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The Diversity and Phylogenetic Relationships of a *Chaetopterus* Symbiont Community in Djibouti, with Redescription of *Chaetopterus djiboutiensis* Gravier, 1906 Stat. Nov. (Annelida: Chaetopteridae)

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Abstract: The tubes of polychaetes of the genus *Chaetopterus* (Annelida: Chaetopteridae) provide habitat for cryptic, symbiotic organisms that are often overlooked when examining diversity. Our study employed molecular phylogenetics to examine the diversity of symbiont species associated with *Chaetopterus djiboutiensis* stat. nov., collected from the Gulf of Tadjoura, Djibouti. A total of 15 *Chaetopterus* hosts and their associated symbionts were collected from nine coastal sites. Four genetic regions were targeted for PCR amplification: the mitochondrial cytochrome oxidase subunit I and 16S rDNA and the nuclear 18S rDNA and Histone H3. *Chaetopterus djiboutiensis* was redescribed from topotypic material and elevated to species rank, and a neotype specimen was designated. Phylogenetic and morphological analysis confirmed five species associated with *C. djiboutiensis* in Djibouti: two porcelain crabs, *Polyonyx pedalis* and *Polyonyx socialis*; one nudibranch, *Tenellia chaetopterana*; one fish, *Onuxodon* sp.; and one amphipod, *Leucothoe* sp. A. As only the fourth comprehensive study on *Chaetopterus* symbionts, our study highlights the diversity and community patterns of symbionts associated with these unique tubicolous marine polychaetes.

Keywords: Polychaeta; symbiotic communities; Indo-Pacific; benthic diversity

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Academic Editor: Michael Wink

Received: 20 March 2025
Revised: 30 April 2025
Accepted: 12 May 2025
Published: 21 May 2025

Citation: Brown, S.D.; Terraneo, T.I.; Moore, J.M.; Paulay, G.; White, K.N.; Berumen, M.L.; Benzoni, F. The Diversity and Phylogenetic Relationships of a *Chaetopterus* Symbiont Community in Djibouti, with Redescription of *Chaetopterus djiboutiensis* Gravier, 1906 Stat. Nov. (Annelida: Chaetopteridae). *Diversity* **2025**, *17*, 366. https://doi.org/10.3390/d17050366

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1. Introduction

Coral reefs are diverse, productive marine ecosystems that account for a high percentage of the ocean's biodiversity [1,2]. Research on these ecosystems often focuses on free-living, conspicuous, easily sampled organisms. However, small symbiotic taxa are highly diverse in coral reefs and contribute to hidden diversity [3]. Larger invertebrates, such as echinoderms and polychaetes, are known to live in association with cryptofauna. Recent studies have begun examining the diversity of these symbiont communities [4–7]. In [7], for instance, the authors examined crinoids and their symbionts in Vietnam and found

70 symbiont species associated with 33 species of crinoids. A review on echiurans [5], an unusual group of unsegmented annelids, highlighted diverse infaunal organisms, such as crustaceans, polychaetes, and mollusks, hidden within the worms' burrows. While studies focused on symbiont communities have recently increased, cryptofauna continue to be overlooked due to their small size and limited dedicated sampling efforts [8]. As coral reefs face ongoing decline due to global and local stressors [2,9], the loss of symbiotic relationships will accelerate biodiversity loss [10,11], highlighting the need to close this knowledge gap.

Chaetopterus (Annelida: Chaetopteridae) is a genus of filter-feeding polychaetes, and benthic species frequently harbor symbionts within their tubes. The genus is found worldwide from intertidal to abyssal depths (down to 2200 m) [12,13]. All but one species are tubiculous and infaunal or found attached to hard substrata [12,14]. The one exception, *Chaetopterus pugaporcinus* Osborn, Rouse, Goffredi, & Robison, 2007 [13], is a round, free-floating *Chaetopterus* sampled from mesopelagic waters off Monterey Bay, California. Infaunal *Chaetopterus* species have U-shaped parchment tubes with two or more openings, and their tubes are recognized as a favorable environment for symbionts as they provide protection from predators and a flow-through of oxygenated, food-rich water [15–17].

The taxonomy of the genus remains poorly resolved and in need of revision [13,14], with many problems stemming from a series of synonymies in the late 19th and early 20th centuries, which culminated in the total synonymy of all nominal species under the Mediterranean species *Chaetopterus variopedatus* (Renier, 1804) [18,19]. Recent morphological analyses and partial revisions [20–22], as well as molecular analyses [13,14,23,24], have consistently recovered *C. variopedatus* as a species complex, and 23 species are currently recognized in the genus [25]. A complete systematic revision of the genus is underway (Moore and Osborn, in preparation) and is beyond the scope of this paper.

Information on *Chaetopterus* symbionts is scanty, and even in areas of high biodiversity, such as the Indo-Pacific, symbiont data are available for only a few species (Table S1, [16,21,26–50]). In the Indo-Pacific, only 15 macroinvertebrate genera have been documented in association with *Chaetopterus* species as of this study, with crustacean and polychaete symbionts accounting for 67% and 20% of these genera, respectively (Table S1). In many cases, few details are available about these associations and the overall community composition of these tube habitats. Eight of the symbiont genera from the Indo-Pacific were found in association with hosts identified as *C. variopedatus*, although the host identification is likely inaccurate. Further examination of the hosts' taxonomy is required to understand more about symbiont association patterns.

Only three studies have exclusively studied the symbiont communities of *Chaetopterus* to date [16,28,51]. In [51], the author examined the abundance of two commensal crab species in the tube of *C. variopedatus*, which has since been identified as *Chaetopterus pergamentaceus* Cuvier, 1830 [52], by [21]. When sampling for a commensal polychaete associated with *Chaetopterus cautus* Marenzeller, 1879 [53], in the Sea of Japan, ref. [28] uncovered three additional polychaete symbionts and two crustacean symbionts. In Nhatrang Bay, Vietnam, [16] sampled two closely related species, *Chaetopterus* sp. and *Chaetopterus* cf. *appendiculatus*, and found seven symbionts in total: one polychaete, *Ophthalmonoe pettiboneae* Petersen & Britayev, 1997 [21]; four crabs, *Eulenaios cometes* (Walker, 1887) [54], *Polyonyx* cf. *heok* (Osawa & Ng, 2016) [43], *Polyonyx* sp., and *Tetrias* sp.; one nudibranch species, *Tenellia chaetopterana* Ekimova, Deart, and Schepetov, 2017 [55]; and one fish, *Onuxodon fowleri* (Smith, 1955) [56]. Ref. [16] is the only publication to date examining the symbiotic community composition of two *Chaetopterus* species comparatively.

Our work employed morphological study and molecular phylogenetics to examine the diversity of symbiont species associated with *Chaetopterus djiboutiensis* Gravier, 1906 stat. nov., collected from near its type locality in the Gulf of Tadjoura, Djibouti. The diverse

marine invertebrates off Djibouti's coastline have been scarcely studied [57], and initial exploratory efforts in the region were performed before molecular phylogenetics in systematics and biogeography became widely used [58,59]. As only the fourth study focused on the symbiont community of *Chaetopterus*, we also provided a comparison with the symbiont communities studied in Vietnam [16].

2. Materials and Methods

2.1. Sample Collection

In March 2020, 15 *Chaetopterus* specimens and their tubes were collected from nine coastal sites off the Republic of Djibouti in the Gulf of Tadjoura (Figure 1, Table S2). *Chaetopterus* were collected by SCUBA at depths of 10 to 30 m. They form fragile tubes in the sediment; therefore, they were excavated from the sediment, and the tubes were placed in plastic bags for later examination. After collection, each *Chaetopterus* specimen was transferred to a glass aquarium, the tube was delicately opened, and the host and all associated symbionts were extracted and photographed with a Canon EOS 5D Mark III (Figure 2; Table 1). For worms and crabs, tissue samples were obtained soon after collection from each specimen and preserved in 99% ethanol for further molecular analysis. The voucher specimens were preserved in either formalin (i.e., annelids) or 75% ethanol (i.e., crabs). All crabs were subsampled and vouchered, whereas due to storage constraints, only five of the 15 *Chaetopterus* hosts were kept for molecular analysis. The identification of all worms was visually confirmed before any disposal and later verified with the photographs. All smaller symbionts, such as amphipods and nudibranchs, were stored in ethanol and later subsampled in the laboratory. Voucher specimens were deposited in the Invertebrate Collection at the Florida Museum of Natural History, University of Florida (UF). One of the three collected fish was subsampled, and the voucher specimen was deposited into the California Academy of Sciences Ichthyology Collection (CAS).

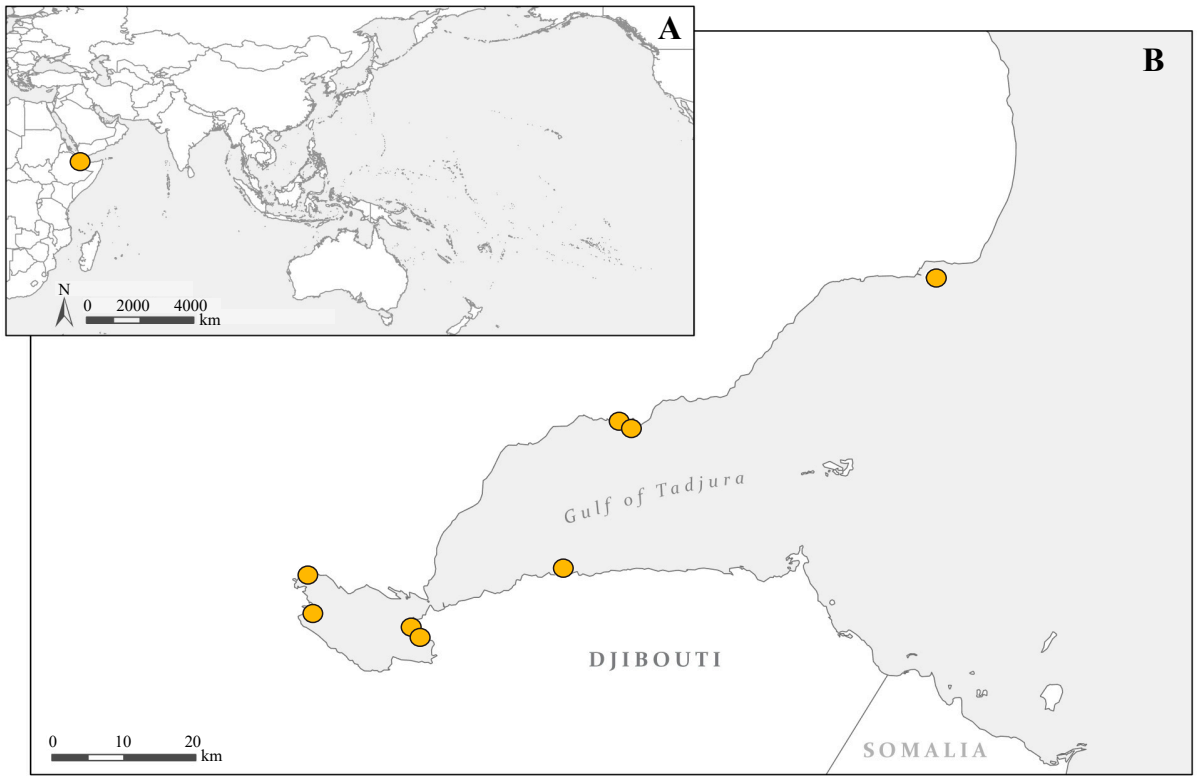


Figure 1. (**A**) Map of Indo-Pacific region. The dot corresponds to our sampling locality in Djibouti. (**B**) Zoom-in of Djibouti area. Yellow dots correspond to samping sites.

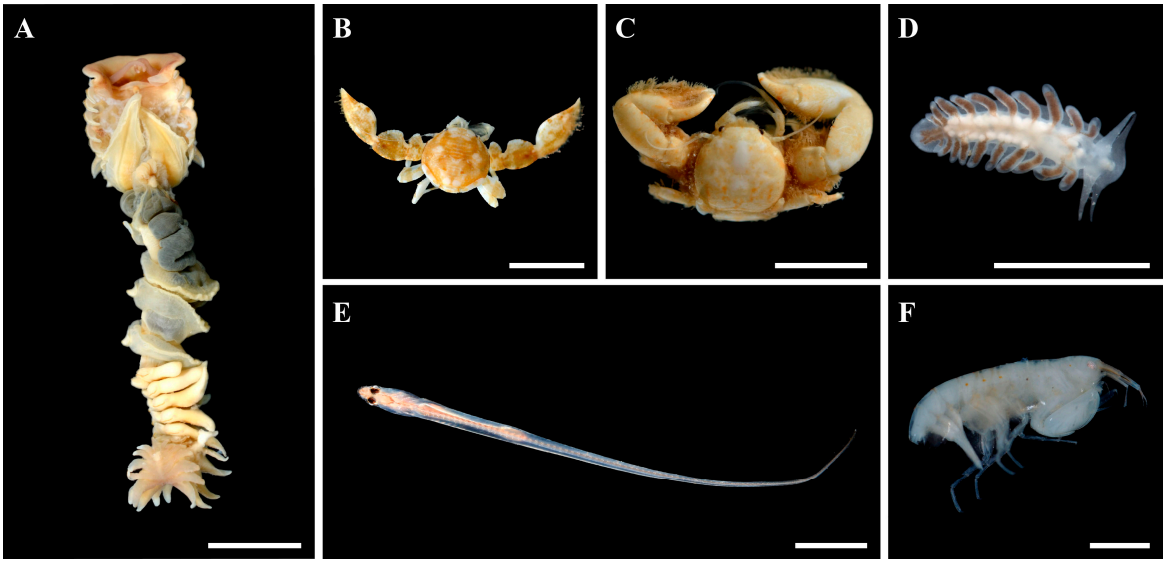


Figure 2. Photographs of the host species and five symbiotic organisms: (**A**) *Chaetopterus djiboutiensis*, host; (**B**) *Polyonyx socialis*; (**C**) *Polyonyx pedalis*; (**D**) *Tenellia chaetopterana*; (**E**) *Onuxodon* sp.; and (**F**) *Leucothoe* sp. (missing urosome, post-subsampling). *Chaetopterus*, *Tenellia*, and *Onuxodon* were photographed live; other organisms were photographed after preservation. Scale bars: (**A**,**E**) 10 mm, (**B**–**D**) 5 mm, and (**F**) 1 mm.

Table 1. List of host specimens sampled and their respective symbiotic community composition. Number of individuals found is denoted in parentheticals if greater than one. The sample site name and host field ID # are provided.

Sample Site	Host ID #	Symbionts
Obock	DJI-CH-01	None
Obock	DJI-CH-02	*Polyonyx pedalis* (2)
Obock	DJI-CH-03	*Polyonyx pedalis*, *Tenellia chaetopterana* (2), *Leucothoe* sp. A
Obock	DJI-CH-04	*Polyonyx pedalis*
Obock	DJI-CH-05	None
Obock	DJI-CH-06	*Polyonyx pedalis* (3) and *Tenellia chaetopterana*
Sable Blanc East	DJI-CH-07	*Polyonyx socialis*
Sable Blanc West	DJI-CH-08	*Polyonyx socialis* and *Leucothoe* sp. A
Ghoubet East	DJI-CH-09	*Polyonyx pedalis* (2)
Ghoubet	DJI-CH-10	None
Devil Island	DJI-CH-11	*Polyonyx pedalis*
Ghoubet East	DJI-CH-12	*Polyonyx pedalis*
Ras Eiro	DJI-CH-13	*Leucothoe* sp. A
The Crack	DJI-CH-14	None
Obock	DJI-CH-15	*Onuxodon* sp. (3) and *Tenellia chaetopterana* (2)

2.2. Morphological Analyses

The host specimens of *Chaetopterus* collected in this study were identified by J.M. using comparison with original descriptions, morphological examinations of specimens from Djibouti, and other comparative material at UF and the Los Angeles County Museum of Natural History (LACM). The species studied here was originally described as a variety, *Chaetopterus variopedatus djiboutiensis* Gravier, 1906 [58], and was first synonymized by [60]. To avoid ambiguity in the identity of the species under study, it is formally redescribed here as *Chaetopterus djiboutiensis* stat. nov., based on a newly designated neotype, and elevated to species rank. For this description, preserved specimens were examined without staining using a Leica stereo dissecting microscope, and measurements were performed using a small

ruler. For examination of the uncini (comb-like neurochaetae), small tissue samples were obtained from the neuropodia, and the uncini were teased from the surrounding tissues using fine needles onto a glass microscope slide and then allowed to air dry. Permanent whole mounts were prepared using Euparal mounting media and allowed to cure before study. Uncini were photographed under 40× magnification using a Nikon D7000 digital camera (Tokyo, Japan) mounted on a compound light microscope. A stage micrometer was photographed and used for measurement calibrations, and the longest length (excluding the stalk) and widest width of slide-mounted uncini were measured from photographs using the software ImageJ v1.6 [61]. Uncini teeth were counted from photographs, excluding the tooth-like structure at the anchor point of the stalk, following [62].

Original descriptions and type material were used for morphological identification of the symbiont specimens, including *Polyonyx* specimens by Bernd Werding and Alexandra Hiller [43,47], *Onuxodon* by Luiz Rocha [pers. comm], *Leucothoe* by K.W. [63–65], and *Tenellia* by S.B. [55,66]. Both preserved specimens and photographs were used.

2.3. Molecular Analyses

DNA was extracted from all subsampled individuals. In addition, for the amphipods specifically, for a more robust dataset, DNA was extracted from the eggs, and an immature juvenile was pulled from the brood pouch of two specimens (Table S2). Twenty-six specimens of Indo-Pacific congeners of the studied symbionts deposited at UF were also extracted to provide a more comprehensive phylogenetic placement for our Djibouti specimens (Table S2). The additional taxa included crab and nudibranchs that were only occasionally recorded as symbionts and were instead selected due to their Indo-Pacific collection locality and desire to increase the size of the phylogenetic comparative dataset. Amphipod extractions were performed using a guanidine extraction protocol [67]. DNA was extracted from all other specimens using DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. Four genomic regions were targeted for PCR amplification in this study: the mitochondrial cytochrome oxidase subunit I (COI) and 16S rDNA and the nuclear 18S rDNA and Histone H3. However, not all regions could be amplified for each organism—*C. djiboutiensis* (COI only), crabs (COI and 16S rDNA), nudibranchs (COI, 16S rDNA, and Histone H3), fish (COI only), and amphipods (COI and 18S rDNA). Primers and PCR protocols are listed in Table S3 [68–74]. All PCR products were checked for amplification via gel electrophoresis, and successful amplifications were sent to TACGen (Richmond, CA, USA) for Sanger Sequencing.

2.4. Phylogenetic Analyses

Forward and reverse sequences were assembled using Geneious Prime® 2021.1.1 (Biomatters Ltd., Auckland, New Zealand). Multiple alignments were performed using the E-INS-i option in MAFFT v7.475 [75] and manually checked with BioEdit v7.2.5 [76]. All sequences were deposited in GenBank (Table S2). Sequences for an additional 16 polychaetes from the genus *Chaetopterus*, 12 crabs from the genus *Polyonyx*, 67 nudibranchs from the genus *Tenellia*, 56 fish from the subfamily Carapinae, and 113 amphipods from the genus *Leucothoe* were downloaded from GenBank or Barcode of Life Data (BOLD) Systems and used for analyses (Table S2). Sixteen *Chaetopterus* sequences from [16] were included to determine how *C. djiboutiensis* was related to *C.* sp. and *C.* cf. *appendiculatus* from Vietnam. In addition, sequence data for *C. djiboutiensis* from the Red Sea (*Chaetopterus* sp. 6 of [14]) were downloaded from GenBank for comparison. Intraspecific and interspecific uncorrected *p*-distances were calculated based on individual gene datasets with MEGA v10.2.4 [77].

Separate phylogenetic reconstructions were performed for the host and each symbiont for each DNA locus. Outgroups were selected for the host and each symbiont based on recent topologies from the literature [16,66,78,79]. Phylogenetic reconstructions were performed under two criteria: maximum likelihood (ML) using the GTRCAT model in RAxML-HPC2 8.2.12 [80] and Bayesian inference (BI) using MrBayes on XSEDE 3.2.7 [81] on the CIPRES Science Gateway [82]. The ML topologies were executed with default CIPRES parameters including a multiparametric bootstrap analysis of 1000 bootstrap replicates. For the BI runs, the best-fit nucleotide substitution model parameters were determined with MrModeltest 2.4 [83]. The Akaike information Criterion (AIC) was used to select best-fitting models. On the CIPRES server, BI reconstructions were performed using four Markov chain Monte Carlo (MCMC) chains for 10 million generations, sampling every 100 generations with a burn-in of 25%. Phylogenetic trees were visualized with FigTree v1.4.4 [84]; ML bootstrap support (BT_{ML}, >70%) and Bayesian posterior probability (PP_{BI}, >0.9) values were plotted onto the phylogenies.

3. Results

3.1. Taxonomic Account

Family: Chaetopteridae Audouin & Milne-Edwards, 1833

Genus: *Chaetopterus* Cuvier, 1830

Chaetopterus djiboutiensis Gravier, 1906 reinstated, stat. nov. Figure 3.

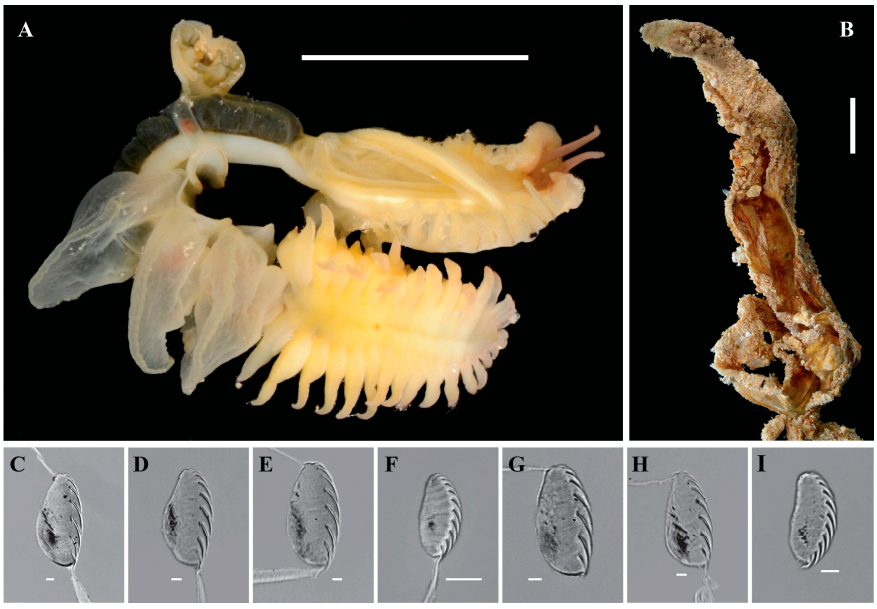


Figure 3. *Chaetopterus djiboutiensis* Gravier, 1906, neotype, UF 2835: (**A**) habitus, lateral view; (**B**) tube fragment; (**C**) A9 uncinus; (**D**) B1 anterior uncinus; (**E**) B1 posterior uncinus; (**F**) B3 piston torus uncinus; (**G**) B3 ventral uncinus; (**H**) C1 lateral lobe uncinus; and (**I**) C1 ventral lobe uncinus. Scale bars: (**A**,**B**) 10 mm and (**C**–**I**) 20 µm.

Chaetopterus variopedatus var. *djiboutiensis* Gravier, 1906 [58]: 186–191, Pl. III, Figs. 205–208, text-Figs. 349–357. Type locality: Recif du Météore, Gulf of Tadjoura, Djibouti; here modified to east of Obock, Djibouti, based on neotype.

Chaetopterus variopedatus: Hartman, 1959 [60]: 396; [85]: 27; [44]: 1, 4, Fig. 4.

Chaetopterus sp. 6: [14]: Figs. 1–3; [24]: 11, Fig. 5.

Type material: The original type material was not located after information requests to the Muséum National d'Histoire Naturelle (NMHN) in Paris and other French collections. See the remarks. A neotype has been designated below for the sake of nomenclatural stability.

Material examined: Neotype (here designated): Djibouti • 1; NE Gulf of Tadjoura, E of Obock, reef slope; 11.9737° N, 43.3358° W; 4–6 m; 28 September 2012: G. Paulay leg.; Genbank COI: [PV216705]; UF 2835.

Other material: Red Sea • 1 f#, specimen with tube; Saudi Arabia, Farasan Islands, Mahama Island; 16.4892° N, 41.9443° E; 4–17 m; 9 March 2013; A. Anker, P. Norby & G. Paulay leg.; UF 3495 • 1 m#; Saudi Arabia, off Thuwal, Abu Shosha Reef; 22.2044° N, 39.0470° E; 7–8 m; 23 March 2013; J. Moore, J. Bouwmeester, A. Anker & P. Norby leg.; UF 3610 • 1; Saudi Arabia, Al Lith, Whale Shark Reef; 20.1170° N, 40.2149° E; 10 m; 22 March 2013; A. Anker, P. Norby & J. Moore leg.; UF 3628 • 2, specimens with tube; same collection data as for preceding; UF 3629 • 1, specimen with tube; Saudi Arabia, off Thuwal, El Fahal, southern point; 22.2227° N, 38.9677° E; 12.2 m; 20 March 2013; J. Moore & C. Braun leg.; UF 3663 • 1, specimen with tube; Saudi Arabia, Yanbu, Ras Majiz; 23.7725° N, 38.2761° E; 10 m; 3 March 2014; G. Paulay leg.; UF 4197 • 1, specimen with tube; Saudi Arabia, Abu Sahim; 22.6587° N, 38.8844° E; 5–9 m; 7 March 2014; G. Paulay leg.; UF 4221 • 1 f#, specimen with tube; Saudi Arabia, Farasan Islands, N Ghorab Island; 17.1095° N, 42.0679° E; 10 m; 18 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; Genbank COI: KX896505, 28S: KX896547, 18S: KX896464; UF 4678 • 1 m#?; same collection data as for preceding; UF 6584 • 1, specimen with tube; Saudi Arabia, Farasan Islands, Baglah; 16.9789° N, 41.3852° E; 0–25 m; 24 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 5604 • 1, specimen with tube; same collection data as for preceding; UF 5605 • 1, specimen with tube; same collection data as for preceding; UF 5608 • 1; same collection data as for preceding; UF 5609 • 1; same collection data as for preceding; UF 6587 • 1; Saudi Arabia, N Ghorab Island; 17.1086° N, 42.0688° E; 4–7 m; 22 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 5606 • 1; Saudi Arabia, Farasan Islands, Dhi Dahaya; 16.8729° N, 41.441° E; 0–25 m; 25 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 5607 • 1; Saudi Arabia, Wasaliyat Shoals, Matbakhaiyan; 17.4656° N, 41.7852° E; 0–25 m; 23 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 5610 • 1 m#; Saudi Arabia, Wasaliyat Island; 17.7828° N, 41.4358° E; 10 m; 17 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 6585 • 1; Saudi Arabia, Wasaliyat Shoals, Mamali Kabir; 17.6066° N, 41.6703° E; 23 October 2014; D. Uyeno, R. Lasley & J. Moore leg.; UF 6586 • 1; same collection data as for preceding; UF 6583. Madagascar • 1; Tuléar, International Indian Ocean Expedition; 10 August 1964; LACM uncatalogued specimen AB363Z.

Diagnosis: Medium- to large-bodied, infaunal *Chaetopterus* inhabiting a U-shaped, tan to brown, relatively thin, flexible parchment-like tube with externally affixed sand and shell debris, segmental distribution 9–12A + 5B + 10–30C. Eyes small, black, and round. With 5–17 large, black cutting notochaetae on segment A4, in a conspicuous ventral fascicle. Parapodia of B1 not posteriorly displaced. Segment B2 very long, dorsal surface not fleshy and elaborated. Region C with short, round, rudimentary dorsal cirri on lateral neuropodial lobes. Tooth distribution of neuropodial uncini as follows: A9 and B1 with 5–8 teeth, B3 piston tori with 7–10, B3 ventral and C1 lateral lobes with 6–7 teeth, C1 ventral lobes with 8–10 teeth.

Description: Based on neotype, with mean and range values in parentheses for all examined specimens. A summary is provided in Table S4.

Gross morphology and presegmental structures. Medium-sized to large infaunal *Chaetopterus*, 40 mm total body length (mean 52 mm, range 30–112 mm, n = 22). Peristomium ventrally broad, horseshoe-shaped in dorsal view, with broad dorsolateral lobes that obscure A1 notopodia, with pink to brown pigment on dorsal surface of ventral lip.

Grooved palps inserted at dorsal inner margins of peristomium, 5.0 mm in length (mean 5.3 mm, range 2.5–9.0 mm, *n* = 20). Eyespots small, black, and round, positioned laterally near the outer bases of the palps and obscured by dorsolateral lobes of peristomium.

Region A. Anterior region with 12 segments (9–12 in other specimens); 13 mm in length (mean 13 mm, range 6–23, *n* = 22) and 11 mm in width (mean 12 mm, 7.0–20.0 mm, *n* = 22), slightly longer than wide. Region A notopodia shortest at A1 or A-last, increasing in length to a maximum at A6–A8 and decreasing again to A-last; A4 notopodia not shorter than neighboring notopodia. Small swellings visible at dorsal base of A notopodia. Segment A4 notopodia bearing 10 (left) and 11 (right; mean 10, range 5–17, *n* = 22) dark brown to black, relatively large, apically blunt cutting chaetae, with a distinct ventral tooth, in conspicuous ventral fascicle. Region A chaetigers with notopodia only, except A-last, A12 also with broad, fan-shaped neuropodia, 3.0 mm in width (mean 3.6 mm, range 2.0–6.0, *n* = 21) furnished with uncini on posterior margin. Ventral surface of A with broad, rectangular glandular shield, 6.5 mm wide (mean 6.4 mm, range 4.5–9.0, *n* = 22).

Region B. Middle body region 17.0 mm in length (mean 25.5 mm, range 14–56 mm, *n* = 20). B1 with long, aliform notopodia, 13 mm in length (mean 12 mm, range 7–16 mm, *n* = 22), reaching anteriorly to peristomium. B1 and B2 neuropodia bilobed, laterally fused with medial separation of lobes, sucker-like, with uncini on anterior and posterior margins. Parapodia of segment B1 not posteriorly displaced, close to the margin of region A. Segment B2 very long, 11 mm (mean 11 mm, range 5–23 mm, *n* = 22) or 27.5% of total body length (mean 22%, range 17–33%, *n* = 22), dorsal surface not fleshy and elaborated. B3 notopodium 8 mm in height (mean 9 mm, range 4–12 mm, *n* = 22). B3 neuropodia with a single pair of medially fused and disc-like lobes, with uncini restricted to posterior margins. B4 and B5 neuropodia medially fused, with a single pair of discrete and symmetrical lobes, bearing uncini on posterior margins.

Region C. Posterior body region 14 mm in length (mean 16 mm, range 6–55 mm, *n* = 21), with 18 chaetigers. Live specimens with pale violet pigmentation on dorsal surface of region C, not visible in preserved material. Region C notopodia broad at base and tapering evenly to apex; C1 notopodia 4 mm in length (mean 5 mm, range 3–7 mm, *n* = 20). Neuropodia bilobed, ventral lobes medially fused, C1 ventral neuropodial lobes broader than those in succeeding segments; lateral lobes of C neuropodia bearing very short, rounded dorsal cirri, 1.0 mm in length in C1 (mean 1.0 mm, range 0.5–1.5 mm, *n* = 19); ventral cirri absent. Neuropodial lobes bear a row of uncini on distal margin.

Uncini. Uncini measurements performed from neotype specimen (UF 2835). A9 neuropodial uncini ovate to pyriform, with 6–7 free teeth (*n* = 24), mean length 97.1 μm (91.4–103.9 μm; *n* = 24), mean width 48.9 μm (44.2–53.2 μm; *n* = 24). B1 uncini of anterior lobe ovate to pyriform with 6–8 free teeth (*n* = 60), mean length 82.6 μm (76.1–90.6 μm; *n* = 61), mean width 42.0 μm (36.1–47.3 μm; *n* = 61). B1 posterior lobe uncini ovate to pyriform, with 5–7 free teeth (*n* = 72), mean length 89.6 μm (78.5–100.7 μm; *n* = 72), mean width 45.2 μm (40.2–49.6 μm; *n* = 72). B3 uncini of piston tori asymmetrically ellipsoid to ovate, with 7–10 free teeth (*n* = 61), mean length 47.0 μm (42.6–53.6 μm; *n* = 58), mean width 24.9 μm (21.3–27.9 μm; *n* = 58). B3 ventral lobe uncini ovate to pyriform, with 6–7 free teeth (*n* = 38), mean length 75.4 μm (66.5–85.5 μm; *n* = 38), mean width 37.5 μm (33.4–43.2 μm; *n* = 37). C1 uncini of lateral lobe pyriform to ovate, sometimes slightly reniform, with 6–7 free teeth (*n* = 101), mean length 84.5 μm (76.7–94.1 μm; *n* = 101), mean width 41.8 μm (33.4–47.3 μm; *n* = 102). C1 ventral uncini rounded ellipsoid to D-shaped, with 8–10 free teeth (*n* = 65), mean length 47.0 μm (42.8–51.1 μm; *n* = 65), mean width 24.1 μm (21.5–26.5 μm; *n* = 65).

Tube. Tube U-shaped, infaunal in soft sediments. Tube tan to brown, opaque, composed of laminated parchment-like proteinaceous material, with externally affixed sand and shell debris.

Remarks: *Chaetopterus djiboutiensis* was described in detail by Gravier [58] as a variety of *C. variopedatus* from Djibouti. Type material may never have been designated for this species, as it is not present in the collections of the MNHN in Paris, where Gravier worked, and could not be located through information requests to other French collections. New collections from near the type locality in Djibouti allowed us to assess the status of this name, and the specimens examined here conform well to the original description.

A specimen from the Red Sea identified as "*Chaetopterus* sp. 6" in [14] matches the neotype morphologically and with >99.5% COI genetic identity. Molecular phylogenetic analyses therein confirmed that it is genetically distinct from *Chaetopterus variopedatus* from the Mediterranean Sea. A comprehensive revision of *Chaetopterus* is forthcoming, but to avoid ambiguity in the identification of the species studied in this paper, we here elevate the variety *Chaetopterus variopedatus djiboutiensis* to the rank of species, an act allowable for variety names published before 1961 under ICZN Article 45.6.4 governing the availability of infrasubspecific names. We also designate a neotype specimen for this species from near the type locality, as well as providing its COI barcode sequence, which allows both morphological and genetic fixation of the species name.

Chaetopterus djiboutiensis is morphologically similar to *Chaetopterus pacificus* Nishi, 2001 [22], described from Japan. *Chaetopterus djiboutiensis* is only known from the western Indian Ocean and Red Sea, and *C. pacificus* is known from Japan and the Philippines. No morphological characters can reliably separate these two species. Genetic characterization of *C. pacificus* from its type locality in central Japan and further sampling of this clade in additional localities may allow further clarification of the limits of these species.

Ecology and distribution: Known from Djibouti and central to southern Red Sea.

3.2. Morpho-Molecular Results

3.2.1. Host: *Chaetopterus djiboutiensis*

The COI alignment for *Chaetopterus* included 23 sequences: 6 from newly collected Djibouti specimens, including the neotype, and 17 from GenBank (Table S2). The COI sequence alignment length was 582 bp with 236 polymorphic sites, 205 of which were parsimony informative. For COI, the analysis under AIC selected the evolutionary model HKY+I+G. The ML and BI phylogenetic trees had largely congruent topologies, recovering six highly supported molecular clades (Clades CH.I–CH.VI) (Figures 4 and S1). The trees did not agree on the relationship of *Chaetopterus* cf. *luteus* (Stimpson, 1855) [86], *Chaetopterus sarsi* Boeck in Sars, 1860 [87], and *C. pugaporcinus*, shown in the basal polytomies in the BI tree (Figure S1). The five sequences from the host *C. djiboutiensis* nested into a highly supported lineage (Clade CH.II: BP_{ML} = 100, PP_{BI} = 1) together with the previously sequenced *C. djiboutiensis* from the Red Sea (*C.* sp. 6 in [14]) and the neotype with very low intraspecific genetic distances (0.2 ± 0.1%). In contrast, the genetic distances between clades were much higher, for example, 9.8 ± 22.3% between Clade CH.II and Clade CH.III and 14.0 ± 41.7% between CH.II and CH.V (Table S5A). *Chaetopterus djiboutiensis* was sister to *C.* sp. (Clade CH.III: BP_{ML} = 100, PP_{BI} = 1), a species collected in Vietnam and found with similar symbionts [16].

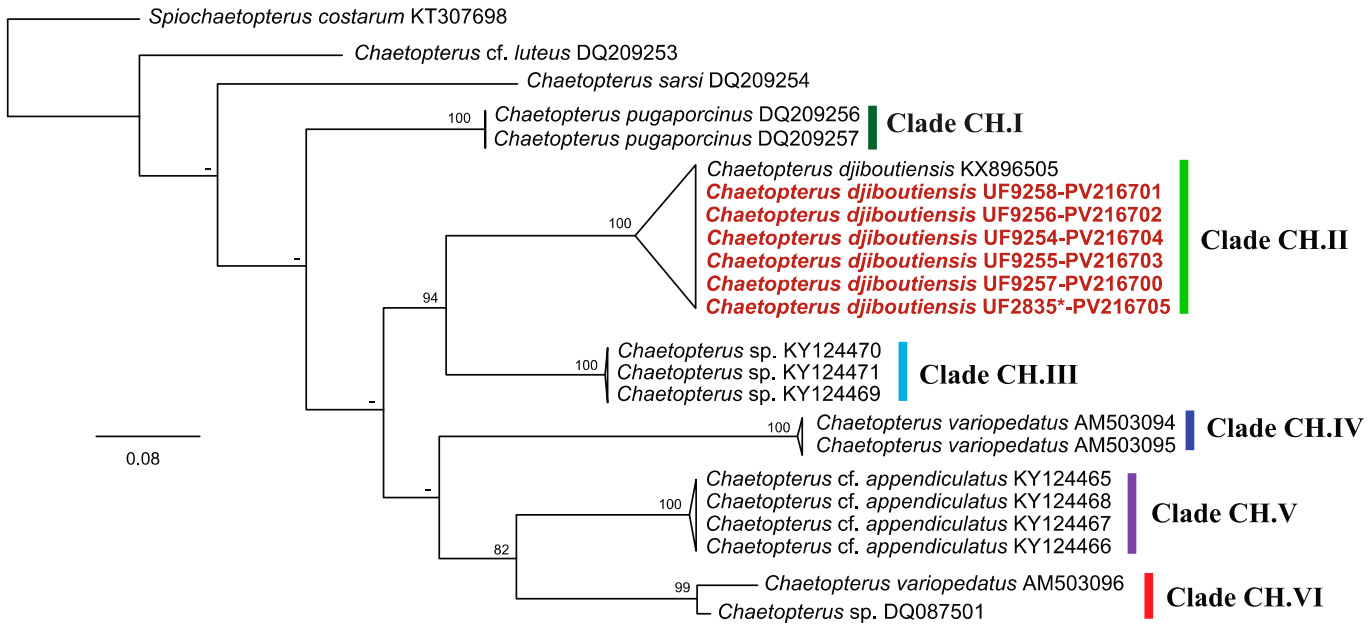


Figure 4. RAxML phylogenetic reconstruction of *Chaetopterus* from the Indo-Pacific based on the COI marker. Newly produced sequences of *Chaetopterus djiboutiensis* from Djibouti are in bold red. Museum specimens' numbers and sequences accession numbers are included for newly collected material, while sequences previously deposited are reported only with their accession numbers. * Neotype. Node values represent ML bootstrap scores (>70%).

3.2.2. Symbionts: Crabs

The smaller crab, *Polyonyx socialis* Werding and Hiller, 2019 [47] (Figure 2B), had a narrow light brown carapace with symmetrical white markings and broadened but flattened chelipeds and walking legs. The morphology aligned with the description provided by [47]. The larger crab, *Polyonyx pedialis* Nobili, 1906 [59] (Figure 2C), had a broader whitish brown carapace with a V-shaped median cleft and a distinguishable rostrum. These morphological characteristics, among others, aligned with the redescription provided by Osawa and Ng, 2016 [47].

The COI alignment for *Polyonyx* included 24 sequences: 13 newly produced (5 from Djibouti and 8 from Saudi Arabia, New Caledonia, and the Philippines (UF codes)), 9 from the BOLD database, and 2 from GenBank (Table S2). The 16S rDNA alignment for *Polyonyx* included 26 sequences: 23 newly produced (10 from Djibouti and 13 from Saudi Arabia, New Caledonia, Madagascar, Scattered Islands, Papua New Guinea, and Australia (UF codes)) and 3 from GenBank (Table S2). The resulting alignments were 658 bp long for COI, with 141 polymorphic and 120 parsimony informative sites, and 524 bp long for 16S, with 128 polymorphic and 103 parsimony informative sites. MrModeltest selected the evolutionary models GTR+I+G and GTR+G. Topologies derived from ML and BI for COI and 16S were mostly in agreement, with the COI trees recovering six highly supported molecular clades (Clades P.I–P.II, P.IV, P.VI) (Figures 5A and S2A), and 16S trees recovering five highly supported molecular clades (Clades P.I–P.V + P.VII) (Figure S3A,B). All clades had previous representatives in GenBank or BOLD, except *P. socialis* (Clade P.I). The conflicting clade numbers between the ML and BI phylogeny could be attributed to the missing species representatives in the 16S phylogenetic tree, specifically, *Polyonyx triunguiculatus* Zehntner, 1894 [88], and *Polyonyx obesulus* Miers, 1884 [89] (Clade P.III and Clade P.V, respectively, in the COI reconstruction). At the same time, *P. biunguiculatus* sequences formed two closely related clades (Clades P.IV and P.VII) in the 16S tree.

The *P. socialis* COI and 16S sequences (Clade P.I: BP_{ML} = 100, PP_{BI} = 1) from Djibouti did not align with any previously deposited sequences but were closely related to sequences of *Heterpoloyonyx biforma* and *P.* sp. A in both topologies. For *P. pedalis* (Clade P.II), four COI sequences (Clade P.II: BP_{ML} = 100, PP_{BI} = 1) and eight 16S sequences (Clade P.II: BP_{ML} = 95, PP_{BI} = 1) from Djibouti were grouped with other *P. pedalis* from Djibouti, Saudi Arabia, and the Philippines (Table S2). Intraspecific genetic distances were low for *P. pedalis* (Clade P.II): 0.4 ± 0.2% for COI. *Polyonyx socialis* (Clade P.I) and *P. pedalis* (Clade P.II) were 17.2 ± 4.9% (COI) divergent (Table S5B).

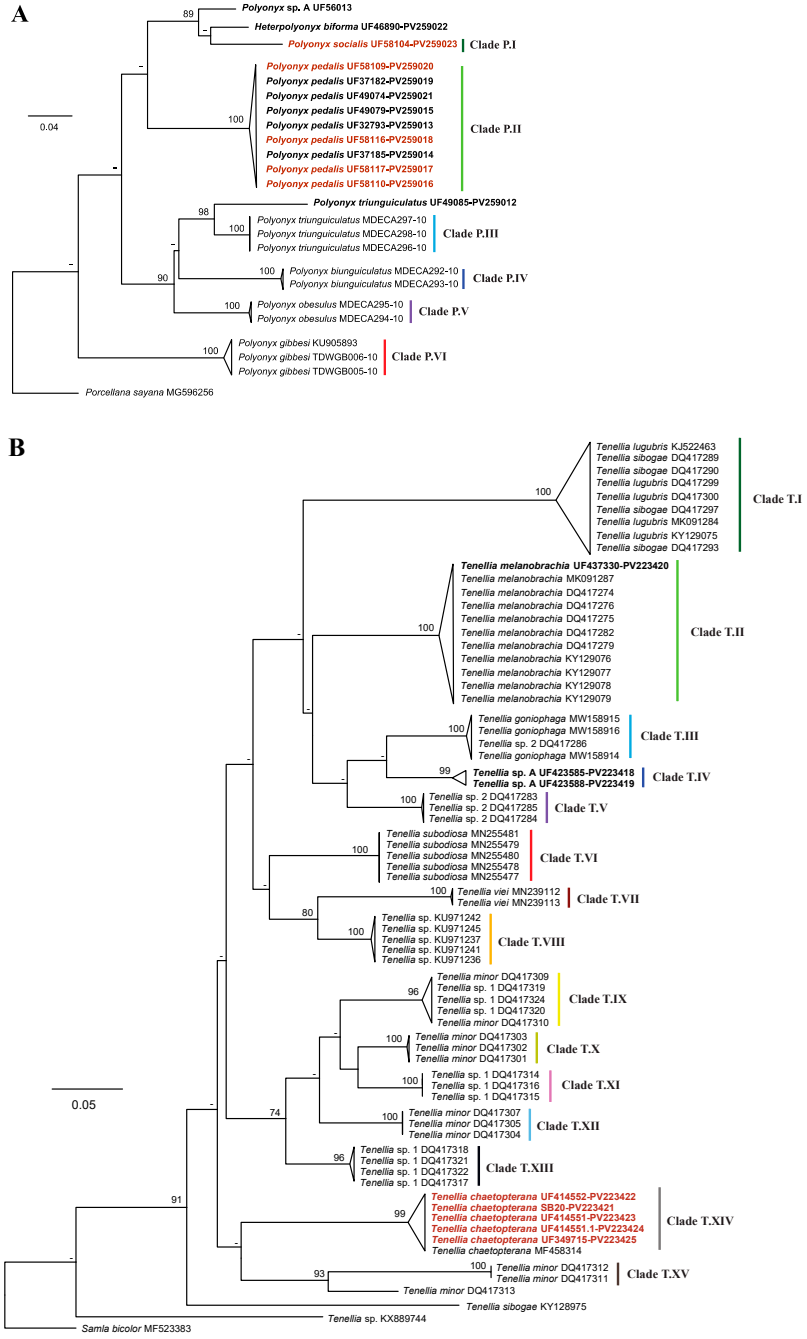


Figure 5. *Cont.*

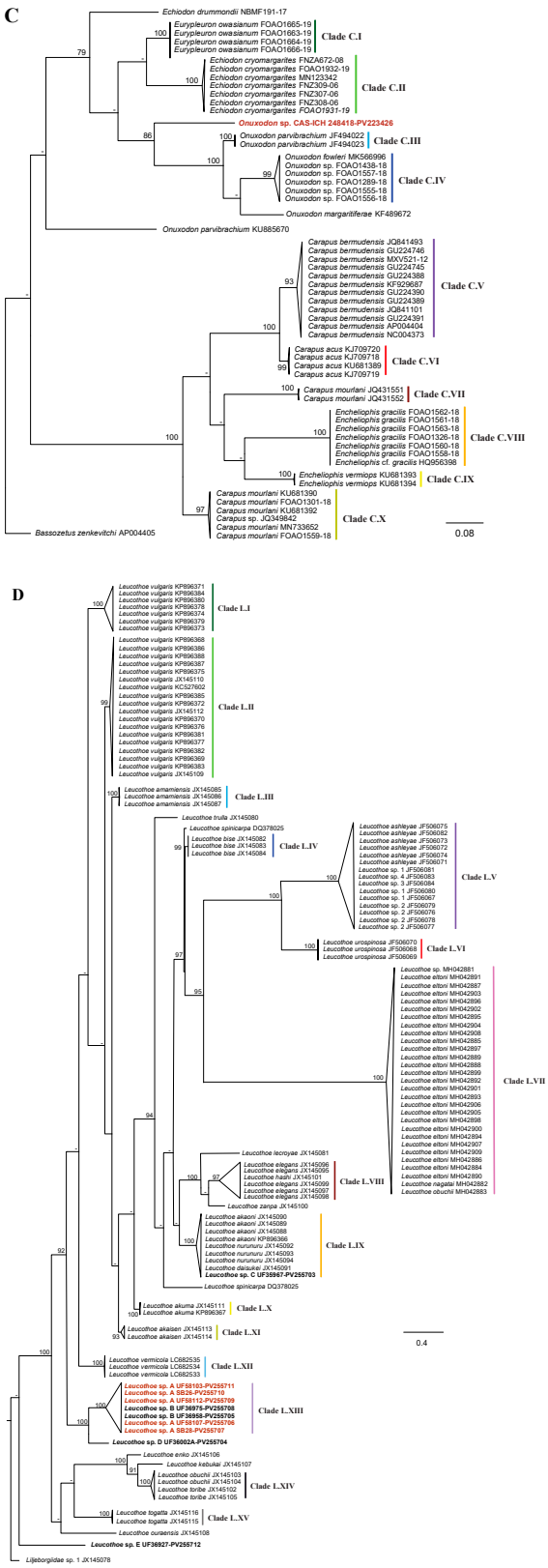


Figure 5. RAxML phylogenetic reconstruction of symbiotic genera associated with *Chaetopterus djiboutiensis* based on COI (**A**–**C**) and 18S regions (**D**). (**A**) *Polyonyx*, (**B**) *Tenellia*, (**C**) *Onuxodon*, and (**D**) *Leucothoe*. Newly produced sequences from specimens from Djibouti are highlighted in bold dark-red, while those from other localities are in bold. Museum specimens' numbers (when available) and sequences accession numbers are included for newly collected material, while sequences previously deposited are reported only with their accession numbers. The numbers on the branches represent support values corresponding to ML bootstrap values (>70%).

3.2.3. Symbionts: Nudibranch

The symbiotic nudibranch (Figure 2D) collected in Djibouti was dorso-ventrally flattened with a wide, slightly rounded foot. Cerata were extended laterally and arranged in two distinct rows of 9–12. Similarly sized rhinophores and conical oral tentacles were distinguishable anteriorly. The specimen had a translucent white body color with internal structures visible through the transparent body wall. The external morphology of our specimen aligned with the detailed description of *Tenellia chaetopterana* by [55].

The COI alignment for *Tenellia* included 71 nudibranch sequences: 8 newly produced (5 from Djibouti and 3 from Madagascar and Australia (UF codes)) and 63 from GenBank (Table S2). The H3 alignment for *Tenellia* included 30 nudibranch sequences: 7 newly produced (4 from Djibouti and 3 from Madagascar and Australia (UF codes)) and 23 from GenBank (Table S2). The resulting alignments were 648 bp long for COI, with 225 polymorphic and 209 parsimony informative sites, and 337 bp long for H3, with 70 polymorphic and 49 parsimony informative sites. The PCR product for 16S was contaminated and removed from the analysis. For COI and H3 BI topologies, MrModeltest selected the evolutionary model GTR+I+G. The ML and BI phylogenetic trees generated using COI recovered 15 highly supported molecular clades (Clades T.I–T.XV) (Figures 5B and S2B), whereas the H3 tree recovered only 6 out of the 15 clades (Clades T.II, T.IV, T.VI, T.VII, T.XIV, T.XV; Figure S3B). The difference in the number of clades was the result of the limited H3 sequence recovered for *Tenellia*. Interspecific genetic distances ranged from 8.7% ± 2.1% (Clades T.X–T.XI) to 31.2% ± 7.7% (*Tenellia* sp.—Clade T.I) for COI and 3.3% ± 1.1% (Clade T.IV—*Tenellia lugubris* (Bergh, 1870)) to 11.6% ± 2.6% (*Tenellia sibogoae* Bergh, 1905—Clade T.II) for H3 (Table S5C,D). The COI sequences from Djibouti nested in a highly supported lineage (Clade T.XIV: BP_{ML} = 99, PP_{BI} = 1) with *T. chaetopterana* from Vietnam [55], as did the H3 sequences (Clade T.XIV: BP_{ML} = 99, PP_{BI} = 1). The intraspecific distances were low, 1.0 ± 0.3% for COI and 0.0 ± 0% for H3. The divergence of *T. chaetopterana* from other clades was high: COI (≥17.2%) and H3 (≥5.8%) (Table S5C,D).

3.2.4. Symbionts: Fish

The symbiotic pearlfish (Figure 2E) collected in Djibouti was a laterally compressed, elongated species with a uniformly translucent coloration. Like the other four *Onuxodon*, the specimen lacked pelvic fins and the process of symphysial fangs on their jaws [90,91]. It was morphologically recognized as an unknown species within the genus *Onuxodon*.

Due to limited sequence availability for the genus *Onuxodon*, a family-level comparison was performed. The COI alignment for the family Carapidae included 57 fish sequences: 1 newly produced from Djibouti, 26 from BOLD, and 30 from GenBank (Table S2). The final alignment consisted of 655 bp with 206 polymorphic sites, 195 of which were parsimony informative. The GTR+I+G was the most suitable evolutionary model. The ML and BI phylogenetic trees had largely congruent topologies, recovering ten highly supported molecular clades (Clades C.I–C.X) (Figures 5C and S2C). The phylogenetic trees were not in agreement about the position of the single sequence of *Onuxodon parvibrachium* (Fowler 1927) [92], which was basal to the family in the BI tree. The *O.* sp. from Djibouti did not cluster with any previously sequenced species but was nested within the *Onuxodon* group. Our specimen was divergent from all within the *Onuxodon* group, as highlighted by the interspecific genetic distances, 21.9 ± 6.4% with Clade C.III (BP_{ML} = 100, PP_{BI} = 0.99), 24.9 ± 7.4% with Clade C.IV (BP_{ML} = 99, PP_{BI} = 1), and 22.5 ± 6.5% with *Onuxodon margaritiferae* (Rendahl, 1921) [93] (Table S5E).

3.2.5. Symbionts: Amphipods

Leucothoe sp. A (Figure 2F) collected in Djibouti has a rounded anteroventral head margin; gnathopod 2, carpus with rounded apex, and propodus with three tubercules; wide pereopod 5–7 bases; and a simple cusp on the posteroventral corner of epimeron 3. The specimen could not be identified to the species level within *Leucothoe*.

The 18S alignment for *Leucothoe* included 124 sequences: 10 newly produced (5 from Djibouti and 5 from Saudi Arabia (UF codes)) and 114 from GenBank (Table S2). The alignment length was 1257 bp with 83 polymorphic sites, 51 of which were parsimony informative. MrModeltest selected the evolutionary model GTR+I+G as the most suitable. The ML and BI phylogenetic trees recovered 15 highly supported molecular clades (Clades L.I–L.XV). The majority of the clades were highly supported and in agreement, except for the position of *Leucothoe bise* White & Reimer, 2012 (Clade L.IV), which was nested together with Clade L.V in the BI tree. In addition, the BI phylogenetic tree had a large, unresolved basal polytomy, which contributed to the disagreement in clade position between the two analyses (Figures 5D and S2D). Five 18S sequences from the examined *L.* sp. A from Djibouti nested within a highly supported lineage (Clade L.XIII: BP_{ML} = 100, PP_{BI} = 0.97) with two morphologically unidentified UF *L.* sp. B collected from Saudi Arabia. The intraspecific genetic distance for Clade L.XIII was very low at 0.3 ± 0.1%. The divergence of this species from nearby clades was 18.6 ± 2.4% from Clade L.VII and 14.1 ± 1.6% from *L.* sp. D (Table S5F).

3.3. Symbiont Community Structure

Four of the 15 individuals of *C. djiboutiensis* were found with no symbionts. The number of species inhabiting the same tube varied from zero to three, and 60% of the tubes sampled contained a unique symbiotic community composition (Figure 6). *Polyonyx pedalis* had the highest prevalence among the symbionts and was usually found in female–male pairings. In contrast, the single *P. socialis* was found alone. The two species of *Polyonyx* were never found together. *Tenellia chaetopterana* was the only symbiont co-occurring with all other symbiotic species, at least once.

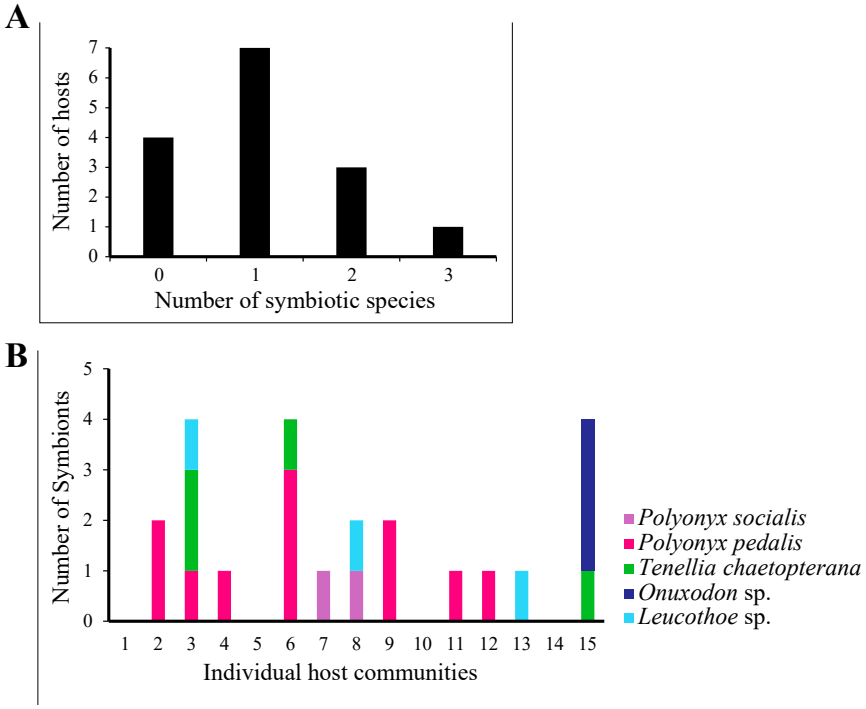


Figure 6. Patterns of the symbiotic communities associated with the host, *Chaetopterus djiboutiensis*, in the individuals associated with symbionts: (**A**) number of symbiotic species per host and (**B**) symbiotic community composition per host.

4. Discussion

Cryptic and symbiotic organisms are often overlooked when examining marine biodiversity, especially in understudied regions such as Djibouti. Our study is the first to examine the symbiotic community composition of *C. djiboutiensis* in detail. We documented five species associated with *C. djiboutiensis*: two porcelain crabs, *P. pedalis* and *P. socialis*; one nudibranch, *T. chaetopterana*; one fish, *Onuxodon* sp.; and one amphipod, *Leucothoe* sp. A.

4.1. Host and Symbionts Identification

The host, *C. djiboutiensis*, was morphologically and genetically distinct from *C. variopedatus* and other sequenced *Chaetopterus* species and conformed to the original description of Gravier [58]. The phylogenetic placement of this species within Chaetopteridae has already been examined [14]. The host species was sister to an undescribed *Chaetopterus* from Vietnam (Figures 4 and S2), with whom it shared two symbionts, *T. chaetopterana* and *P. socialis* [16]. Further work on the phylogeny of the family will provide additional detail on the position of these species.

The porcelain crab symbionts were identified as *P. socialis* and *P. pedalis* and were morphologically and genetically distinct from the other representatives of the genus. *Polyonyx*, a genus in the family Porcellanidae, was divided into three morphological groups: the *Polyonyx biunguiculatus* (Dana, 1852) [94], *Polyonyx denticulatus* Paulson, 1875 [95], and *Polyonyx sinensis* Stimpson, 1858 [96] groups, by Johnson [97]. *Polyonyx socialis* and *P. pedalis* are both members of the *P. sinensis* group. *Polyonyx socialis* was recently recorded with *Chaetopterus* sp. in Vietnam [16,47], and the collection in Djibouti marks the first sampling of the species outside Vietnam, whereas *P. pedalis* has been collected in association with *Chaetopterus* in the Red Sea and is documented from the Red Sea, Gulf of Aden, and western Indian Ocean [43]. The molecular phylogeny of *Polyonyx* and other porcellanid genera remains poorly examined [78].

The nudibranch symbiont from Djibouti was morphological and genetically identified as *T. chaetopterana*, a known associate of *Chaetopterus* [31,66] previously known only from Vietnam. *Tenellia* is a genus of primarily corallivorous nudibranchs in the family Trinchesiidae [66,98–101]. While *T. chaetopterana* showed considerable morphological differences from other *Tenellia* species, our molecular analyses were consistent with previous studies [66,101,102] and supported [66] decision to place *T. chaetopterana* within *Tenellia*.

The fish symbiont was a genetically new *Onuxodon* species. *Onuxodon* is a genus of commensal pearlfish in the family Carapidae that includes only four species: *Onuxodon albometeori* Koeda, 2019 [91]; *Onuxodon fowleri*; *O. margaritiferae*; and *O. parvibrachium*. Based on morphological features, Williams [103] deemed *Onuxodon* and *Echiodon* sister taxa that together formed a sister group to *Carapus* and *Echeliophis*. Our molecular analysis supported these relationships and nested our *Onuxodon.* sp. within the *Onuxodon* group (Figures 5C and S2C). The Djibouti samples were distinct from *O. fowleri*, the only known fish species associated with *Chaetopterus* [16]. The translucent bodies of pearlfish and their cryptic, highly specialized nature [90] suggested hidden diversity within the genus. This hypothesis was supported by the recent description of *O. albometeori*, opportunistically sampled from commercial trawl bycatch in Taiwan [91].

Three amphipod symbionts were morphologically and genetically confirmed to be the same species of *Leucothoe*. Morphologically, our symbiont was most similar to *Leucothoe richiardii* Lessona, 1865 [63], a species previously observed in *Chaetopterus* in the Mediterranean Sea. The similarity was attributed to the shared existence of a cusp on the posteroventral corner of epimeron 3. In addition, our *Leucothoe* specimens shared a similar color pattern with *L. richiardii* (orange and red bands on the pereon, red coxa 4, and red antennae) [104]. Based on the morphological examination, another possibility

was them belonging to *Leucothoe furina* (Savigny, 1816) [64], a species previously observed in *Chaetopterus* tubes in Phuket [105] and throughout the Indo-Pacific, including the Red Sea [33]. However, unlike our specimen, *L. furina* lacks a cusp on the epimeron 3; in addition, the cosmopolitan distribution of *L. furina* calls for further examination. Sequences are unavailable for either of these morphologically similar *Leucothoe* species, but *Leucothoe* collected from *C. djiboutiensis* in the Red Sea and deposited at UF formed a highly supported clade with our symbionts (Figures 5D and S2D).

4.2. Symbiont Distribution and Association Patterns

Polyonyx species are widely distributed across the Indo-Pacific and are found in association with sponges, corals, and tube-dwelling polychaetes [29,43,47]. *Polyonyx socialis* was originally described from Vietnam, and our results represent the first record outside Vietnam and contribute an important range extension into the Indian Ocean. In Vietnam, the species shared the tube of *Chaetopterus* sp. with a larger crab, *Polyonyx heok* Osawa & Ng, 2016 [43], which is restricted to the western Pacific and belongs to the *P. pedalis* complex [16]. In Djibouti, *P. socialis* were not found in association with other crabs (Figure 6B), which may be the result of limited sampling. *Polyonyx pedalis* has previously been sampled in the Red Sea in association with *C. djiboutiensis* [43]; therefore, its extension into Djibouti as a symbiont of the same species demonstrates a potential host specificity in the region. Heterosexual pairs of *P. pedalis* were observed, in addition to one solitary individual and one female–female–male pairing (Figure 6B). Our observations were comparable to those of [43], who also observed *P. pedalis* as heterosexual pairings.

Tenellia chaetopterana was recently described from Vietnam [16,66]. Our results provide a substantial range extension into the Indian Ocean and suggest a potentially broad Indo-Pacific distribution. *Tenellia chaetopterana* has only been found in association with *Chaetopterus,* which is substantially different from other *Tenellia* species that inhabit coral [16]. *Tenellia chaetopterana* was solitary or found in pairs (Figure 6B), as in Vietnam [16]. *Tenellia chaetopterana* was the only symbiont that co-occurred with all other symbionts (Figure 6B), which suggests that interspecific interactions have little impact on this nudibranch species. Both intraspecific and interspecific interactions can influence symbiont occurrence [106,107]. Crustaceans, for example, have been observed to avoid conspecifics or to act aggressively toward individuals of different species sharing their habitat [108,109]. Further studies on *Chaetopterus* and their symbionts could reveal the overall influences of these factors on association patterns.

Onuxodon species, distributed throughout the Indo-Pacific, are recognized as commensal symbionts. Except for *O. albometeori*, which was found without a host [91], the three other known species have been found in association with bivalves, sea stars, and sea cucumbers [56,110,111]. Recently, *O. fowleri* was recovered from *Chaetopterus* sp. in Vietnam [16]; therefore, our symbiont is only the second known *Onuxodon* association with an annelid. Further sampling is required to deduce the ecological implications of this association, especially with a genus with so few species. *Onuxodon* symbionts are commonly found with other individuals of the same species [16,110,112]; however, cohabitation between *Onuxodon* species is uncommon [110]. The *Onuxodon* sp. associated with *C. djiboutiensis* in Djibouti was observed as a trio (Figure 6B), which is comparable to the association patterns of *O. fowleri* [16]. Due to the limited sampling of *Onuxodon* species, further sampling is required to draw ecological conclusions about the species.

Leucothoe are symbiotic associates of large, filter-feeding sessile invertebrates worldwide, especially in coral reefs, mangroves, and seagrass beds [33,113]. They are found in pairs or groups and often in male/female pairings. Five *Leucothoe* species have been found in association with polychaetes, three in *Chaetopterus* [114], only one of which was found

in the Indo-Pacific (Table S1). While our *Leucothoe* symbiont is not identified to species, it is the first reported association of the genus with *C. djiboutiensis*, and the molecular results indicate that the distribution of this amphipod species and the specificity to its Chaetopterus host extends to the Red Sea (Figure 5D). While *Leucothoe* sp. A were found solitary in association with *C. djiboutiensis* (Figure 6B), this may be the result of sampling bias as the mobility and size of *Leucothoe* allows for easy escape during sampling.

In our study, 63% of *C. djiboutiensis* sampled in Djibouti were found with symbionts, with zero to three species and zero to five individuals per tube (Table 1, Figure 6). Many factors may influence the structures of these communities, including the host and symbiont size, interspecific and intraspecific interactions, and species density [16,31,108,109,115,116]. Ref. [109] studied the symbiont communities of sea cucumbers (Echinodermata: Holothuroidea) and noted that intraspecific relationships impacted territorial symbiont species (e.g., crabs and fish). In our study, intraspecific competition was predicted between the two crab symbionts, *P. pedalis* and *P. socialis*, which were never found together. The number of symbionts tends to increase with the host size, as demonstrated in hermit crabs, sea cucumbers, and brittle stars [31]. While host measurements were not performed in this study, our largest symbiont, *Onuxodon* sp., was found only in association with the second smallest symbiont, *P. chaetopterana*; thus, space availability and niche partitioning likely influenced the community structure. Further surveys of *Chaetopterus* hosts and symbionts are required to unveil more about community structure and the forces driving it.

Chaetopterus djiboutiensis was a sister species of *Chaetopterus* sp. [16], sampled in Djibouti and Vietnam, respectively, and they shared two symbiont species and a third belonging to the same species complex, which could relate to the phylogenetic position of the two species. While Djibouti and Vietnam are ~10,000 km apart, both have diverse, fringing coral reef ecosystems [117,118], and the overlap between these symbionts also might suggest host specificity for several of these symbiont genera.

Cryptic symbiont diversity in marine invertebrates likely plays an important yet poorly understood role in shaping ecosystem functions, such as nutrient cycling and stress tolerance. These unknown ecological dynamics are likely important in maintaining ecosystem balance and adaptability, especially in complex marine environments. Uncovering the diversity and symbiotic relationships is a first step toward unraveling the intricate ecological roles and processes that may be pivotal for conservation strategies, particularly in under-sampled regions such as Djibouti.

As only the fourth detailed study of *Chaetopterus* symbionts, more research is needed to learn about their community patterns and cryptic symbiont diversity. From our dataset, the relationship between chaetopterids and their associated symbionts points toward commensalism. For example, all the symbionts collected in this study benefited from the shelter, food, and oxygenated water provided by the chaetopterid, and upon visual inspection during collection, no physical damage was observed on the worms as a result of their symbionts. Nevertheless, further research is necessary to characterize the ecological nature of such association. Additional phylogenetic analysis of *Chaetopterus* is also necessary to classify these associations. The majority of *Chaetopterus* species were previously synonymized into a single, morphologically variable, "cosmopolitan" species, *C. variopedatus* [14]. As a result, information about symbiont community composition on a species level was lost. Our study and [16] highlight the importance of examining communities on a species level as comparative analyses provide valuable insights about the ecology and biogeography of the symbionts.

5. Conclusions

This study highlights the critical ecological and conservation significance of cryptic symbiont diversity, particularly in understudied regions such as Djibouti. The documentation of several symbiotic species associated with *Chaetopterus djiboutiensis* provides a glimpse into the complexity of symbiotic relationships that are often overlooked. The observed host specificity and shared symbionts between geographically distant populations suggest that these associations could be shaped by evolutionary and ecological factors and may be especially vulnerable to host decline or environmental change.

Conservation efforts must recognize that the loss of a single host species may cascade into the disappearance of multiple dependent, and often undescribed, organisms. To safeguard marine biodiversity, it is essential to adopt integrative approaches that include cryptic symbionts and emphasize taxonomic precision. Finally, by focusing on these hidden components of biodiversity, particularly in regions with limited baseline data, conservation strategies can become more effective and inclusive.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d17050366/s1: Figure S1: *Chaetopterus* Bayesian inference tree; Figure S2: Symbiont Bayesian inference trees (A–D); Figure S3: RAxML *Polyonyx* 16S tree (A) and *Tenellia* H3 tree (B); Table S1: List of known *Chaetopterus* symbionts in the Indo-Pacific; Table S2: List of specimens used for molecular analysis; Table S3: Primer list and PCR protocols used; Table S4: Summary of morphological characters for *Chaetopterus djiboutiensis*; Table S5: (A–F): Pairwise comparison of genetic distance values within and between species examined.

Author Contributions: Conceptualization, S.D.B. and F.B.; data curation, S.D.B., T.I.T. and G.P.; formal analysis, S.D.B., T.I.T. and J.M.M.; funding acquisition, M.L.B. and F.B.; investigation, S.D.B., T.I.T., G.P. and F.B.; methodology, S.D.B., T.I.T. and J.M.M.; project administration, F.B.; resources, F.B.; software, S.D.B. and T.I.T.; supervision, T.I.T. and F.B.; validation, S.D.B., T.I.T., J.M.M., G.P., K.N.W., M.L.B. and F.B.; visualization, S.D.B. and T.I.T.; writing—original draft, S.D.B. and T.I.T.; writing—review and editing, T.I.T., J.M.M., G.P., K.N.W., M.L.B. and F.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by KAUST baseline research funds to F.B. and M.L.B.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Newly produced sequences are available at NCBI.

Acknowledgments: This research was undertaken in accordance with the policies and procedures of the King Abdullah University of Science and Technology (KAUST). Permission relevant for KAUST to undertake the research was obtained from the applicable governmental agencies in the Kingdom of Saudi Arabia.

Conflicts of Interest: The authors declare no conflicts of interest.

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