Title Under the Sea: Investigation of telson morphology and cryptic diversity within *Eucopia sculpticauda*, a deep-sea lophogastrid from the Gulf of Mexico (Peracarida: Lophogastrida)

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Abstract

The field of phylogenetics employs a variety of methods and techniques to study the evolution of life across the planet. Understanding evolutionary relationships is crucial to enriching our understanding of how genes and organisms have evolved throughout time and how they could possibly evolve in the future. *Eucopia sculpticauda* Faxon, 1893, is a deep-water peracarid in the Order Lophogastrida Boas, 1883, which can often be found in high abundances in pelagic trawls. The species can be found along the Mariana Trench, in the Mid-Atlantic Ridge, west Atlantic and east Pacific Oceans, and in the Gulf of Mexico and as deep as 7,526 meters. Recent collections of *E. sculpticauda* in the Gulf of Mexico have revealed putative cryptic diversity within the species based on both molecular and morphological evidence. Previous studies have documented two different morphotypes of the telson, the terminal part of the pleon (abdomen) and part of the tail fan. In adults, the morphotypes can be distinguished by lateral constrictions in the telson. This evidence, combined with a previous barcoding study, led to speculation that telson morphology may be a distinguishing character useful to define cryptic diversity within *E. sculpticauda*. This study presents additional molecular data from the mitochondrial genes cytochrome c oxidase subunit I (COI), the large ribosomal subunit (16S), and the nuclear histone 3 gene (H3) to investigate telson morphotypes in relationship to evolutionary history within this species. Molecular data identified two strongly supported clades, lending support for potential cryptic diversification within the Gulf of Mexico. Investigations into telson morphology suggest that this character may be informative, but the morphotypes were sometimes ambiguous and additional characters could not be found that discriminate clades. At present, our data suggests early evidence for cryptic diversification

within Gulf of Mexico populations, but additional morphological characters and geographic sampling are needed before a new species can be described.

Introduction

The field of phylogenetics employs a variety of methods and techniques to study the evolution of life across the planet. Most often, these include the use of morphological and/or genetic data to establish evolutionary relationships among groups of organisms (Costello et al. 2013). Understanding these relationships is crucial to help enrich our understanding of morphological and genetic differences within and among cryptic species. As technology and methodology have advanced over recent decades, the study of these relationships has become more accessible, particularly in the field of molecular phylogenetics. Molecular phylogenetics has the power to identify instances of *cryptic diversity*, where species are morphologically identical or nearly identical but genetically distinct (Knowlton et al. 1993; Bracken-Grissom et al. 2014; Novo et al. 2010) and are subject to the reproductive isolation that is necessary to facilitate speciation (Coyne and Orr 2004; Yang and Rannala 2012; Kulmuni et al. 2020). Previous studies have used molecular phylogenetics to study population differentiation in marine species, which might be early indications of speciation events (Duran et al. 2004; Bracken-Grissom et al. 2014).

Eucopia sculpticauda (Order Lophogastrida Boas, 1883; Family Eucopiidae G.O. Sars, 1885; Faxon, 1893) is a bathy- to mesopelagic crustacean with known habitat ranges reaching as deep as 7,526 meters, with a global distribution including the Pacific and Indian Oceans, the Arctic Circle, and the Gulf of Mexico (Faxon, 1893; Hansen, 1912; Tattersall 1951; Müller, 1993; Kou et al. 2019) (Figure 1). A previous study using DNA barcoding techniques provided evidence for two potentially genetically divergent populations within the Gulf of Mexico (Varela

et al. 2021). In Varela et al. (2021), six individuals from the northern region of the Gulf of Mexico were included and two distinct clades were recovered. These observations provided an opportunity to investigate the species using more genetic data with the addition of a morphological investigation. Existing literature on *E. sculpticauda* is sparse, in part due to its extreme and inaccessible habitat, however, it is documented that several morphological characters make *E. sculpticauda* unique. For example, *E. sculpticauda* does not have reduced eyes as seen in the other seven species of *Eucopia,* and even though most lophogastrids have gills on the 8th thoracopod, they are absent in all species of *Eucopia* except *E. sculpticauda* (Casanova et al. 1998).

Early descriptions of *Eucopia sculpticauda* included two morphotypes with variations in the shape of the telson (Kathman et al. 1986), defined as the terminal segment of the abdomen that makes up the tail fan in combination with the uropods. It has been stated that juveniles possess a telson that is anteriorly broad with an eventual smooth transition to a narrow rounded posterior point, identified as “morphotype A” in this study (Figure 2; Figure 3). As individuals of this species age, adults develop two lateral constrictions, giving the telson an “hourglass-like” appearance with “honeycomb ridges", identified as “morphotype B” (Hansen 1912; Kathman et al. 1986; Casanova et al. 1998) (Figure 2; Figure 3). In Varela et al. (2021) the clades also corresponded to the “A” and “B” morphotypes, which prompted investigation into telson morphology and its potential for differentiating cryptic diversity within *E. sculpticauda*.

This study investigates telson morphology and cryptic diversity within *Eucopia sculpticauda.* The mitochondrial genes for the cytochrome c oxidase subunit I (COI) and the small ribosomal subunit rRNA (16S), and the nuclear histone 3 gene (H3) were used for phylogenetic analysis and the resulting topologies were compared against telson morphotypes.

Our main objective was to increase sampling of *E. sculpticauda* in an effort to provide further and more powerful evidence for cryptic diversity and establish if the lateral constrictions of the telson could be used to differentiate a potentially new *Eucopia* species. This study contributes to the understanding of deep-sea biodiversity and highlights the need to combine molecular techniques and morphological techniques for identification.

Materials and Methods

Specimen Collection

In this study 56 individuals of *Eucopia sculpticauda* were included (Supplemental Table 1). The specimens were collected over the course of six research expeditions into the Gulf of Mexico (GOM) with a combined total of 79 days at sea aboard the R/V Point Sur in the northern GOM. The expeditions were funded by the Gulf of Mexico Research Initiative (GOMRI) as part of the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) consortium.

During the DEEPEND expeditions, sampling occurred twice daily at each sampling site: once at noon and once at midnight, and each sampling occurred at 0-1,500 Meter (M) depths. The expeditions occurred biannually in 2015 and 2016, once in May (regional dry season) and once in August (regional wet season), and once per year in May 2017 and May 2018 as ship time funding allowed. The DEEPEND expedition employed a six-net Multiple Opening/Closing Net and Environmental Sensing System (MOC-10) rigged with six 3-millimeter (mm) mesh trawling nets. The system allowed for the opening and closing of each net at discrete depth ranges, allowing for collected samples to be separated by the depth range they were collected at (0 - 200M, 200 - 600M, 600 - 1,000M, 1,000 - 1,200M, 1,200 - 1,500M, with the sixth net sampling the water column from 0 - 1,500M).

After trawl retrieval, specimens from each net were sorted into large trays and identified to the lowest taxonomic level, as determined by morphology and dichotomous keys during each expedition. After identification, samples were cataloged and preserved in either 70 or 80% ethanol, and immediately stored at -20˚C onboard the vessel. Samples were transported from the vessel in Gulfport to the CRUSTOMICS lab on dry ice, where they were stored at -80˚C until muscle tissue was plucked from each specimen. The specimens and corresponding tissue samples were assigned individual voucher numbers and cataloged into the Florida International Crustacean Collection (FICC) database. The specimens were preserved in 80% ethanol and stored in the FICC Museum for further molecular and morphological studies.

Morphological Observations

The telson of each specimen was examined and measured under a Wild M5 Dissection Scope (Wild Heerbrugg, Switzerland) and photographed under a SW-2 Series Super Widefield Stereo Microscope with 1.3 MP camera (AmScope, Irvine, CA, USA). Body length measurements to the whole millimeter were taken using Mitutoyo CD-8 ASX Digimatic Calipers (Mitutoyo Corporation, Kanagawa, Japan). Based on previous descriptions (detailed in Kathman et al.1986; Kou et al. 2019) specimens were either determined to be morphotype “A” or morphotype “B” if the telson features were distinct and they could confidently be assigned without reservation (i.e. “A” or “B”). Lower case “a” or “b” were used if the telson features were ambiguous and they could not confidently be categorized. The lowercase “a” and “b” indicate that they resembled the telson morphology that matches the letter assigned but we could not be completely certain. Interestingly, early studies documented this shape but identified that as the “juvenile” form before reaching adulthood (Kathman et al. 1986; Hansen 1912; Casanova et al. 1998). To investigate this, every individual in this study was identified as male or female,

measured, assigned a telson morphotype and checked for sexual maturity. Mature females in the order Lophagastrida possess a marsupium pouch formed by seven pairs of plate- like oostegites on the thoracopods (Haupt and Ritcher, 2008; Wittman and Ariani, 2010; Meland et al. 2015; Castellani et al. 2017). All individuals were examined for the presence of large, thin, angled plates with a medial line fringed and setae, that starts from the bases and coxae of the thoracopods. Because only the females have the reproductive characters that allowed us to assign maturity (i.e. oostegites), males were less informative. At first, we assumed that all individuals that were assigned a telson morphotype of "a" or "b" were juveniles, however some did possess oostegites although they were not significantly smaller than those individuals with confident telson morphology assignments ("A" or "B"). Morphological determinations, presence of oostegites and body length are documented in Supplemental Table 1.

DNA Extractions

Genomic Deoxyribonucleic acid (gDNA) was extracted from abdominal muscle tissue with Qiagen DNeasy® Blood and Tissue Kits (Cat. No. 69504). DNA extraction quality and quantity were assessed using 1% gel electrophoresis and a dsDNA High Sensitivity Assay kit with a Qubit 2.0 Fluorometer (Invitrogen, Life Technologies, CA, USA), respectively, following manufacturers protocols. DNA extractions were preserved at -20˚C for downstream molecular work.

Sanger Sequencing

Three partial genes were selected for phylogenetic analysis based on their reliability in resolving taxonomic relationships: the nuclear H3 protein-coding gene, the mitochondrial protein-coding gene, COI, and the mitochondrial 16S gene. Sequencing of the DNA barcoding genes, 16S and COI have been used extensively in species identification studies because they are

variable enough to detect species level differences (Hebert et al. 2003; Wilson-Wilde et al. 2010; Waterborg 2012) with 16S being just as informative as COI in most decapod crustaceans that have been studied (Varela et al. 2021). These genes were amplified via PCR using Promega's GoTaq ® Green Master Mix Protocol (Promega, M7122) and primers listed in Table 1. PCR amplifications were performed using a thermal cycler (Pro-Flex PCR system). Gene fragments were amplified using the following thermal profiles: initial denaturing for 2 minutes at 94˚C; annealing for 35 cycles: 30 seconds at 95˚C, 30 seconds at 37-57˚C (depending on the gene and individual being amplified), 1 minute at 72˚C; final extension 3 minutes at 72˚C, with the respective primers for each target gene region. Details can be found in Table 1. Amplification success for all PCRs was verified using 2% gel electrophoresis. PCR products were sequenced through GENEWIZ® Sanger Sequencing services (Genewiz, Boston, MA, USA) to produce forward and reverse strand reads.

New 16S primers were designed for this study (Euco_16S_Rev1, Euco_16S_Rev2, Euco_16S_For) due to difficulties in PCR amplification with current universal primers (Table 1). These primers were designed using a combination of *Eucopia* 16S partial gene sequences already acquired through Sanger sequencing, mtGenome sequencing, and available sequences published on NCBI's Genbank nucleotide database in May 2019. These sequences were aligned in Geneious Prime v2024.0.3 using the MAFFT E-INS-I with default settings. Conserved 5' end and 3' end regions of the alignment were selected for forward and reverse primers, respectively. Melting temperatures of the primers were calculated using Oligo Calculator version 3.27. Primers were manufactured by Integrated DNA Technologies. All gene sequences used in this dataset are publicly available in NCBI's GenBank Database.

Phylogenetic Analysis and Pairwise Distances

Forward and reverse strands from Sanger sequencing were assembled with the program Geneious Prime v2020.0.3, using the de novo assembly function at the "highest sensitivity / slow" setting (Kearse et al. 2012), and then trimmed manually. Consensus sequences were extracted and screened for pseudogenes and other contamination manually by assessing all six reading frames. Consensus sequences for each gene were aligned using MAFFT E-INS-I with default settings using Geneious Prime, gaps in the 16S alignment were addressed with Gblocks (Castresana 2000). The alignments were 529, 516, and 341 base pairs in length for 16S, COI and H3 respectively. IQtree v2.0.4 was used with the edge-unlinked branch lengths to determine models of evolution and to build maximum likelihood (ML) single-gene trees for each gene alignment using Rapid Bootstrapping with 10,000 replicates (Nguyen et al. 2014).

The single-gene alignments were then concatenated using Geneious Prime into a single dataset, and one maximum likelihood phylogeny was constructed. For that, IQ-TREE again was used to first determine models of evolution, and then using the edge-proportional branch lengths as recommended by the user manual for multi-gene analyses, with Rapid Bootstrapping for 10,000 replicates, and otherwise default settings. Single gene trees were first constructed to examine congruence. To construct a 16S gene tree, 54 *E. sculpticauda* individuals were used (Supplemental Figure 1). *Eucopia unguiculata* (Willemoes-Suhm, 1875) and *Eucopia grimaldii* (Nouvel, 1942), were used as the outgroups, as they are closely related Eucopia species (Varela et al. 2021) and 16S sequences for them were available in the GenBank database. The COI gene tree included 36 *E. sculpticauda* individuals (Supplemental Figure 2) with *Gnathophausia zoea* (Willemoes-Suhm, 1873), *E. unguiculata*, *E. grimaldii*, and *Eucopia australis* (Dana, 1852),

selected as the outgroups, because COI sequences for those species were available in the GenBank database. In the H3 gene tree, 26 *E. sculpticauda* individuals were used (Supplemental Figure 3) with *Cuapetes amymone* (De Man, 1902) selected as the outgroup, as it was the closest species related to *E. sculpticauda* with an available H3 sequence. To construct the concatenated tree (16S, COI, and H3), 56 individuals of Eucopia sculpticauda were included with *G. zoea*, *E. unguiculata*, *E. grimaldii*, and *E. australis* as the outgroups (Figure 3).

Molecular variation of the morphotypes was compared using 16S, COI and H3 sequence pairwise distances calculated using p-distance and pairwise deletion of gaps in MEGA ver. 7 (Kumar et al. 2016).

Results

Morphological Characters

In total, 23 individuals were coded as morphotype "A", 13 as morphotype "B", 11 as morphotype "a" and 9 as morphotype "b". The data can be found in Supplemental Table 1.

Pairwise Distances

The pairwise distances between morphotype "A" and morphotype "B" are 8.7% for 16S, 7.2% for COI, and 2.8% for H3. See Supplemental material (Supplementary Tables 2, 3, and 4, respectively) for comprehensive genetic distance tables.

Phylogenetic Analyses

In total, 116 new sequences were generated, 54 for 16S, 36 for COI, and 26 for H3. The 16S tree recovered two monophyletic groups, with morphotypes "A" and "B" in separate clades (Supplemental Figure 1). For COI, 2 clades were recovered, and morphotypes "A" and "B" formed two reciprocal monophyletic groups (Supplemental Figure 2). In the H3 tree, as in the two other gene trees, 2 monophyletic clades were recovered (Supplemental Figure 3).

In the concatenated tree Morphotypes "A" and "B" formed two monophyletic groups, however only clade "B" was significantly supported by both SH-aLRT and UFBoot analyses (97.6 and 100 respectively). Clade "A" is significantly supported by SH-aLRT analysis (96.9) (Figure 3). In all single gene trees and the concatenated trees, telson morphotype "b" appeared scattered within both clades, while morphotype "a" was consistently recovered within morphotype "A".

Discussion

Cryptic Diversity within Eucopia sculpticauda

The objective of this study was to investigate cryptic diversity within *Eucopia sculpticauda* using telson morphology and phylogenetics. A previous study that barcoded 82 different species of crustaceans, including three species of *Eucopia* had found preliminary evidence for population structure, or potentially cryptic diversity within *E. sculpticauda* (Varela et al. 2021). In this study, six individuals from the northern region of the Gulf of Mexico were included and two distinct clades were recovered that corresponded to telson differences, with both morphotypes being found sympatrically and across similar depths. Our study recovered similar results with the inclusion of significantly more individuals targeted from the same geographic region and depth gradients. We consistently recovered two clades based on three single gene trees and one concatenated dataset for 16S, COI and H3. For the phylogeny built with the concatenated dataset, two of the clades are significantly supported (Figure 3). The branch lengths found in the concatenated tree reveal the occurrence of cryptic diversity, as the lengths for the *E. sculpticauda* clades are similar to those of other species of *Eucopia* (*E. unguiculata and E. australis*) that have been studied (Kou et al. 2019; Varela et al. 2021). Often, pairwise distances can be calculated to lend support for cryptic diversity, however, it can be

difficult to assign a precise percentage to speciation as evolutionary rates differ from species to species. In the case of *E. sculpticauda*, pairwise distances are relatively high, also lending evidence for cryptic speciation. For example, COI divergence between the two clades is 7.2 %, and COI intraspecies divergence is rarely greater than 2% (Hebert et al. 2003) and comparatively in one study it was documented that COI divergence among *Eucopia sculpticauda* individuals was only 0.3% (Kou et al. 2019). 16S divergence was recovered to be 8.7% and interspecies divergence for 16S typically falls between 5-20% (Bartos et al. 2024). It is well known that nuclear genes are more conserved than mitochondrial genes (Kartavtsev et al. 2018), and the H3 distance recovered was also substantial (2.8%). Based on the phylogeny and COI pairwise distance there is evidence of cryptic diversity within *E. sculpticauda*.

Telson Morphology

It is important that morphological characters be used in combination with molecular evidence to discriminate and describe new species. Although scarce, the literature does describe some variation in telson morphology in *Eucopia* (Kathman et al. 1986) and our observations based on research cruises and microscopy confirm previous findings. The original description of *Eucopia sculpticauda* describes the telson as having concave lateral margin much like an hourglass and being "beautifully ornamented with a network of ridges like honeycomb" (Faxon, 1893, morphotype "B", in this study), which is a character we often use to identify this species. However, upon continued examination, we started to see a telson morphology that seemed to lack the typical "hourglass" shape, with the lateral margins narrowing, but not concave (morphotype "A", in this study). When we compare telson morphology against the molecular phylogeny it seems that a well-developed telson ("A" or "B") does have phylogenetical significance, since morphotype "A" and "B" form reciprocal monophyletic clades. However,

this becomes problematic when telson morphology becomes more ambiguous, such as the case for morphotype "b", which can be found scattered across both clades. In conclusion, it appears that well-developed telson can be used to discriminate cryptic diversity, however this character appears unreliable in less developed telsons, and additional morphological characters must be investigated before the description of a new species can be completed.

Conclusions and Recommendations for Future Research

A combination of both morphological and molecular approaches is useful in distinguishing species and populations (Cánovas et al. 2016; Ballou et al. 2021). Evidence from this study suggests that a certain degree of cryptic diversity occurs in the Gulf of Mexico for *E. sculpticauda*. However, although well-developed telson morphology may be a good indicator for species discrimination, this can be a confusing and ambiguous character, and we conclude additional morphological investigation is needed. To date, our investigations have not revealed a reliable morphological character that can be used to discriminate species, but it is possible a more vigorous investigation of their morphologies could reveal one. One limitation to our study is that the material was collected using a MOC10 midwater trawl, which often results in animals being damaged upon retrieval due to long trawl times and collection of many specimens within cod ends. *Eucopia sculpticauda* are extremely fragile and this method of collection is not ideal for detailed morphological investigations, because the individuals can be missing thoracopods or other segments that can help with identification. Future sampling should use methods that preserve the morphology, which may include tucker trawls. This study highlights the necessity of integrating molecular methods, such as phylogenetic analysis, with morphological investigations, to accurately assess biodiversity in the deep sea.

Data Availability Statement

Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) https://data.gulfresearchinitiative.org; https://doi.org/10.7266/N70P0X3T and doi 10.7266/n7-1xs7-4n30 and on NCBI GenBank.

Conflict of Interest Declaration

The authors declare no conflicts of interest.

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Table 1. Primer pairs and annealing temperatures associated with PCR amplification of genes targeted for DNA barcoding of samples.

Target Gene	Forward Primer	Reverse Primer	Annealing Temperature
16S	16S_Euco_F1 5'- GTAAAACGACGGCCAGTGG GCTGCAGTATTTTAACTGTG C-3' (This study)	16S_Euco_R1 5'- CAGGAAACAGCTATGAC CCACCGGTCTGAACTCA AATCATG-3' (This study)	37-56.1°C
		16S_Euco_R2 5'- CAGGAAACAGCTATGAC CTCAACATCGAGGTCGC AAGC-3' (This study)	37-56.1°C
COI	COI_Crusty_F 5'- YTCHWSDAAYCAYAARGAY ATTGG-3' (Varela et al. 2021)	COI_Crusty_R 5'- TANACYTCNGGRTGNCC RAARAAYCA-3' (Varela et al. 2021)	37°C
H3	H3_aF 5'- ATGGCTCGTACCAAGCAGA CVGC-3' (Colgan et al. 1998)	H3_aR 5'- ATATCCTTRGGCATRATR GTGAC-3' (Colgan et al. 1998)	38-40°C

Figure 1. Lateral view of *Eucopia sculpticauda* Faxon, 1893 from the Gulf of Mexico. Photo Credit: Danté Fenolio.

Figure 2. Photographs of the telson of Eucopia Sculpticauda Faxon, 1893. A) HBG 8967 *Eucopia sculpticauda* Faxon, 1893 (Morphotype "A") B) HBG 7361 *Eucopia sculpticauda* Faxon, 1893 (Morphotype "B").

Figure 3. Maximum-likelihood (ML) phylogeny including 23 individuals of the species *Eucopia sculpticauda* morphotype "A", 13 of morphotype "B", 10 of morphotype "a" and 9 of morphotype "b" and outgroup species *Gnathophausia zoea* (Willemoes-Suhm, 1873), *Eucopia*

unguiculata (Willemoes-Suhm, 1875), *Eucopia grimaldii* (Nouvel, 1942) and *Eucopia australis* (Dana, 1852) based on the mitochondrial genes, 16S and COI, and the nuclear gene, H. Shimodaira–Hasegawa-like approximation ratio likelihood test (SH-aLRT) and ultrafast bootstrap (UFBoot) values, respectively, indicated on branches. SH-aLRT support values =>80 and UFB values => 95 indicate strong support. Individuals identified by their voucher number, in the Florida International Crustacean Collection (FICC) catalogue number and by their telson shape.

Supplemental Table 1. Taxonomy, voucher catalog numbers, localities, GenBank (GB) accession numbers for gene sequences used in the study, length of individual in millimeters, presence of oostegites in specimen, Depth of each sample found in meters (M), and longitude and latitude of each sample ; N/A, missing sequence data; GOM, Gulf of Mexico; MAR, Mid Atlantic Ridge; EPO, East Pacific Ocean; IPO, indo-Pacific Ocean.

Supplemental Table 2. Estimates of Evolutionary Divergence between Sequences for the gene 16S. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Kimura 2-parameter model (Kimura 1980; Kumar et al. 2016). This analysis involved 54 nucleotide sequences.

Supplemental Table 3. Estimates of Evolutionary Divergence between Sequences for the gene COI. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Kimura 2-parameter model (Kimura 1980; Kumar et al. 2016). This analysis involved 36 nucleotide sequences.

Supplemental Table 4. Estimates of Evolutionary Divergence between Sequences for the gene H3. The number of base substitutions per site from between sequences are shown. Analyses were

conducted using the Kimura 2-parameter model (Kimura 1980; Kumar et al. 2016). This analysis involved 26 nucleotide sequences.

Supplemental Figure 1. Maximum-likelihood (ML) phylogeny including outgroups (*Eucopia grimaldii* and *Eucopia unguiculata*), based on the mitochondrial gene 16S, with an alignment length of 529 base pairs. The number along the branches represent Shimodaira–Hasegawa-like approximation ratio likelihood test (SH-aLRT) and ultrafast bootstrap (UFBoot) values, respectively. SH-aLRT support values =>80 and UFB values => 95 indicate strong support. Individuals are identified in the tree by their voucher number, which corresponds to their identification number in the Florida International Crustacean Collection (FICC), and by their telson morphotype.

Supplemental Figure 2. Maximum-likelihood (ML) phylogeny including outgroups (*Eucopia grimaldii*, *Eucopia unguiculata*, *Eucopia australis*, and *Gnathophausia zoea*) based on the mitochondrial gene COI, with an alignment length of 516 base pairs. The number along the branches represent Shimodaira–Hasegawa-like approximation ratio likelihood test (SH-aLRT) and ultrafast bootstrap (UFBoot) values respectively. SH-aLRT support values =>80 and UFB values => 95 indicate strong support. Individuals are identified in the tree by their voucher number, which corresponds to their identification number in the Florida International Crustacean Collection (FICC), and by their telson morphotype.

Supplemental Figure 3. Maximum-likelihood (ML) phylogeny including outgroup (*Cuapetes amymone,* De Man, 1902) based on the nuclear gene H3, with an alignment length of 341 base pairs. The number along the branches represent Shimodaira–Hasegawa-like approximation ratio likelihood test (SH-aLRT) and ultrafast bootstrap (UFBoot) values respectively. SH-aLRT support values =>80 indicate strong support. UFB values => 95 indicate strong support.

Individuals are identified in the tree by their voucher number, which corresponds to their identification number in the Florida International Crustacean Collection (FICC), and by their telson morphotype.

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Figure 1. Lateral view of *Eucopia sculpticauda* Faxon, 1893 from the Gulf of Mexico. Photo Credit: Danté Fenolio.

139x84mm (600 x 600 DPI)

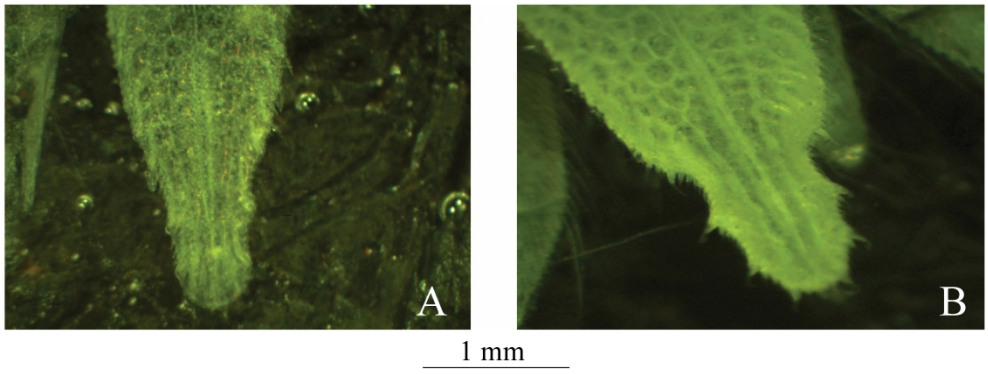

Figure 2. Photographs of the telson of *Eucopia sculpticauda* Faxon, 1893. A) HBG 8967 *Eucopia sculpticauda* Faxon, 1893 (Morphotype "A") B) HBG 7361 *Eucopia sculpticauda* Faxon, 1893 (Morphotype "B").

2464x954mm (72 x 72 DPI)

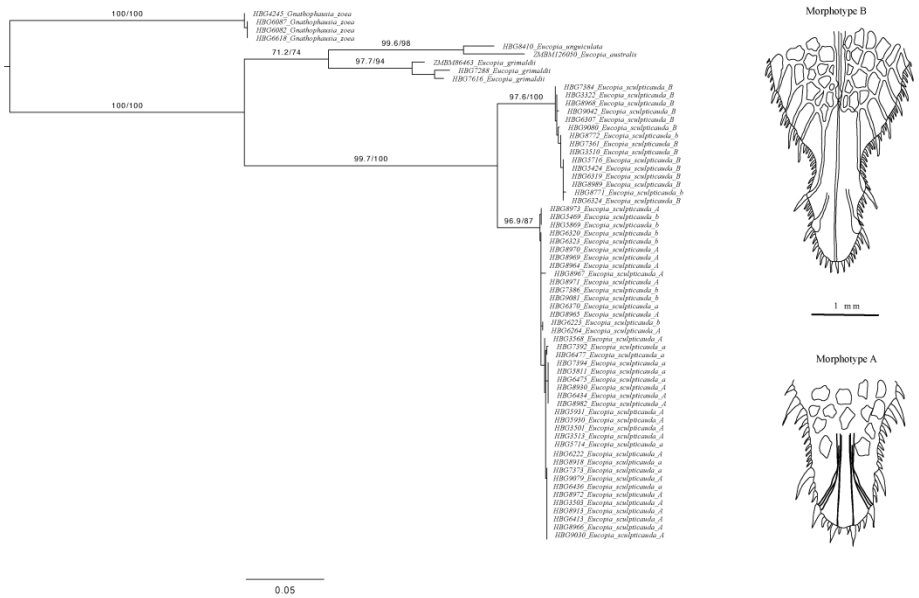

Figure 3. Maximum-likelihood (ML) phylogeny including 23 individuals of the species *Eucopia sculpticauda* morphotype "A", 13 of morphotype "B", 10 of morphotype "a" and 9 of morphotype "b" and outgroup species *Gnathophausia zoea* (Willemoes-Suhm, 1873), *Eucopia unguiculata* (Willemoes-Suhm, 1875), *Eucopia grimaldii* (Nouvel, 1942) and *Eucopia australis* (Dana, 1852) based on the mitochondrial genes, 16S and COI, and the nuclear gene, H. Shimodaira–Hasegawa-like approximation ratio likelihood test (SH-aLRT) and ultrafast bootstrap (UFBoot) values, respectively, indicated on branches. SH-aLRT support values =>80 and UFB values => 95 indicate strong support. Individuals identified by their voucher number, in the Florida International Crustacean Collection (FICC) catalogue number and by their telson shape.

711x441mm (144 x 144 DPI)