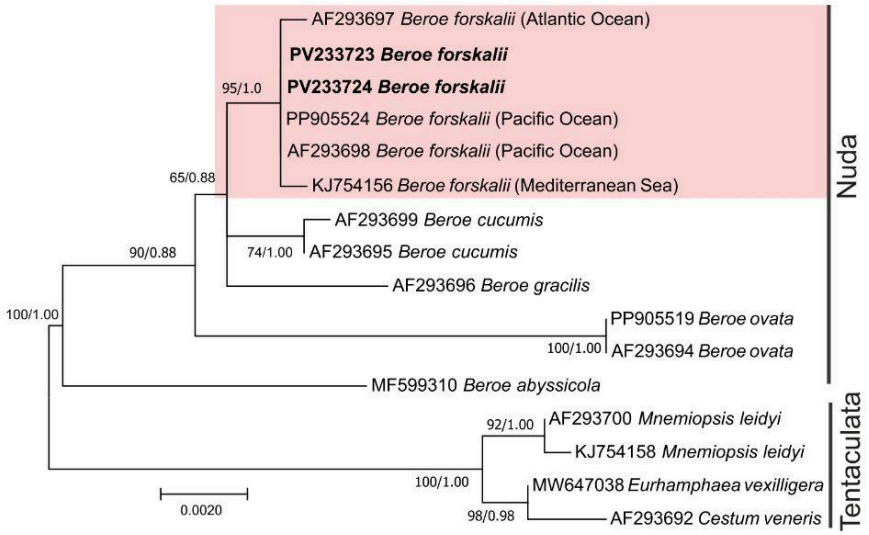

Fig. 3. Systematic position of *Beroe forskalii* determined through an analysis of maximum likelihood (ML) and Bayesian inference (BI) using sequences of the ribosomal 18S rDNA gene. The pink boxes represent the set of *B. forskalii* sequences, while those from organisms in the Central Mexican Pacific are indicated in bold. The values associated with each node correspond to bootstrap values and posterior probability (ML/BI). The tree is unrooted.

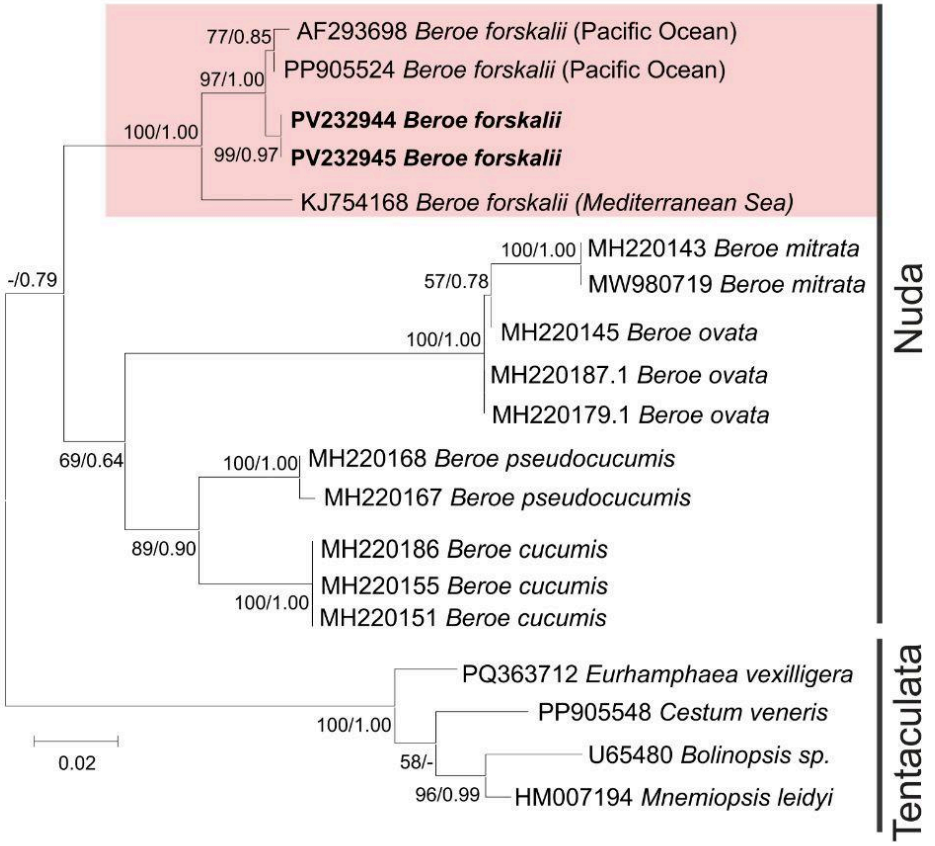

Fig. 4. Systematic position of *Beroe forskalii* determined through an analysis of maximum likelihood (ML) and Bayesian inference (BI) using partial sequences of the internal transcribed spacer (ITS) gene. The pink boxes represent the set of *B. forskalii* sequences, while those from organisms in the Central Mexican Pacific are indicated in bold. The values associated with each node correspond to bootstrap values and posterior probability (ML/BI). The tree is unrooted.

(NC038065 and MG655622). However, genetic variation was observed in other organisms from the same and nearby localities (Table S3). The CMP sequences showed greater divergence from the Gulf of California sequence (0.044 ± 0.010). The interspecies genetic distances analysis determined that of the species we included, the most distant relative of *B. forskalii* was *B. abyssicola* (18S = 0.011 + 0.002; COI = 0.183 + 0.016).

4. Discussion

This study provides new insights into the morphology, genetic differentiation, and distribution of *B. forskalii,* with a particular focus on its occurrence in the CMP. These findings highlight the importance of integrating molecular and morphological data to better understand *Beroe* diversity and biogeography.

4.1. Morphological and molecular evidence for species differentiation

While the general morphology of the CMP specimens aligned with descriptions from other regions (see Table 2), some notable differences were observed. In particular, the tropical Atlantic and Mediterranean exhibit long, extensively branched papillae, whereas our specimens exhibited short papillae. We also identified a stable, narrow band of macrocilia running along the inner lip, which has not been reported previously in *B. forskalii* (Tamm and Tamm, 1993; Oliveira and Migotto, 2014). Given that macrociliary traits such as size, tooth number, and arrangement are proposed as diagnostic traits for *Beroe* species identification (Tamm and Tamm, 1993), these differences may indicate regional morphological variation or potential taxonomic divergence. We did not examine the number of teeth on the macrocilia, nor the macrociliary pattern inside the mouth to determine whether it formed stripes or a continuous carpet, as described by Oliveira and Migotto (2014). However, based on the narrow macrociliary band observed on our specimens, if macrociliary patterns are indeed species-specific, then the *B. forskalii* population in the CMP exhibits a divergence from the tropical Atlantic population.

These morphological differences are further supported by molecular analyses. Previous studies have reported varying degrees of genetic divergence between Mediterranean (France) and Pacific (California, USA) *B. forskalii* populations. Simion et al. (2015) include ITS sequences from Mediterranean specimens in a broad phylogenetic study of ctenophores but may not have had sufficient resolution to detect finer-scale genetic distinctions. In contrast, Johansson et al. (2018) use the same two ITS sequences as an outgroup in their study of European *Beroe* species and find only 84.6 % sequence identity between the Mediterranean and Pacific specimens. Those authors recommend COI and ITS as appropriate barcoding regions for *Beroe* species, given the apparent lack of fine-scale phylogenetic signal in 18S sequences.

In fact, although the 18S gene has been used for establishing broad phylogenetic relationships across Ctenophora (e.g., Podar et al., 2001), it is highly conserved and often fails to discriminate between closely related or even distinct species. For instance, species within genera such as *Bathocyroe*, *Deiopea*, and *Eurhamphaea* share nearly identical 18S sequences (Christianson et al., 2022), underscoring its poor utility in species delimitation within this phylum. In contrast, research has found that ITS regions in Ctenophora exhibit considerably higher interspecific variability (Simion et al., 2015), as demonstrated by studies in *Beroe*, where ITS sequence identities between different species typically range

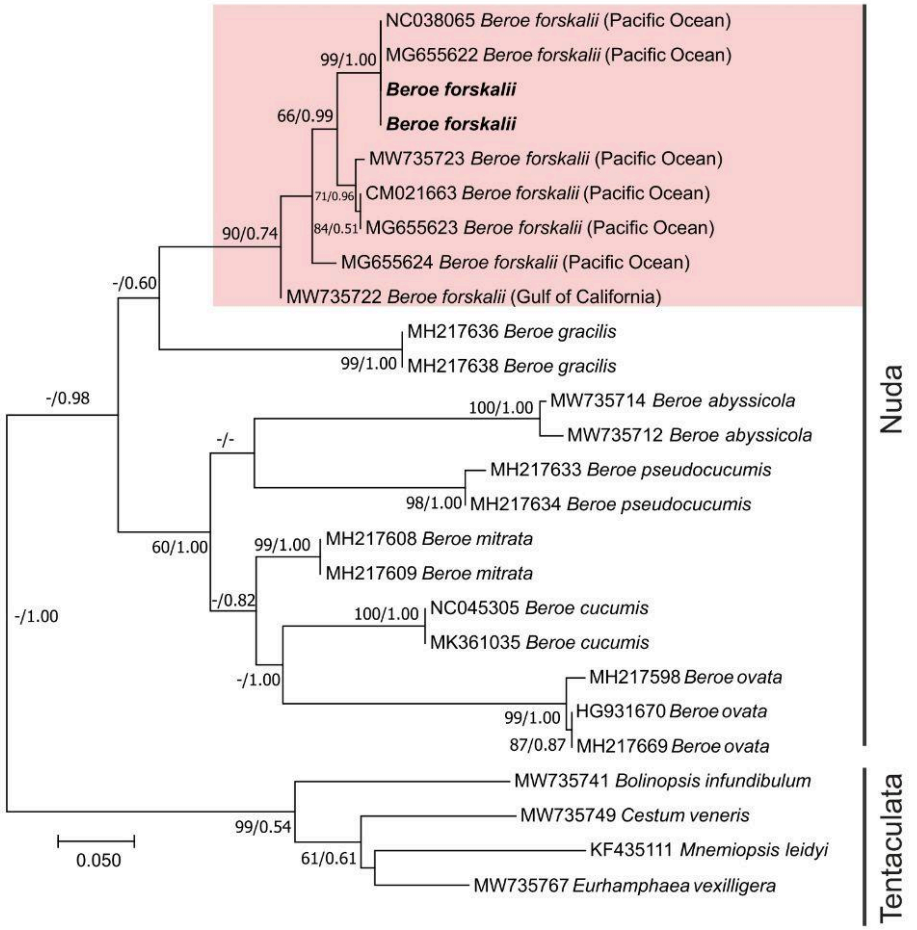

Fig. 5. Systematic position of *Beroe forskalii* determined through an analysis of maximum likelihood (ML) and Bayesian inference (BI) using sequences of the mitochondrial Cytochrome C Oxidase subunit 1 (COI) gene. The pink boxes represent the set of *B. forskalii* sequences, while those from organisms in the Central Mexican Pacific are indicated in bold. The values associated with each node correspond to bootstrap values and posterior probability (ML/BI). Values less than 50 were replaced by (-). The tree is unrooted.

Table 1
Genetic sequence data of *Beroe forskalii,* listing the unique sequence ID, geographic location, associated water body, targeted gene, base pair length (Bp), and original data source. Sequences include mitochondrial, COI, 18S, and ITS genes from various marine regions.

ID	Location	Water body	Gene	Bp	Source
MG655622	Monterey Bay (USA)	Pacific	mitochondrion	13338	Schultz et al. (2020)
MG655623	Monterey Bay (USA)	Pacific	mitochondrion	13339	Schultz et al. (2020)
MG655624	Monterey Bay (USA)	Pacific	mitochondrion	13357	Schultz et al. (2020)
NC038065	Monterey Bay (USA)	Pacific	mitochondrion	13338	Schultz et al. (2020)
CM021663	Near Big Sur (USA)	Pacific	mitochondrion	13339	Unpublished (BioSample: SAMN08156691)
MW735722	Gulf of California (MEXICO)	Gulf of California	COI	748	Christianson et al. (2022)
MW735723	Central California Coast (USA)	Pacific	COI	748	Christianson et al. (2022)
PV233714	Bahía de Navidad (MEXICO)	Pacific	COI	524	This study
PV233715	Bahía de Navidad (MEXICO)	Pacific	COI	524	This study
KJ754156	Villefranche-sur-mer (FRANCE)	Mediterranean	18 s	1755	Simion et al. (2015)
AF293697	Gulf Stream, Florida (USA)	Atlantic	18 s	1802	Podar et al. (2001)
PV233723	Bahía de Navidad (MEXICO)	Pacific	18 s	1801	This study
PV233724	Bahía de Navidad (MEXICO)	Pacific	18 s	1801	This study
PV232944	Bahía de Navidad (MEXICO)	Pacific	ITS	713	This study
PV232945	Bahía de Navidad (MEXICO)	Pacific	ITS	713	This study
KJ754168	Villefranche-sur-mer (FRANCE)	Mediterranean	ITS	674	Simion et al. (2015)
AF293698	Santa Barbara, CA (USA)	Pacific	18 s - ITS	1802	Podar et al. (2001)
PP905524	California Coast (USA)	Pacific	18 s - ITS	6093	Unpublished (BioSample: SAMN03579477)

Note: All accessions were downloaded from the NCBI database.

Table 2
Morphological characteristics of *Beroe forskalii* reported across various regional water bodies. Superscript numbers indicate the source of each characteristic as detailed in the footnotes. A dash (-) indicates the absence of reported data for a given characteristic. There were no original descriptions from the Gulf of California.

Characteristic	Present Study	California Pacific	Mexican Pacific	Tropical Atlantic	Mediterranean
Body length (mm)	40	150[1]	50–125[3,4]	25 (juvenile)[5]	150–200[6]
Color (alive)	Transparent pink-brown, darker along comb rows	Rose pink, darker red along comb rows[1]	Pinkish[3,4]	Pinkish[1]	Pinkish in juveniles, becoming pink in adults[6]
Body shape	Long, conical	Long, conical[1]	Long, conical in aboral half[3]	Sack-shaped, conical aboral extremity[5]	Conical, pointed aboral end[6]
Flattened on lateral plane	Flattened	Flattened[1]	Flattened[3]	Flattened[5]	Flattened[6]
Mouth structure	Wide and rounded	Wide[1]	Wide[3]	Wide[5]	Wide and rounded[6]
Ciliary combs	¾ body length, starting at aboral pole	¾ body length, starting at aboral pole[1]	66/7 body length, starting at aboral pole[3]	Aboral 2/3 of body[5]	¾ body length starting at aboral pole[6]
Diverticula	Anastomosed in ¾ body, blind ends close to aboral pole	Anastomosed[1]	Anastomosed[3]	Anastomosed in oral half, blind ends in aboral half[5]	Dense with frequent anastomosis[6]
Aboral papillae	Short and branched	-	Two sets, branched papillae[3]	Prominent and branched[5]	Long and branched[6]
Bioluminescence	Not observed	Observed in anastomosing canals[1]	-	-	-
Stable macrociliary band inside lips	Present	-	-	Not present[5]	-
Macrociliary field	-	Arranged in long rows from the lip edge into the stomodeal cavity[2]	-	Broad stripes running from the lips, tapering towards the inner stomodeum[5]	-
Macrocilium length	40–60 μm	80–100 μm[2]	-	40–55 μm[5]	-
Macrocilium diameter	-	12–15 μm[2]	-	7–9 μm[5]	-
Tooth number	-	16–24[2]	-	18–26[5]	-
Tooth size	-	Equal, short[2]	-	2–3 μm length[5]	-

[1]Mills and Haddock, 2007
[2]Tamm and Tamm, 1993
[3]Ruíz-Escobar et al., 2015
[4]Cruz-González et al., 2018
[5]Oliveira and Migotto, 2014
[6]Shiganova and Malej, 2009

from 82.5 % (*B. cucumis* vs. *B. ovata*) to 95.4 % (*B. anatoliensis* vs. *B. ovata*), while intraspecific variation consistently exceeds 98.8 % (Johansson et al., 2018). These thresholds provide valuable reference points for interpreting divergence within *B. forskalii*.

Our study expanded upon this by incorporating new ITS and 18S rDNA sequences from the CMP, which revealed substantial nucleotide differences between Mediterranean and Pacific *B. forskalii* specimens with the ITS. The combined morphological and molecular evidence indicates that the Mediterranean and Pacific *B. forskalii* populations could represent distinct species. Genetic analyses reveal significant divergence, aligning with observed morphological differences, particularly in aboral papillae structure and the presence of a macrociliary band in our specimens. The similarity between Mediterranean and tropical Atlantic papillae suggests a closer relationship between these populations, though macrociliary traits remain unexamined in the Mediterranean. Given the diagnostic value of macrociliary patterns and tooth arrangement, further investigation is needed to clarify species boundaries and assess the extent of diversity within *B. forskalii*.

4.2. COI Insights: Pacific and Gulf of California populations

Although COI sequences of *B. forskalii* are currently available only from the Pacific and Gulf of California, limiting the scope of a broader comparison, our COI-based phylogenetic reconstruction revealed an unexpected pattern. The well-supported clade grouped specimens from the California coast and the CMP together, while the Gulf of California specimen formed a separate branch. This result suggests a potential genetic divergence within the population, possibly indicating ongoing speciation. However, given that only a fragment of the COI gene was analyzed, this pattern could also be an artifact of the inherent variability within the gene rather than true lineage separation, as seen in other ctenophores (Johnson et al., 2022). Previous studies have noted that COI in ctenophores exhibits a high mutation rate and rapid evolution, leading to difficulties in amplification and phylogenetic interpretation. Additionally, the mitochondrial genome of ctenophores is highly divergent, with atypical gene order and high AT content, which also complicates the use of COI as a barcoding marker (Christianson et al., 2022). In *B. forskalii*, genetic differentiation has been more successful using ITS rather than COI, due to these limitations (Shiganova and Abyzova, 2022).

Some mechanisms of speciation in ctenophores, especially in those populations with partial geographic overlap (such as is the case of the Gulf of California and eastern Pacific) can include self-fertilization, reproductive isolation through asymmetrical spawning, and oceanic circulation patterns which can reduce interaction (Johnson et al., 2022). For example, a clear geographic break in zooplankton community structure in between Monterey Bay and the Gulf of California was attributed to latitudinal variability in environmental variables such as temperature and salinity (Pitz et al., 2020).

Behaviorally, our specimens exhibited swimming behaviors consistent with that reported off the California coast (Monterey Bay) by Wrobel and Mills (1998); specifically, a spiraling motion with the mouth opening wide to capture prey (see Video S1). There is no record of swimming patterns from specimens in the Gulf of California, or morphological characteristics. Further investigation is needed to determine whether differences in environmental conditions, feeding strategies, or other ecological factors are contributing to genetic and morphological differentiation within the species. Additional samples from the Atlantic, Pacific, Mediterranean and Gulf of California, with amplification of mitochondrial and nuclear markers are also needed to clarify this question.

4.3. Phylogenetic note on Beroe abyssicola

Although not the focus of this study, the results of our analyses aligned with the findings of Simion et al. (2015), who report that *B. abyssicola* falls outside a well-supported clade containing all species other *Beroe* species when 18S sequences are included in the phylogenetic analysis. They hypothesize that *B. abyssicola* evolved independently from the rest of the *Beroe,* suggesting a paraphyletic relationship. Similarly, our 18S trees positioned *B. abyssicola* outside the *Beroe* clade, whereas COI analysis placed it within. However, there is not enough data currently available to determine if these claims are valid, and further investigation is needed to clarify the phylogenetic position of this species within Ctenophora.

4.4. Limitations and future directions

Our findings highlight key questions regarding *B. forskalii,* leading to several recommendations for future research. Sampling of organisms and amplifying the COI and ITS genes across all regions where the species has been reported is essential to assess the extent of genetic differentiation and clarify whether these populations represent isolated lineages or distinct species. The use of degenerate primers, particularly those proposed by Christianson et al. (2022), will help resolve the difficulty of amplifying COI. Additionally, further evaluation of the limitations of the 18S rDNA gene for species delimitation should be carried out.

It is important to note that given their gelatinous structure, these organisms can lose their body shape and other distinctive characteristics upon fixation. Describing the external morphology of these organisms while alive is recommended to ensure that potentially important diagnostic characteristics are included in the identification. Additionally, investigating environmental drivers of genetic divergence, such as oceanographic barriers (e.g., vertical distribution, water characteristics), could provide insights into the mechanisms shaping population structure (Pitz et al., 2020). Lastly, a comparative analysis of macrociliary structures between Pacific and Gulf of California *Beroe* populations would be valuable in determining whether functional differences correlate with genetic divergence.

5. Conclusions

Our integrative analysis of *B. forskalii* provides new evidence supporting the hypothesis that populations from the Mediterranean and Pacific may represent distinct species. Morphological comparisons revealed regional differences between Pacific, Mediterranean-Atlantic groups, and molecular data, particularly from ITS sequences, indicate greater divergence than previously recognized. Additionally, COI data suggest that the Gulf of California population is genetically distinct from the broader Pacific group. These findings offer a tantalizing preview to a larger population genetic dataset for *B. forskalii* and underscore the need for multi-gene approaches to resolve taxonomic uncertainties in Ctenophora, a phylum with challenging molecular characteristics. Comprehensive morphological documentation, in both live and preserved specimens, will also be essential to building a robust taxonomic framework and improving our understanding of ctenophore diversity and biogeography.

CRediT authorship contribution statement

Ruth Percino-Daniel: Writing – review & editing. **Eva R. Kozak:** Writing – original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jeimy Denisse Santiago-Valentín:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Montserrat Arevalo-Nucamendi:** Writing – original draft, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2025.104397.

Data availability

The gene sequences were deposited in the National Center for Biotechnology Information

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