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PHYLOGENETIC ANALYSIS OF *RIDGEWAYIA* (COPEPODA: CALANOIDA) FROM THE GALAPAGOS AND OF A NEW SPECIES FROM THE FLORIDA KEYS WITH A REEVALUATION OF THE PHYLOGENY OF CALANOIDA

Diego F. Figueroa

(DFF, figuerod@onid.orst.edu) College of Oceanic and Atmospheric Sciences, 104 COAS Admin Bldg, Oregon State University, Corvallis Oregon 97331-5503

ABSTRACT

The mitochondrial gene cytochrome-c oxidase subunit 1 (COI) and the nuclear ribosomal DNA region known as Internal Transcribed Spacer 1 (ITS1) are used in a phylogenetic analysis of *Ridgewayia* from the Galapagos Islands and of a new species, *Ridgewayia tortuga*, from the Florida Keys. In addition, the phylogeny of Calanoida is reconstructed based on the 18S ribosomal RNA gene. The following characters exclude *R. tortuga* from the three recognized species groups of *Ridgewayia*: the presence of only 7 setae on the terminal endopod segment of leg 2; a 20-segmented male right antennule with two geniculations, the first between segments 9 and 10 and the second between segments 16 and 17; and details of the male fifth leg, in particular the elongate, unarmed, right endopod with a bifurcated tip. The molecular analysis shows that the first half of the COI gene not only fails to differentiate the various species of *Ridgewayia*, but it also fails to differentiate between the families Ridgewayiidae and Pseudocyclopidae. The second half of this gene and the ITS1 region are species specific. Molecular and morphological evidence suggest that the Galapagos ridgewayiids are the result of one colonization event and that the current phylogeography of these animals can be explained by a combination of vicariance and active migration models. The 18S ribosomal RNA gene proves successful in the reconstruction of the phylogeny of Calanoida with the following main conclusions: 1) Centropagoidea is the sister branch to all other Calanoida; 2) Ridgewayiidae and Pseudocyclopidae likely share a common ancestor with Augaptiloidea; 3) Ridgewayiidae and Pseudocyclopidae should be included in the same superfamily, the Pseudocyclopoidea; and 4) Spinocalanoidea likely needs to be included in Clausocalanoidea to recover the monophyly of the latter superfamily.

KEY WORDS: Calanoida, *Ridgewayia*, phylogeny, biogeography, molecules

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INTRODUCTION

Members of Ridgewayiidae are demersal calanoid copepods found worldwide in subtropical and tropical waters. Its species have a high degree of endemism with strictly localized distributions. These near-shore animals have been found associated with sea grass, actinarians, oysters, coral rubble, and in anchialine habitats (Wilson, 1958; Humes and Simth, 1974; Ferrari, 1995; Razouls and Carola, 1996; Barthélémy et al., 1998, 1999; Ohtsuka et al., 2000; Boxshall and Halsey, 2004; Figueroa and Hoefel, 2008). Due to their bottom-dwelling nature, ridgewayiids are rarely found in plankton tows and are difficult to collect. I present here a description of a new species of *Ridgewayia* from the Florida Keys, along with a molecular analysis including two species of *Ridgewayia* from anchialine pools in the Galapagos Islands. Additionally, I demonstrate the close phylogenetic relationship of Ridgewayiidae and Pseudocyclopidae and their placement within the phylogeny of Calanoida.

Two molecular markers were used, one from mitochondrial genes, cytochrome-c oxidase subunit 1 (COI) and the other a nuclear ribosomal DNA region known as Internal Transcribed Spacer 1 (ITS1). Mitochondrial genes have proved useful in resolving copepod phylogenies (Edmands, 2001; Rocha-Olivares et al., 2001; Bucklin et al., 2003; Machida et al., 2006). Folmer et al. (1994) developed a set of universal primers to amplify the first half of the mitochondrial Cytochrome Oxidase I gene. This 5′ end of the COI gene is typically referred to as the ''Folmer'' region and it has become the most popular marker in molecular systematics. It is the genetic marker used by the Barcode of Life initiative that is developing DNA ''bar-coding'' as a global standard for the identification of all species. This particular gene has provided much effective insight regarding systematic distinctions and relationships in copepods (Folmer et al., 1994; Bucklin et al., 2003; Goetze, 2005; Lee, 2007; Newer et al., 2008). Custom primers were also designed for this study to sequence the second half of the COI gene.

The ribosomal DNA region known as ITS1 has also been used as a successful marker for phylogenetic and population analyses in crustaceans (Chu et al., 2001), including copepods (Schizas et al., 1999; Rocha-Olivares et al., 2001; Elvers et al., 2006; Ki et al., 2009). This is a non-coding region acting as a spacer between ribosomal genes. Because it is not transcribed, it lacks selective constraints, following a neutral model of evolution (Marinucci et al., 1999). In addition to these two markers, sequences were obtained from GenBank (Benson et al., 2005) of the highly conserved 18S ribosomal RNA gene in a wide range of species, and they are used to reconstruct the phylogeny of Calanoida.

Materials and Methods

Field Sampling

Copepods were collected from two anchialine pools on two different islands in the Galapagos Archipelago, Ecuador, and from coral rubble washings from the Florida Keys. The first site, known locally as Grietas Delfin (00°45.426′S 90°18.932′W), is located on the island of Santa Cruz, Galapagos; it is described in detail by Iliffe (1991). Samples were taken on 30 January, 6 February and 16 February 2005, using two simple nets, one with 333 µm mesh and a mouth opening of 30 cm and one with 102 µm mesh and a mouth opening of 60 cm. These nets were towed at various depths by swimming with snorkeling equipment or by pulling a line from shore. Numerous specimens of *Ridgewayia delfine* Figueroa and Hoefel, 2008 and of two undescribed species of *Pseudocyclops* (Figueroa in preparation) were collected at this site.

The second site (00°57.565′S 90°59.417′W) is located on the southeastern side of the Island of Isabela, near the town of Puerto Villamil. The pool is inside a lava tunnel, formerly known as Cueva de la Cadena; and it is described in detail by Peck and Peck (1986) and by Montoriol-Pous and Escola (1978). Samples from this site were taken on 23 March 2005, and 10 April 2005. A simple net of 333 µm mesh with a 30 cm mouth opening was used to collect the samples. The net was towed, with the aid of snorkeling equipment, through the pool at various depths, including near the bottom and at the surface, for a distance of about 25 m into the tunnel. Numerous specimens of *Ridgewayia tunela* Figueroa and Hoefel, 2008 were collected from this site.

Specimens of an undescribed species of *Ridgewayia* were collected from Garden Key in the Dry Tortugas, Florida Keys (24°37.6′N 82°52. 3′W) on 16 November 2006. Coral rubble was collected from the intertidal zone, at a depth of approximately one-half meter. Sea water was used to wash the rubble and the washings were strained through a 225 µm mesh. The samples recovered from all three sites were immediately split and preserved after collection, one half placed in a 10% buffered formalin solution and the other half in 97% ethanol. Morphological terminology is based on Boxshall and Halsey (2004) with the exception of the interpretation of the structure of the maxilla and maxilliped, which is based on Ferrari and Ivanenko (2008) and Ferrari (1995), respectively.

Molecular Analysis

Copepods preserved in ethanol were re-hydrated in Milli-Q water and DNA extraction was accomplished by standard proteinase-K digestion, using Qiagen's DNAeasy kit. Polymerase chain reaction (PCR) primers LCO 1490 (5′GGTCAACAAATCATAAAGATATTGG 3′) and HCO 2198 (5′TAAACTTCAGGGTGACCAAAAAATCA 3′; Folmer et al., 1994) were used to obtain sequences from the Folmer Region of the COI gene. Custom primers were designed to sequence the second half of the COI gene; these were based on preliminary sequences obtained from universal primers for specimens of both *Ridgewayia* and *Pseudocyclops*. These custom primers are H2612-COI (5′AGGCCTAGGAAATGTA TAGGGAAA 3′) and L592-RCOI (5′AACCTTAATACATCTTTTTAT GATG 3′). Primers F1665-18S (5′CCGTCGCTACTACCGATTGAACG 3′) and R73-5.8S (5′GTGTCGATGTTCATGTGTCCTGC 3′) (Dr. Riyuji J. Machida, University of Tokyo, personal communication) were used to obtain the ITS1 region of ribosomal DNA. PCR was carried out in a 50 µl reaction with the following reagents: 3 µl of Accuprime *Taq* polymerase, 10 µl purified DNA, 2.5 µl of each primer, 5 µl of Accuprime Buffer II (includes dNTPs and MgCl2), and 28 µl water. Thermocyler conditions were: 3 minutes at 94°C; followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1.5 minutes at 68°C; followed by 5 minutes at 68°C and then cooling to 4°C. The PCR product was cleaned using the standard Montage PCR product cleaning kit. Both the forward and reverse strands were sequenced at the Center for Genome Research and Biocomputing at Oregon State University.

The presence of nuclear mitochondrial pseudogenes (numts) can be problematic as they can be amplified instead of the target mitochondrial genes. Typical evidence of numts contamination includes PCR ghost bands and sequence ambiguities (Bensasson et al., 2001), neither of which were observed. PCR product was loaded on a gel and, after electrophoresis, it was checked under UV light, bands for all specimens were clear and of consistent size. The chromatogram for each sequence was visually inspected and double peaks were not present. Also, the forward and reverse sequences for each specimen were consistent with each other and both were used to generate a strict consensus sequence.

Sequences for a region of the 18S RNA gene were obtained for 32 copepod species from GenBank. This set includes representatives from 16 Calanoid families comprising 7 of the 10 Calanoid superfamilies. A complete list of specimens with accession numbers is included in Table 1.

Sequences were aligned using ClustalW 1.4 and visually inspected for optimality. Phylogenetic analyses were performed with PAUP*4.0 (ver. 4.0b10; Swofford, 2009) using neighbor-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) methods. A transitional model with gamma distribution (TIM + G) was selected by Modeltest 3.7 (Posada and Crandall, 1998) as the best fitting model of molecular evolution based on the Akaike Information Criterion (AIC). This model was applied in PAUP*4.0 (ver. 4.0b10; Swofford, 2009) to reconstruct phylogenies by all three methods (MP, ML and NJ) with the following settings: MP- heuristic search with 10,000 replicates, branch swapping with tree-bisection-reconnection, bootstrap values from 10,000 replicates; ML- heuristic search with 100 replicates, branch swapping with tree-bisection-reconnection, bootstrap values from 100 replicates; NJ- Hasegawa-Kishino-Yano-85 (HKY85) distance measure, heuristic search with 10,000 replicates, branch swapping with tree-bisection-reconnection, bootstrap values from 10,000 replicates. A Bayesian analysis was also performed with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Mrmodeltest 2.2 (Nylander, 2004) was used to select the best fitting model of evolution based on AIC scores, and chose the HKY85 model with invariant sites (HKY + I). The analysis was carried out for 1,000,000 generations, sampling every 100th generation. As suggested by Nylander (2004), the initial 25% (2500) of sampled generations were omitted from the analysis.

Systematics

Subclass Copepoda H. Milne Edwards, 1830
Order Calanoida G. O. Sars, 1903
Ridgewayiidae Wilson, 1958
Ridgewayia Thompson and Scott, 1903
Ridgewayia tortuga n. sp.
(Figs. 1–3)

Material Collected.—Some 5 specimens (2 females, 1 male and 2 fifth stage copepodites) collected on 16 November 2006, in coral rubble washings at Garden Key in the Dry Tortugas, Florida. Two females and one male were used for analysis, including dissection and measurements.

Body Length.—Female Holotype. Total length = 0.78 mm; prosome length = 0.59 mm; urosome length = 0.19 mm. Female Paratype. Total length = 0.77 mm; prosome length = 0.54 mm; urosome length = 0.23 mm (urosome segments severely distended giving the appearance of a longer urosome than holotype). Male Paratype. Total length = 0.68 mm; prosome length = 0.50 mm; urosome length = 0.18 mm.

Types.—Deposited in the Smithsonian Institution National Museum of Natural History, Washington. Holotype: adult female, USNM 1140470; allotype: 1 adult male, USNM 1140471, and paratype 1 adult female, USNM 1140472. All collected on 16 November 2006 (24°37.6′N 82°52. 3′W).

Description.—Female (holotype). Body (Fig. 1A, B) light red in life, slender, prosome 6-segmented. Cephalosome clearly separate from first pedigerous somite. Posteriolateral angles of prosome rounded and extending along the genital double somite to one third of its length, with a small notch in distal ventral margin. Large eye present in anterior section of cephalosome, red pigmented even after preservation in formalin. Rostrum a simple process

Table 1. List of species used for the 18S ribosomal RNA phylogenetic analysis.

Species	Family	Superfamily	GenBank accession number
Exumella mediterranea	Ridgewayiidae	Epacteriscoidea	AY629259
Pseudocyclops sp.	Pseudocyclopidae	Pseudocyclopoidea	AY626994
Metridia lucens	Metridinidae	Augaptiloidea	AY118072
Gaussia princeps	Metridinidae	Augaptiloidea	GQ325591
Haloptilus ocellatus	Augaptilidae	Augaptiloidea	AY118069
Heterorhabdus farrani	Heterorhabdidae	Augaptiloidea	AY118065
Skistodiaptomus pygmaeus	Diaptomidae	Centropagoidea	AY339161
Leptodiaptomus sicilis	Diaptomidae	Centropagoidea	AY339155
Mastigodiaptomus nesus	Diaptomidae	Centropagoidea	AY339156
Onychodiaptomus sanguineus	Diaptomidae	Centropagoidea	AY339157
Eudiaptomus graciloides	Diaptomidae	Centropagoidea	AY339149
Pseudodiaptomus annandalei	Pseudodiaptomidae	Centropagoidea	AY629258
Tortanus sp.	Tortanidae	Centropagoidea	AY626995
Candacia armata	Candaciidae	Centropagoidea	AY446899
Calanoides acutus	Calanidae	Megacalanoidea	AY118071
Mesocalanus tenuicornis	Calanidae	Megacalanoidea	AF367716
Nannocalanus minor	Calanidae	Megacalanoidea	AF367715
Neocalanus tonsus	Calanidae	Megacalanoidea	AF367713
Calanus pacificus	Calanidae	Megacalanoidea	L81939
Ctenocalanus citer	Clausocalanidae	Clausocalanoidea	AY118078
Microcalanus pygmaeus	Clausocalanidae	Clausocalanoidea	AY118068
Pseudocalanus moultoni	Clausocalanidae	Clausocalanoidea	AF367717
Drepanopus forcipatus	Clausocalanidae	Clausocalanoidea	AF462321
Clausocalanus ingens	Clausocalanidae	Clausocalanoidea	AF367718
Scolecithricella dentate	Scolecitrichidae	Clausocalanoidea	AY118070
Scaphocalanus magnus	Scolecitrichidae	Clausocalanoidea	AY446895
Gaetanus tenuispinus	Aetididae	Clausocalanoidea	AY118075
Stephos longipes	Stephidae	Clausocalanoidea	AY118073
Paraeuchaeta antarctica	Euchaetidae	Clausocalanoidea	AY118064
Euchaeta norvegica	Euchaetidae	Clausocalanoidea	AY446898
Spinocalanus abyssalis	Spinocalanidae	Spinocalanoidea	AY118074
Tigriopus californicus	Harpacticidae	Outgroup	AF363306

produced ventrally with single sharp tip. Urosome (Fig. 1C) four segmented. Genital double somite symmetrical with genital operculum midventral. Caudal rami symmetrical bearing six setae (Fig. 1C): a small, blade-like seta on the outer distal corner, followed medially by a longer seta covered in small setules, two long, plumose median setae jointed basally, a small plumose seta extending dorsally (Fig. 1A), and a longer plumose seta on the distal inner margin.

Antennules (Fig. 1D) 25-segmented, barely reaching genital double somite. Armature of articulated segments as follows: 1-2 (setae) + ae (aesthetasc), 2-6 + ae, 3-2 + ae, 4-2 + ae, 5-2 + ae, 6-2 + ae, 7-2 + ae, 8-2 + ae, 9-2 + ae, 10-2 + ae, 11-2 + ae, 12-2 + ae, 13-2 + ae, 14-2 + ae, 15-2 + ae, 16-2 + ae, 17-2 + ae, 18-2 + ae, 19-2 + ae, 20-1, 21-1, 22-2, 23-2 + ae, 24-2, 25-5 + ae.

Antenna (Fig. 2A) bearing plumose seta on coxa. Basis bearing two setae of unequal length. Exopod indistinctly 8-segmented, with setal formula 1, 1, 1, 1, 1, 1, 1, 1 + 3. Endopod 2-segmented, first segment bearing 2 subterminal setae. Second segment bilobed; subterminal lobe bearing 8 setae, terminal lobe bearing 4. Two rows of setules along outer margin of second segment.

Mandibular gnathobase (Fig. 2B) bears a long and slender tooth on inner distal margin, followed by several shorter and wider teeth, decreasing in size towards the outer margin. Basis of palp with four setae. Exopod indistinctly 4-segmented with setal formula 1, 1, 1, 3; endopod 2-segmented; first segment bearing 4 setae, second segment bearing 9.

Maxillule (Fig. 2C) well developed; precoxal arthrite bearing 5 spiniform and 3 spinulose distal setae, 1 anterior and 4 posterior slender setae. Coxa bearing 9 setae on epipodite and 4 on endite. Basis with one seta on exite, 4 setae on first endite and 5 on second. Exopod unsegmented, with 3 + 8 setae. Endopod 2-segmented with setal formula 4 + 4, 4.

Maxilla (Fig. 2D) with distal endite of precoxa bearing 3 long setae. Endite of coxa with 3 setae. Proximal and distal endites of basis bearing 3 setae each. Endopod 5-segmented with distal 3 segments partly fused. First segment with 1 sclerotized seta and three long setae. Remaining 4 segments bearing 3, 2, 2, 2 setae respectively.

Maxilliped (Fig. 2E) with syncoxal endites bearing 1, 2, 4, and 3 setae respectively. Basis with 5 setae, 2 of these on distal medial lobe. Patch of long setules along inner margin. Endopod 5-segmented, with setal formula 4, 4, 3, 3 (inner) + 1 (outer), 4. First and second endopod segments with row of setules along inner margin, fourth segment with patch of setules on outer distal margin.

Legs 1–4, with 3-segmented rami (Fig. 3A-D). Seta and spine formulae are given in Table 2. Inner coxal setae present on legs 2-4 absent on legs 1 and 5 (Fig. 3E). Leg 1 (Fig. 3A) with von Vaupel Klein organ (Ferrari and Steinberg, 1993; Barthelemy et al., 1998), a curved inner basal setae with setules and a semi-circular process

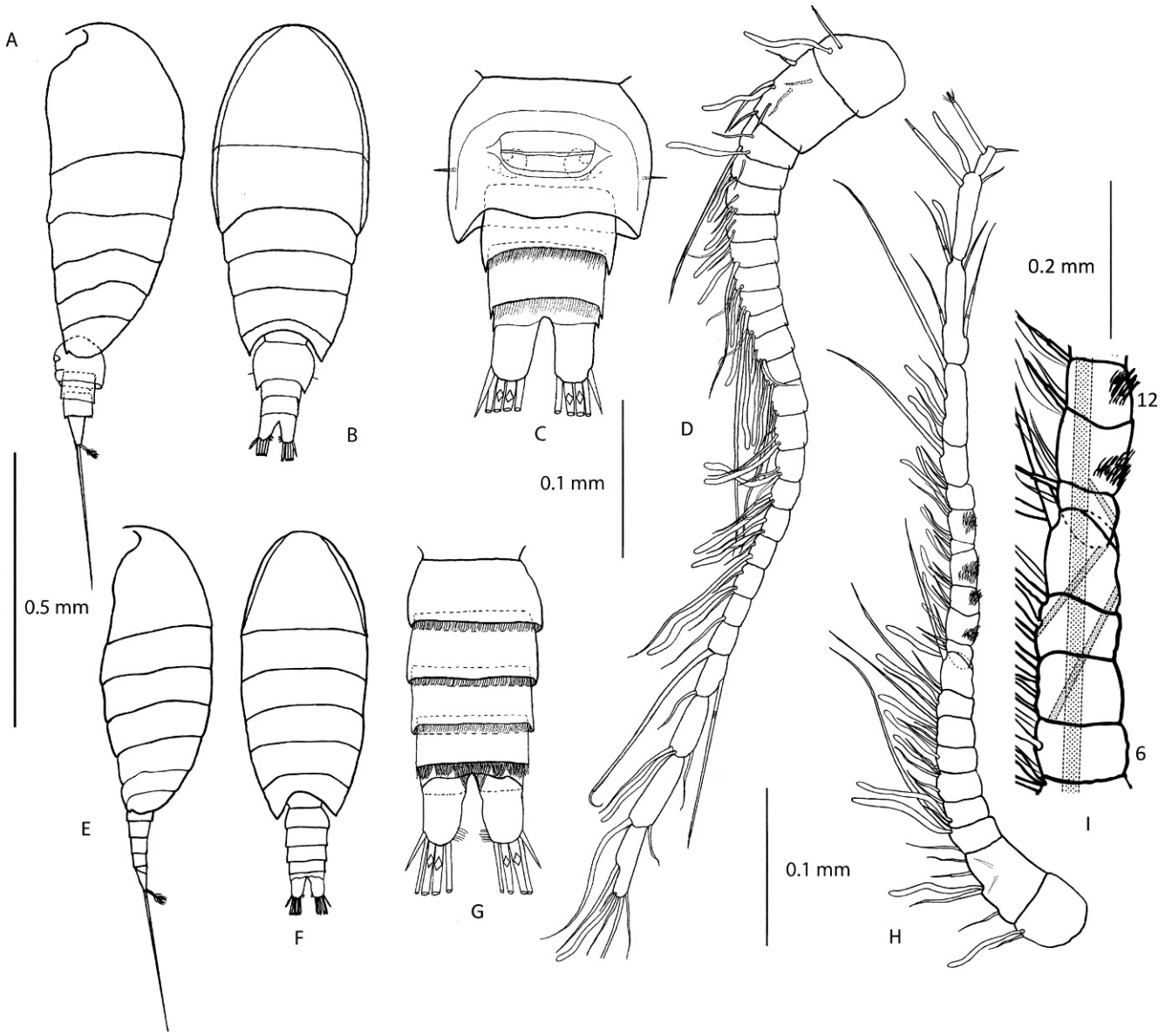

Fig. 1. *Ridgewayia tortuga* n. sp. A, female, habitus, lateral; B, female, habitus, dorsal; C, female, urosome, ventral; D, female, antennule; E, male, habitus, lateral; F, male, habitus, dorsal; G, male, urosome, ventral; H, male, right antennule; I, male, right antennule segments 6-12 with schematic of muscle pattern near first geniculation.

originating anteriorly from first endopod segment and extending just beyond distal margin. Patch of setules present on proximal outer margin of first exopod segment; same segment with row of fine setules on distal margin. Second segment, with 2 lamellate, leaf-like processes at distal outer margin, between outer setae and insertion point of third segment; larger process with row of fine spinules around its entire margin. Leg 2 (Fig. 3B) bearing row of setules on inner distal margin of second exopod segment. Legs 3 and 4 (Fig. 3C-D) bearing rows of spinules on distal margin of exopod and endopod segments 1 and 2. Leg 3 with row of fine spinules on anterior distal surface of third exopod segment. Terminal segments of exopods and endopods of legs 1-4 with large pores on distal anterior surface, slightly smaller than the pores in *Ridgewayia typica* Thompson and Scott, 1903 shown in scanning electron micrographs and termed ''tympani'' by Por (1979). Pores form openings to relatively large sub-cuticular sacs. Leg 5 (Fig. 3E) with three-segmented exopod and a two-segmented endopod. Inner coxal seta absent, basal seta present. Patch of fine setules present along proximal inner margin of second exopod segment. Large pore present on anterior surface of terminal endopod segment.

Male (paratype). Body (Fig. 1E, F) as in female, but slightly smaller. Urosome (Fig. 1G) 5-segmented. Caudal rami similar to female, but with row of setules on distal inner margins. Left antennule same as female.

Right antennule (Fig. 1H) 20 segmented, strongly geniculate between segments 9–10 and weakly geniculate between segments 16–17. Schematic of muscle pattern of first geniculation shown in Fig. 1i. Armature of articulated segments as follows; 1-1 + ae, 2-6 + ae, 3-2 + ae, 4-2 + ae,

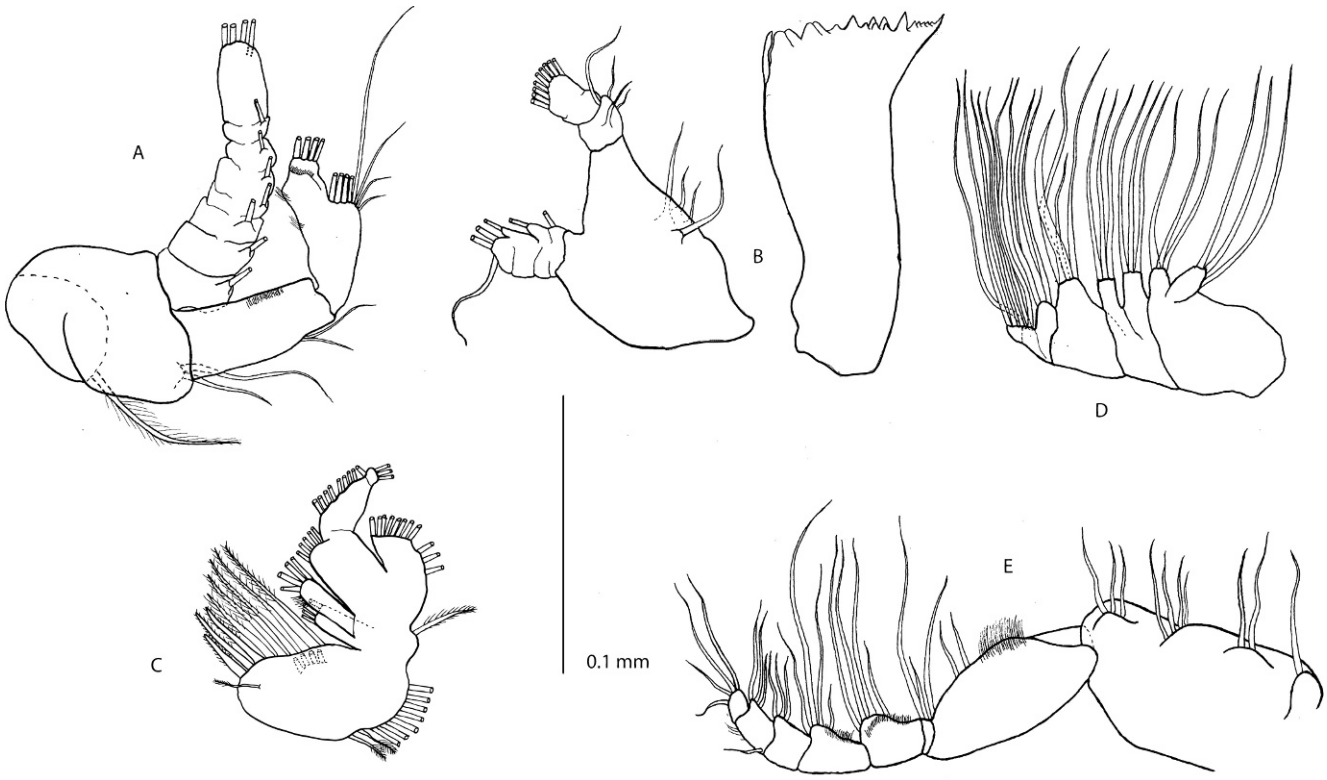

Fig. 2. *Ridgewayia tortuga* n. sp. Female mouthparts. A, antenna; B, mandible; C, maxillule; D, maxilla; E, maxilliped.

5-2 + ae, 6-2 + ae, 7-3 + ae, 8-2 + ae, 9-2 + ae, 10-2 + ae, 11-2 + ae, 12-2 + ae, 13-2 + ae, 14-2 + ae, 15-2 + ae, 16-2 + ae, 17-2 + ae, 18-3 + ae, 19-4 + ae, 20-4 + ae. Rows of spinules present on segments 11, 12, 13, and 15. Segments 10 and 14 constricted.

Legs 1 through 4 same as in female. Leg 5 (Fig. 3F, G) biramous, asymmetrical, and strongly modified. Coxa without setae. Basis unarmed. Right leg (Fig. 3F) with 2-segmented exopod and 1-segmented, elongate, endopod. Exopod segments without setae. First segment with outer spine and patch of setules on posterior inner margin. Second exopod segment with outer spine; distal end of segment unarmed and modified into an elongate finger-like process. Endopod elongate, reaching the tip of the exopod; forked at tip and unarmed. Left leg (Fig. 3G) with 3-segmented exopod. First and second segments with one strong outer spine each. Third segment complex and strongly modified, bearing three elements. In order from inner to outer margin: first element a slender, elongate, finger-like projection near the outer margin; second element leaf-like with laterally directed marginal folds; third element also leaf-like but larger with anteriorly directed marginal folds. Endopod consists of an ovoid segment tapering distally, ending in a conical tooth-like element.

Remarks.—Presently *Ridgewayia* can be divided into three species groups: *marki*, *typica*, and *gracilis* (Ummerkutty, 1963; Ferrari, 1995; Barthelemy et al., 1998; Ohtsuka et al., 2000; Figueroa and Hoefel, 2008). *Ridgewayia tortuga* most closely resembles members of the *typica*-group, sharing two of the three characters used by Barthelemy et al. (1998) to define it: 1) lack of an inner seta on the coxa of leg 1 (this seta is present in members of the *marki*- and *gracilis*-groups) and 2) presence of only 1 outer spine on the distal exopod segment of the male right fifth leg (two outer spines are present in species of the *marki*- and *gracilis*-groups). The third character, presence of 4-5 setal elements on the endopod of the male right fifth leg, is not found in *R. tortuga*. In this species, the right endopod is elongate, unarmed and forked at the tip, more similar to that found in the *gracilis*- and *marki*-groups. There are two key differences that set *R. tortuga* apart from all other *Ridgewayia*: 1) males of *R. tortuga* have a 20-segmented, doubly geniculate, right antennule; a single geniculation is present in all other species with 21-24 articulated segments; and 2) the third endopod segment on the second leg of *R. tortuga* has 7 setae, all other species have 8.

There are two previous descriptions of specifically undetermined specimens of male *Ridgewayia* that are similar to *R. tortuga*. Yeatman (1969) described one male of a *Ridgewayia* sp. from a night haul in St. George's Harbor, Bermuda; and Por (1979) described several males from the Bitter Lakes, in the Suez Canal between the Mediterranean and Red Seas. The fifth legs of the males described by Yeatman and Por are remarkably similar to that of *R. tortuga* (Fig. 3H, I, J). The segmentation and armature of the left fifth leg are the same, except for the presence of a small outer seta on the basis of Yeatman's specimen. This seta is absent in Por's drawing and in *R. tortuga*. The left endopod has the same oblong shape with a distal tooth-like element in all three. The right fifth legs in

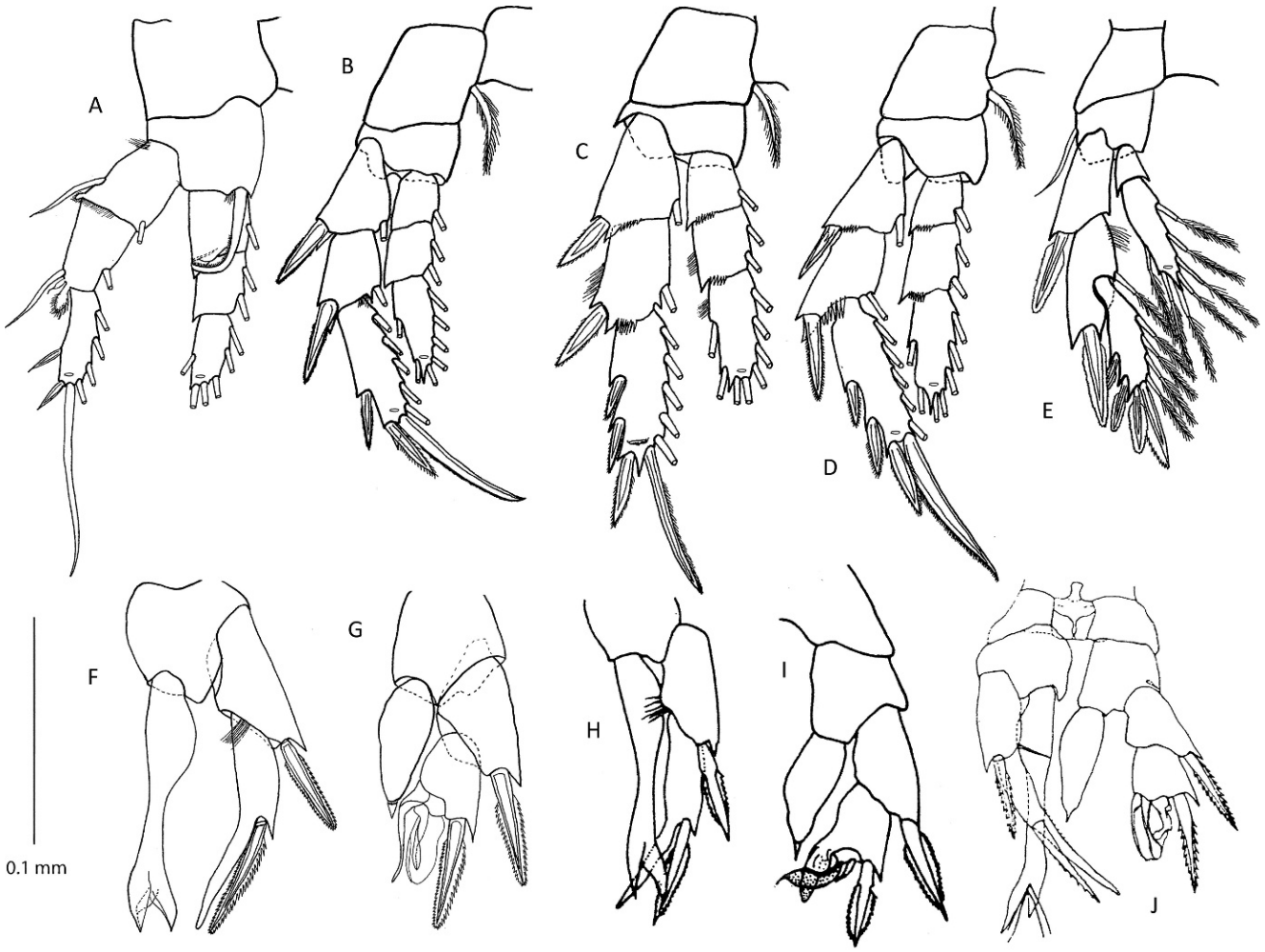

Fig. 3. A-G, *Ridgewayia tortuga* n. sp. A, female, leg 1, anterior; B, female, leg 2, anterior; C, female, leg 3, anterior; D, female, leg 4, anterior; E, female, leg 5, anterior; F, male, right leg 5, posterior; G, male, left leg 5, anterior. H-J, *Ridgewayia* sp. H, male specimen from the Bitter Lakes, right leg 5, posterior, as in Por (1979); I, male specimen from the Bitter Lakes, left leg 5, anterior, as in Por (1979); J, male specimen from Bermuda, leg 5, anterior, as in Yeatman (1969).

R. tortuga and Yeatman's and Por's specimens also have the same segmentation and armature, except for a thin seta present on the inner margin of the first exopod segment as shown on Yeatman's drawing; in *R. tortuga* and Por's specimen there is a patch of fine setae on this inner margin. The right endopod has the same elongated shape with a forked tip at the end in all three males. Yeatman described a set of fine seta-like structures on this forked tip, not present in *R. tortuga* or in Por's drawing. Yeatman's and Por's specimens are depicted as having an unequally bifurcated tip, while *R. tortuga* has a symmetrically bifurcated tip.

Table 2. Setae and spine formulae for legs 1-5 of female *Ridgewayia tortuga*.

			Exopod			Endopod		
	Coxa	Basis	1	2	3	1	2	3
Leg 1	0-0	0-1	I-1	I-1	II, I, 4	0-1	0-2	1, 2, 3
Leg 2	0-1	0-0	I-1	I-1	II, I, 5	0-1	0-2	2, 1, 4
Leg 3	0-1	0-0	I-1	I-1	III, I, 5	0-1	0-2	2, 2, 4
Leg 4	0-1	0-0	I-1	I-1	III, I, 5	0-1	0-2	2, 1, 4
Leg 5	0-0	1-0	I-0	I-1	III, I, 4	0-0	2, 2, 3	

Por (1979) likely incorrectly assumed that these peculiar males belong to *R. typica* (specimens of which were collected in abundance in the nearby Di Sahav Pool in the Gulf of Elat, Red sea) and suggests that the observed differences in fifth leg structure are due to polymorphism within this species. Working under this assumption, Por neglects to describe the males from the Bitter Lakes in detail beyond their fifth leg structure. Yeatman (1969) has a more complete description that includes all swimming legs and antennules. Yeatman's specimen and *R. tortuga*, both have a 25-segmented left antennules. But Yeatman's specimen has a 22-segmented right antennule, while *R. tortuga* has only 20 segments. The swimming legs in both have the same segmentation and armature that include only 7 setae on the terminal exopod segment of leg 2 instead of the 8 found in all other *Ridgewayia*.

The unusual geniculation on the right antennule of the male of *R. tortuga* between articulated segments 9 and 10

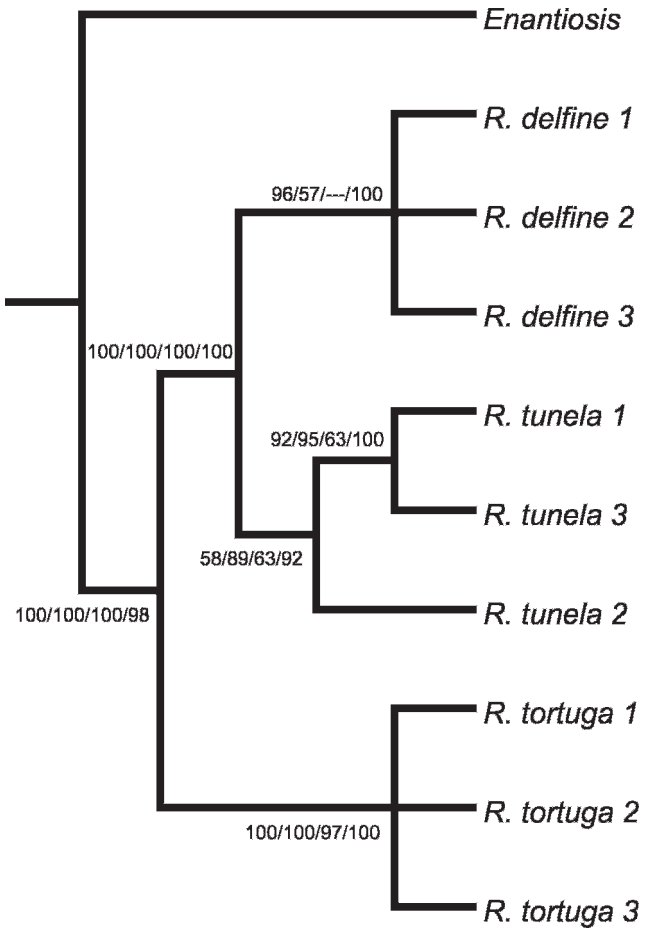

Fig. 4. *Ridgewayia* phylogenetic tree based on a 569 base-pair region of ITS-1. Branch values correspond to bootstrap support for maximum parsimony, maximum likelihood, neighbor joining, and Bayesian posterior probabilities (—, indicates that the branch failed the 50% bootstrap support).

(with 11 segments beyond the geniculation) is not mentioned by Yeatman (1969) nor has it been observed in any other species of *Ridgewayia*. But such a geniculation has been found elsewhere in Ridgewayiidae. Suarez-Morales and Iliffe (2007) describe a new genus and species of Ridgewayiidae, *Hondurella verrucosa*. This particular ridgewayiid inhabits a karstic cave in the Caribbean on the island of Utila, Honduras. The male right antennule of *H. verrucosa* is 23 segmented with a geniculation between segments 13 and 14 (with 11 segments beyond the geniculation). The position of the geniculation in *R. tortuga* and *H. verrucosa* with 11 segments beyond the geniculation contrasts the usual position in the family with only 4 segments beyond the geniculation (Boxshall and Halsey, 2004).

Etymology.—*Ridgewayia tortuga* is named after the type locality, the Dry Tortugas, Florida.

Results

Nuclear ITS1 Region and Mitochondrial COI Gene

A 569 base-pair region of ITS-1 was successfully amplified from 3 specimens each of *R. delfine*, *R. tunela*, and *R. tortuga*, 3 specimens of each of the two undescribed species of *Pseudocyclops* from Galapagos, and 1 specimen of *Enantiosis galapagensis* Fosshagen, Boxshall and Iliffe, 2001 from the closely related family Epacteriscidae, also from Galapagos (collected from a third anchialine pool in the Island of Santa Cruz, Galapagos, 00°45.735′S 90°19.742′W). The *E. galapaguensis* sequence was used as an outgroup. The ITS-1 region for *Ridgewayia* showed between species differences of 2% for *R. delfine* and *R. tunela* and of 30% between *R. tortuga* and either of the Galapagos ridgewayiids. Within species differences ranged from 0–1%.

Only 1 tree was retained for both MP and ML methods, while NJ resulted in 9 trees, which were used to construct a strict consensus tree. These 3 methods and the Bayesian analysis resulted in similar phylogenetic reconstructions (Fig. 4) with well-supported branches, except for one branch in the NJ consensus tree leading to *R. delfine,* which had less than 50% bootstrap support. This branch is well supported by the other 3 methods. The phylogenetic tree shows *R. tortuga* branching separately from the ridgewayiids of the Galapagos. It also shows that *R. delfine* and *R. tunela* are closely related, but belong to separable clades.

Twenty-four specimens each of *R. delfine* and *R. tunela*, 2 of *R. tortuga*, 6 specimens of *Pseudocyclops*, and 6 specimens of the endemic Galapagos neritic calanoid *Acartia levequei* Grice, 1964 were sequenced for the Folmer region of COI. The 620 base-pair sequences obtained from this region were identical for all ridgewayiids and pseudocyclopiids, except for two *Pseudocyclops* sequences which vary by 1 base-pair each, the first at position 381 and the second at position 535, with a substitution from G to A in both cases. The sequence obtained from the *Acartia levequei* specimens show a 30% base-pair difference from that of the specimens of *Ridgewayia* and *Pseudocyclops*. The lack of differentiation between *Ridgewayia* and *Pseudocyclops* was unexpected; therefore, DNA was extracted from new specimens (6 *R. delfine*, 6 *R. tunela*, 6 *Pseudocyclops* sp. and 6 *A. levequei*) to obtain new sequences. Different batches of chemicals and primers were used for all reactions. Results obtained the second time were identical to the earlier results, showing no differences in the Folmer region of COI from *Ridgewayia* and *Pseudocyclops* specimens and a 30% base-pair difference with the *Acartia* specimens.

Because these results were unusual, specimens of *R. delfine*, *R. tunela*, *R. tortuga*, and *Pseudocyclops* sp. were sent for sequencing of the COI gene to Dr. Riyuji J. Machida, the Census of Marine Zooplankton Asia Project Manager and Postdoctoral Fellow at the University of Tokyo. His sequencing results are inconclusive. He obtained the same sequence for one individual of *R. tunela*, 2 of *R. tortuga* and 5 *Pseudocyclops sp.* (with less than 1% base-pair differences). But he also obtained different sequences for 1 individual of *R. tunela* and 5 other specimens of *Pseudocyclops* sp. (these show between 14-28% base-pair variability). He was unable to obtain a COI sequence from any of the specimens of *R. delfine*. The difficulty in obtaining sequences from these specimens was probably due to deterioration of the DNA since they had

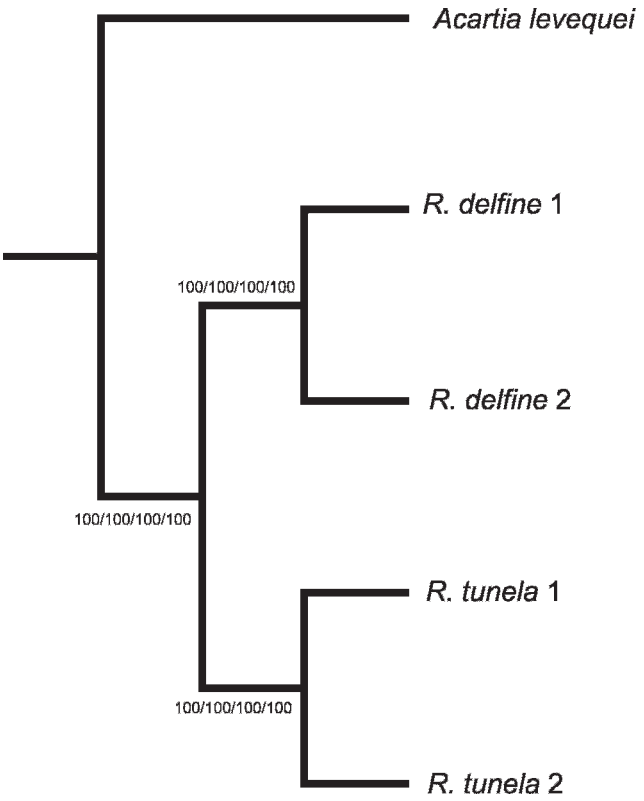

Fig. 5. *Ridgewayia* phylogenetic tree based on the second half of the COI gene, a 656 base-pair region. Branch values correspond to bootstrap support for maximum parsimony, maximum likelihood, neighbor joining, and Bayesian posterior probabilities. (—, indicates that the branch failed the 50% bootstrap support).

been stored for over 4 years. Sub-optimal shipping conditions when sent to Japan probably exacerbated this problem. The various sequences obtained by Dr. Machida suggest that pseudogenes may be interfering in his replication process. Evidence for the presence of pseudogenes was not observed when replicating and sequencing specimens at Oregon State University.

Custom primers were designed based on the *Ridgewayia*/*Pseudocyclops* sequence and used in conjunction with universal primers to sequence the second half of the COI gene. A specimen of *A. levequei* and two each of *R. delfine* and *R. tunela* were sequenced for this second half. A 656 base-pair sequence was obtained, and, as for the Folmer region, *A. levequei* showed a 30% base-pair difference from both *R. delfine* and *R. tunela*. Unlike the Folmer region, *R. delfine* and *R. tunela* had sequence differences of 2–5%, while within species differences ranged from 1–2%. A phylogenetic tree was reconstructed following the same procedures as with the ITS-1 region, resulting in the same tree for all 4 methods, MP, ML, NJ, and Bayesian; all with well supported branches (Fig. 5).

Sequences were submitted to GenBank with the following accession numbers: 1) Folmer region of the COI gene – *R. delfine* (HM481266, HM481267); *R. tunela* (HM481268, HM481269); *R. tortuga* n. sp. (HM481272); *Pseudocyclops* sp. (HM481270, HM481271); *Acartia levequei* (HM481287); 2) Second half of the COI gene – *R. delfine* (HM481273, HM481274); *R. tunela* (HM481275, HM481276); and 3) Internal transcribed spacer 1 region – *R. delfine* (HM481277, HM481278, HM481279); *R. tunela* (HM481280, HM481281, HM481282); *R. tortuga* n. sp. (HM481283, HM481284, HM481285); *Enantiosis galapagensis* (HM481286).

18S Ribosomal RNA Gene

The partial sequences for the 18S small subunit ribosomal RNA gene obtained from GenBank were aligned using ClustalW 1.4 and visually inspected for optimality resulting in a 685 base-pair region. A symmetrical model with invariable sites and gamma distribution (SYM + I + G) was selected by both Modeltest 3.7 (Posada and Crandall, 1998) and Mrmodeltest 2.2 (Nylander, 2004) as the best fitting model of molecular evolution based on the Akaike Information Criterion (AIC). A phylogenetic tree was reconstructed following the same procedures as with the ITS-1 region, resulting in similar trees for all 4 methods, MP, ML, NJ, and Bayesian (Fig. 6) with variable branch bootstrap support. Centropagoidea is the first previously recognized clade that diverges from the rest of Calanoida. This included representatives of Pseudodiaptomidae (1 genus), Diaptomidae (5 genera), Tortanidae (1 genus), and Candaciidae (1 genus). The Pseudodiaptomidae diverge early in this group. Next, Candaciidae and Tortanidae split off together while their sister branch contains all Diaptomidae, recovering the monophyly of this family. The sister branch to Centropagoidea includes all 6 superfamilies remaining in the available data: Augaptiloidea, Epacteriscoidea, Pseudocyclopoidea, Megacalanoidea, Clausocalanoidea, and Spinocalanoidea. Two clades separate on this branch. The first contains the Augaptiloidea, Epacteriscoidea, and Pseudocyclopoidea (strong branch support only in the Bayesian reconstruction), while the second contains the Megacalanoidea, Clausocalanoidea, and Spinocalanoidea.

Ridgewayiidae and Pseudocyclopidae diverge early from Augaptiloidea, forming their own clade. The monophyly of Augaptiloidea is recovered, represented by Augaptilidae (1 genus), Heterorhabdidae (1 genus), and Metridinidae (2 genera). In the sister branch to this clade of Augaptiloidea, Epacteriscoidea, and Pseudocyclopoidea, Megacalanoidea is the first to diverge. Megacalanoidea are represented in this analysis only by members of Calanidae (5 genera); other members of this superfamily (Mecynoceridae, Megacalanidae, and Paracalanidae) need to be analyzed to ascertain the monophyly of this group. The sister branch to Calanidae includes Clausocalanoidea and Spinocalanoidea. Clausocalanoidea are paraphyletic unless Spinocalanidae are subsumed into this superfamily. Clausocalanidae are also paraphyletic, represented by 5 genera in the same branch as members of Scolecitrichidae, Aetididae, Euchaetidae, Stephidae, and Spinocalanidae.

Discussion

Ridgewayiidae is currently composed of ten genera, seven of which are found only in marine caves: *Brattstromia*, *Exumellina*, *Hondurella*, *Normancavia*, *Robpalmeria*, *Stargatia*, and *Badijella* (Fosshagen and Iliffe, 1991, 2003;

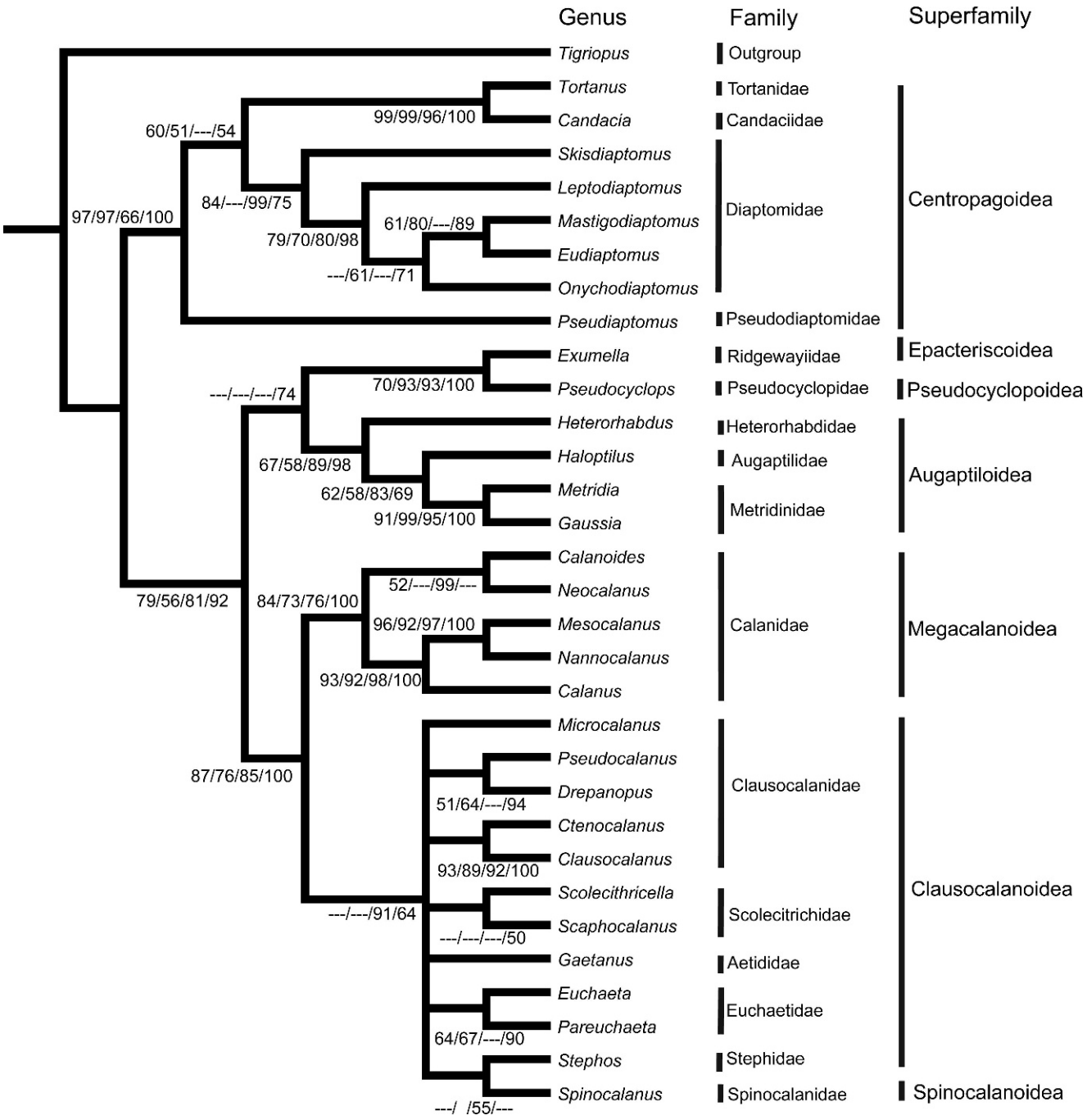

Fig. 6. Calanoida phylogenetic tree based on the 18S ribosomal RNA gene. Branch values correspond to bootstrap support for maximum parsimony, maximum likelihood, neighbor joining, and Bayesian posterior probabilities. (—, indicates that the branch failed the 50% bootstrap support).

Krsinic, 2005; Suarez-Morales and Iliffe, 2007). The remaining three genera, *Placocalanus*, *Exumella,* and *Ridgewayia* are mainly hyperbenthic and/or epibenthic; but *Exumella* and *Ridgewayia* also have members that inhabit marine caves (Wilson, 1958; Fosshagen and Iliffe, 1998; Ohtsuka et al., 2000). The genus *Ridgewayia* was established by Thompson and Scott (1903) and redescribed by Wilson (1958). There are fifteen described species and two subspecies belonging to it, including the new species described in this paper. The species of *Ridgewayia* have disjunct distributions and have been found in tropical and subtropical shallow waters of the Indo-West Pacific, the Caribbean, the Mediterranean, and the Eastern Pacific (Barthelemy et al., 1998, Ohtsuka et al., 2000, Figueroa and Hoefel, 2008).

While *R. delfine* and *R. tunela* from Galapagos clearly belong to the *marki*-group (Figueroa and Hoefel, 2008), *R. tortuga* from Florida cannot be assigned to any of the three defined groups: *marki*, *typica*, or *gracilis*. The characters that define the genus are clearly present in *R. tortuga*, the general body shape, segmentation and size, structure of the urosome, the segmentation and armature of all mouthparts

and legs, including the third exopod segment of the female fifth leg originating halfway along the inner margin of the second segment and the complex and highly modified male fifth leg. There is one character of *R. tortuga* that does not agree with the generic definition of *Ridgewayia* or with the definition of Ridgewayiidae. The right male antennule in Ridgewayiidae as defined by Boxshall and Halsey (2004) is geniculate, with four segments beyond the geniculation. Such geniculation is present in *R. tortuga*, but there is also a stronger geniculation between segments nine and ten, a character only observed in *Hondurella verrucosa* Suarez-Morales and Iliffe, 2007, but it is presumably absent in all other species belonging to Ridgewayiidae. In addition to this unique character, *R. tortuga* has several characters (see remarks) that by themselves do not make it unique, but when combined set it apart from all other species of this genus. The defining characters of the three species groups established by Ummerkutty (1963), Ferrari (1995), Barthelemy et al. (1998), and Ohtsuka et al. (2000) clearly exclude *R. tortuga* from all of them. The unique characters of *R. tortuga* warrant a fourth species group with it as the sole member or, perhaps a rethinking altogether of the characters that should be used to define ''groups'' in *Ridgewayia*. More specimens of Yeatman's (1969) *Ridgewayia* sp. from Bermuda and Por's (1979) *Ridgewayia* sp. from the Bitter Lakes are necessary for a complete description of each. These specimens are closely related to *R. tortuga* and presumably can be included in the same species group with the following characters: 1) Lack of inner seta on coxa of leg 1; 2) presence of only 1 spine on the second exopod segment of the male right fifth leg; 3) an elongate unarmed endopod forked at the tip on the male right fifth leg; and 4) the presence of only 7 setae on the terminal endopod segment of leg 2.

The sequences obtained from the ITS-1 region prove to be species specific, and from them one can reconstruct the phylogeny of *Ridgewayia*. This gene shows that although *R. delfine* and *R. tunela* are closely related, they are genetically isolated, supporting their status as separate species as suggested by their morphology (Figueroa and Hoefel, 2008). The ITS-1 region also shows that *R. tortuga* from Florida is a distant relative of these two sibling species from Galapagos. Analysis of the second half of the COI gene supports these conclusions.

Four models have been proposed to explain the distribution pattern of anchialine organisms: 1) vicariance (Iliffe et al., 1984; Stock, 1993, 1994; Jaume and Boxshall, 1996; Boxshall and Jaume, 2000; Danielopol et al., 2000; Humphreys, 2000), 2) regression (Stock, 1980; Holsinger, 1988; Suarez-Morales and Iliffe, 2007), 3) deep-sea origin (Hart et al., 1985; Manning et al., 1986; Boxshall, 1989; Iliffe, 1990), and 4) active migration (Iliffe, 2000; Kano and Kase, 2004). Among these four evolutionary models, it seems that for *Ridgewayia* a combination of vicariance and active migration processes was responsible for the observed species distributions. Most members of the *marki*-group are found in the Caribbean suggesting a faunal link between this region and the Galapagos. An exchange of *Ridgewayia* between the Caribbean and the Eastern Pacific must have occurred during an open Panamanian Seaway. The Isthmus of Panama emerged, completely closing the Panama seaway, about 3–6 million years ago, while the Caribbean Plate is estimated to have formed about 75–95 million years ago. Therefore, a ridgewayiid exchange between the Caribbean and Galapagos must have occurred during this time frame. The direction of this transfer cannot be determined with the present data. It is likely that the two species of *Ridgewayia* from the Galapagos are a result of one colonization event. Once the Isthmus of Panama emerged closing this seaway, *Ridgewayia* from the Galapagos became isolated from those of the Caribbean, leading to speciation through vicariance.

Active migration and colonization with subsequent speciation are also occurring in the present-day ocean. This is demonstrated by the colonization of *Ridgewayia* on Isabela, a geologically young island. It is also possible that *Ridgewayia* are not only colonizing nearby islands, but are also able to cross vast stretches of ocean. Morphologically the closest relative of the ridgewayiids of the Galapagos is *R. stygia* Ohtsuka, Kase and Boxshall, 2000 found in Palau (Figueroa and Hoefel, 2008). Clearly an exchange also occurred between the Eastern and Western Pacific, though the direction and timing of this exchange are not certain. A phylogenetic analysis that includes more species of *Ridgewayia* from various geographic regions is necessary to determine the evolutionary patterns that shaped the current diversity and distribution of this genus. The ITS-1 molecular marker would be an excellent choice for such an analysis.

My data suggest that the Folmer region of the COI gene cannot be used to distinguish species of *Ridgewayia* and *Pseudocyclops*. More specimens of these two families need to be analyzed for the second half of the COI gene, which shows better potential as a genetic marker, as it differentiated in the two Galapagos species of *Ridgewayia*. A similar result was reported by Erpenbeck et al. (2006) for sponges; they showed that the Folmer region failed to differentiate at the species level, but the second half of the gene proved to be species specific. Low differentiation of the COI gene has also been observed in the sponges *Lubomirskia* and *Baikalospongia* (Schroeder et al., 2003), *Crambe* (Duran et al., 2004), and *Astrosclera* (Worheide, 2006). Evidence is accumulating that the Folmer region, which has become the standard for DNA barcoding of all living organisms, may not be appropriate for some. Minimal to no variation of this gene has also been found in most cnidarian (Hebert et al., 2003) and coral species, suggesting that the rate of evolution of this gene in Anthozoans is very slow (Snell et al., 1998; Medina et al., 1999; Hellberg, 2006). Slow rates are characteristic of fungi and angiosperms, implying that slowly evolving mitochondrial genes are a pleisiomorphic trait in eukaryotes (Hellberg, 2006; Huang et al., 2008).

Through phylogenetic reconstruction, Hellberg (2006) and Huang et al. (2008) arrived independently at the same conclusion that the switch from slow to fast evolution of mitochondrial DNA in animals occurred once in the branch that leads to Medusozoa and separately in the branch leading to Bilateria. The switch from slow to fast evolution of the COI gene has been attributed to the sudden loss of

mitochondrion-specific DNA repair and/or replication genes (Hellberg, 2006; Huang et al., 2008). The extremely low diversity of the Folmer region in *Ridgewayia* and *Pseudocyclops* and the much faster evolution of the nuclear genes (as shown by ITS-1) fit the more primitive pattern having mitochondrial DNA repair. These are the only copepods that have shown such a trait; all other copepods where the sequence of the Folmer region has been analyzed show a rapid evolution (Bucklin et al., 2003; Goetze, 2005; Lee, 2007; Newer et al., 2008). It is conceivable, then, that Ridgewayiidae and Pseudocyclopidae have re-established a mitochondrial DNA repair system that operates with high fidelity in this particular region.

Phylogenetic reconstructions of Calanoida have been solely based on morphology and commonly depicted as a linear progression with each superfamily as an offshoot from one main branch, those with the greatest number of plesiomorphic traits diverging first (Andronov, 1974; Bowman and Abele, 1982; Park, 1986; Andronov, 1991). This method fails to show actual phylogenetic relationships between the various superfamilies. Park (1986) could not determine the placement of Pseudocyclopoidea and Epacteriscoidea within a phylogeny of Calanoida due to the highly specialized traits found in these superfamilies related to their bottom-dwelling nature. Therefore, based on plesiomorphic traits, he assumed that they form a basal branch. The currently accepted phylogeny of Calanoida is largely based on Andronov (1974) and Park (1986), and it places Pseudocyclopoidea/Epacteriscoidea, Augaptiloidea, and Centropagoidea as the first three branches of Calanoida in that order. Those are followed by Megacalanoidea, Bathypontioidea, Eucalanoidea, and Ryocalanoidea. Finally, at the tip of the main stem there is split between Clausocalanoidea and Spinocalanoidea.

The phylogeny obtained from the 18S ribosomal RNA gene suggests that the superfamily Centropagoidea (Diaptomidae, Candaciidae, and Tortanidae), was the first group to diverge, rather than Pseudocyclopoidea and Epacteriscoidea. A similar result was obtained by Braga et al. (1999), who included 6 species of calanoids in a broader phylogenetic analysis of Copepoda based on the 28S ribosomal RNA gene; the resulting tree has Centropagoida as the first branch of Calanoida. Centropagoidea is composed of families primarily found in neritic and freshwater environments; they have a geniculated right male antennule and un-myelinated axons; most species have lost their seminal receptacles, gained a genital operculum and have feeding males (Davis et al., 1999; Ohtsuka and Huys, 2001; Bradford-Grieve, 2002).

The next division in the 18S ribosomal RNA gene phylogeny is between the more ancestral Augaptiloidea, Pseudocyclopoidea, and Epacteriscoidea and the more derived Megacalanoida, Clausocalanoida, and Spinocalanoidea. The former clade consists primarily of deep-ocean (Augaptiloidea) and hyperbenthic (Pseudocyclopoidea and Epacteriscoidea) species, while the latter clade includes the dominant epipelagic copepods. As in Centropagoidea, members of the former clade have feeding males (with a few exceptions in Augaptiloidea) and presumably un-myelinated axons (more research is necessary on this observation since only two species in Augaptiloidea have been examined for the presence of myelination (Davis et al., 1999)); additionally, most members of Augaptiloidea have males with a geniculation on the left antennule (a few species have the geniculation on the right), while all Epacteriscoidea and Pseudocyclopoidea have the geniculation on the right male antennule (Ohtsuka and Huys, 2001; Bradford-Grieve, 2002; Boxshall and Halsey, 2004).

Presently Epacteriscoidea includes Epacteriscidae and Ridgewayiidae, while Pseudocyclopoidea includes Pseudocyclopidae and Boholinidae (Boxshall and Halsey, 2004). However, the membership in these two superfamilies has long been debated among copepodologists. Andronov (1974) and Park (1986) placed Ridgewayiidae in Pseudocyclopoidea. Fosshagen et al. (2001) then removed Ridgewayiidae from Pseudocylopoidea and placed them in Epacteriscoidea. Boxshall and Halsey (2004) supported this move. More recently, Andronov (2007) suggested that all subfamilies in Pseudocyclopoidea and Epacteriscoidea should be placed in the single family, Pseudocyclopidae. His argument for such a move takes into account the increasing number of described genera in Epacteriscidae and Ridgewayiidae that has resulted in shared overlap of formerly diagnostic characters. The present genetic analysis supports Andronov's classification with Ridgewayiidae closely associated with Pseudocyclopidae. These two families have the same sequence for the Folmer region of the COI gene, and they form a well-supported, deep branch, based on the 18S ribosomal RNA gene phylogeny of Calanoida. Unfortunately the Boholonidae and Epacteriscidae had no sequences available for this analysis. Nevertheless, the close genetic relationship between Ridgewayiidae and Pseudocyclopidae suggests that they should at least be included in the same superfamily, Pseudocyclopoidea.

The final branch in the 18S ribosomal RNA phylogeny includes more derived families: Megacalanoidea, Clausocalanoidea, and Spinocalanoidea. Several key characters separate this derived clade from the rest of Calanoida: a single genital operculum covering gonopores and copulatory pores, duplication of antennulary aesthetascs in males (some Augaptiloidea have duplicated aesthetascs in the first few segments of both male and female antennules), and most members have non-feeding males and lack antennular geniculation. More families of Megacalanoidea need analysis of 18S ribosomal RNA to determine the monophyly of this group. Based on the proposed phylogeny, Clausocalanoidea is paraphyletic, since it does not include the monotypic superfamily Spinocalanoidea established by Park (1986). A more comprehensive phylogenetic analysis of these 3 superfamilies is necessary to determine whether Spinocalanidae should be placed back in Clausocalanoidea as originally recommended by Fleminger (1983).

The suggested phylogeny of Calanoida based on the 18S ribosomal RNA gene is similar to the currently accepted phylogeny based on morphologic characters. The similarities include the placement of the three most plesiomorphic families at the base of the tree, though with Centropagoidea as the first branch instead of Epacteriscoidea/Pseudocyclopoidea; followed by the more derived families branching later in the phylogeny. The main difference is that a

phylogenetic tree based on genetic information can better establish ancestral relationships of the various superfamilies, allowing systematists to define synapomorphies that are diagnostic of each. In summary, this particular genetic analysis suggests: 1) that Centropagoidea is the sister branch to all other Calanoida; 2) that Ridgewayiidae and Pseudocyclopidae likely share a common ancestor with Augaptiloidea; 3) that Ridgewayiidae and Pseudocyclopidae should be included in the same superfamily, Pseudocyclopoidea; and 4) that the Spinocalanoidea likely needs to be included in Clausocalanoidea to recover the monophyly of the latter superfamily.

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