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Larval development of the stygobitic shrimp *Creaseria morleyi* (Creaser, 1936) (Decapoda: Caridea: Palaemonidae) from the Yucatán Peninsula, Mexico

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ABSTRACT

Although the larval development of epigean palaemonid shrimps has been studied extensively, only a few investigations deal with stygobitic species. We present the larval development of the cave-adapted *Creaseria morleyi* (Creaser, 1936) from anchialine caves in the Tulum area, Quintana Roo, Yucatán Peninsula, Mexico. Through the discovery of a series of larvae at different stages of development, we constructed a sequence extending through the juvenile stage. The larvae (41) were captured in plankton tows above the halocline at depths ranging between 11 and 15 m during eight surveys conducted between 2013 and 2016. Six larval stages and the first juvenile were identified; however, it is clear from the gradual modification of structures and appendages that more stages exist. The first larvae have a large quantity of vitellum and do not feed, since they have only rudimentary, and possibly non-functional, mouthparts. In the sixth stage and the juvenile, when the stages have no vitellum left, the mouthparts, chelae, and pleopods develop entirely. A comparison with other palaemonid shrimps suggests that *C. morleyi* has a greater affinity with those palaemonid species possessing extended larval development as is seen in species of *Macrobrachium* Spence Bate, 1868.

Key Words: abbreviated development, anchialine caves, cave environment, developmental biology, endemic shrimps, epigean shrimps, freshwater shrimps, plankton

INTRODUCTION

The larval development of many freshwater palaemonid shrimps has been studied, including species of *Macrobrachium* Spence Bate, 1868 (e.g., Bueno & Rodrigues, 1995; Alvarez *et al.*, 2002; Murphy & Austin, 2005), but also species from other genera such as *Palaemon* Weber, 1795, *Pseudopalaemon* Sollaud, 1911, and *Euryrhynchus* Miers, 1877 (formerly in Palaemonidae) (e.g., Magalhães & Walker, 1988; Rodríguez-Almaraz *et al.*, 2010). Species of *Macrobrachium* with an amphidromous life cycle may produce more than two hundred thousand eggs measuring about 0.5 mm in diameter, as is the case in *M. carcinus* (Linnaeus, 1758) (Mejía-Ortíz *et al.*, 2001; Lara & Wehrtmann, 2009). Typically, such species with "prolonged or normal" larval development (Jalihal *et al.*, 1993) pass through ~11 larval stages before reaching the juvenile stage (e.g., Choudhury, 1970; Gomez-Diaz, 1987). In contrast, most of the strictly freshwater species of *Macrobrachium* and *Palaemon* have partially abbreviated larval development, and may produce few (< 100) large (~ 1.5 × 2.2 mm) eggs from which an advanced zoeal stage has been identified (e.g., Alvarez *et al.*, 2002; Mejía *et al.*, 2003). Partially abbreviated development in Palaemonidae as defined by Jalihal *et al.* (1993) occurs when the first larval stage has primordial pleopods appearing as small buds, a subtriangular or rounded telson without uropods, and variable numbers of biramous pereopods. It takes 3–5 molts to reach the juvenile stage in this type of development (Jalihal *et al.*, 1993). In contrast, completely abbreviated larval development is where the shrimp hatches from the egg with fully developed pleopods, chelate pereopods, and a toothed rostrum (Jalihal *et al.*, 1993).

Within the strictly freshwater Palaemonidae, several species of Macrobrachium and *Cryphiops* Dana, 1852 and all the species in *Calathaemon* Bruce & Short, 1993, *Creaseria* Holthuis, 1950, *Neopalaemon* Hobbs, 1973, *Troglocubanus* Holthuis, 1949, and *Troglomexicanus* Villalobos, Alvarez & Iliffe, 1999 are cave-adapted. In several of these cases abbreviated larval development

is assumed due to the large eggs found in ovigerous females and to the absence of plankton in the usually oligotrophic cave environments (Botello & Alvarez, 2013). Only the larval development of the Balcones Cave shrimp, *Palaemon antrorum* Benedict, 1896, from Texas, and that of the Squirrel Chimney cave shrimp, *Palaemon cummingi* (Chace, 1954), from Florida, were known until now (Dobkin, 1971; Strenth *et al.*, 1988). The larval development of *Macrobrachium yui* (Holthuis, 1950) has also been studied, but this species migrates between open and cave streams during its life cycle and has partially abbreviated development with mixed characteristics of epigean and cave species (Kounthongbang *et al.*, 2015). It thus becomes relevant to determine how larval development has been shaped in freshwater palaemonids given the dispersal limitations imposed by their environment.

We have conducted intensive sampling in the anchialine caves of the Yucatán Peninsula over the past six years, with a special interest in the area around the town of Tulum, Quintana Roo, Mexico (Fig. 1). A series of small larvae appeared in plankton samples obtained during the surveys, some with significant amounts of yolk in the dorsal portion of the cephalothorax. We started to recognize a sequence in the size and morphology of the larvae after 30 to 40 specimens had been collected from several surveys in the same area. Once all the larvae were examined and a few of them sequenced, we identified six larval stages and the first juvenile of the Yucatán endemic *Creaseria morleyi* (Creaser, 1936). We assigned the larvae to *C. morleyi* because they had typical palaemonid characteristics and this species is the only anchialine palaemonid present in the Yucatán Peninsula. Their identity was nevertheless confirmed by sequencing the COI mitochondrial gene. We present here a detailed description of the six larval stages and the juvenile of *C. morleyi* obtained from plankton samples and discuss the implications of this type of larval development within the Palaemonidae.

MATERIALS AND METHODS

Field and laboratory work

Sampling was conducted between February 2013 and October 2016 in eight caves around the town of Tulum, Quintana Roo, Mexico (Fig. 1). A total of 16 plankton samples were taken from the water column in total darkness with a 300 µm plankton net and later fixed in 70% ethyl alcohol. The larvae were sorted according to size in the laboratory at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City.

Measurements and drawings of larvae were made using an Olympus SZH10 dissecting microscope (Olympus, Tokyo, Japan) equipped with a *camera lucida*. Prior to dissection and illustration, the larvae were left in glycerin and later fixed in lactic acid for approximately one week; thereafter, dissected parts were mounted on semi-permanent slides. Morphological characters were examined with a compound microscope (Olympus BX50). Photographs were taken with an AXIO Zoom.V16 microscope and AxioCam MRc5 (5 megapixels) camera (Carl Zeiss Light Microscopy, Göttingen, Germany). The ZEN 2012 (blue edition) software was used to finish the photographs.

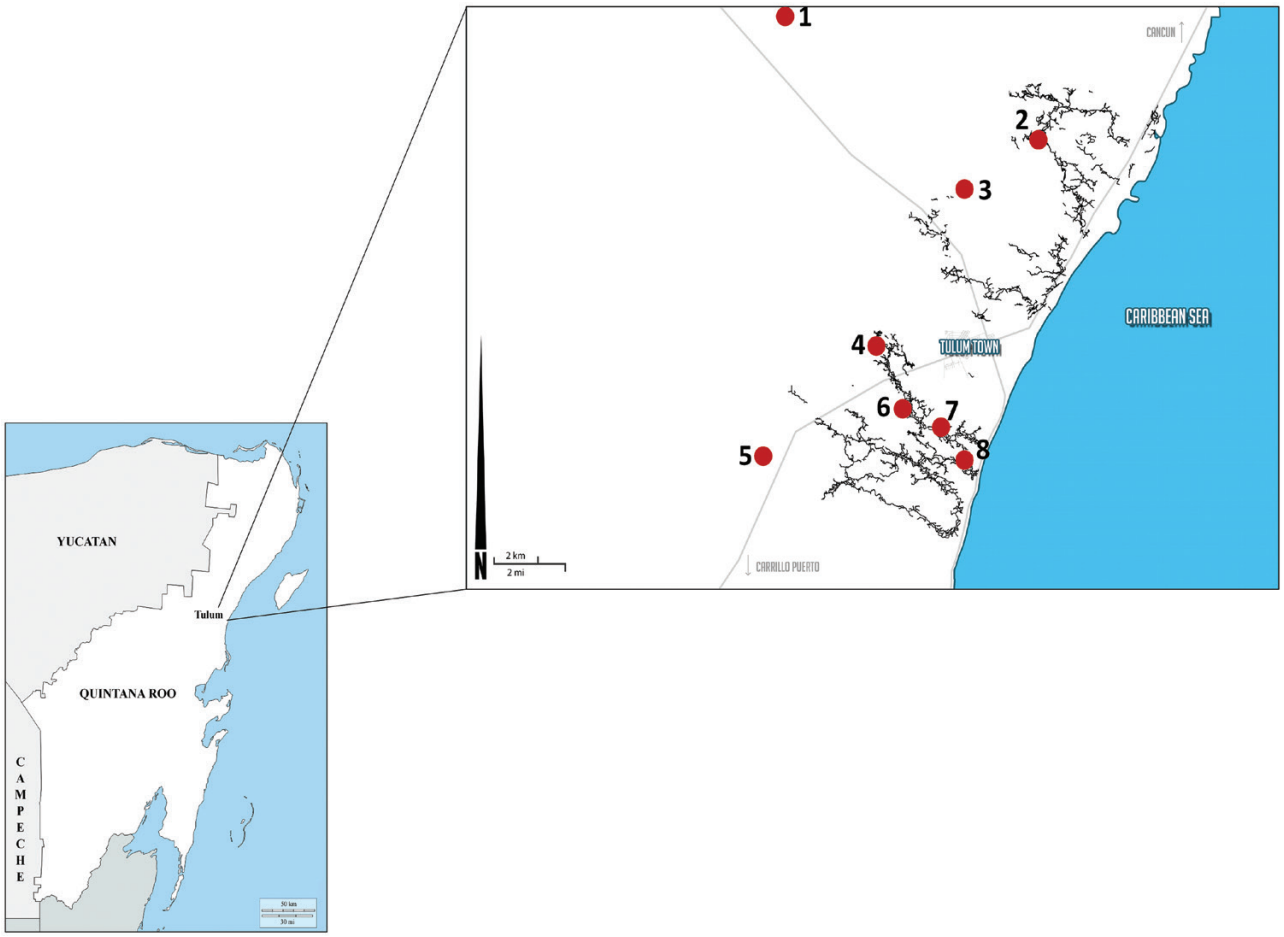

Figure 1. Locations of cenotes around the town of Tulum, Quintana Roo, Yucatán Peninsula, Mexico, where the larvae of *Creaseria morleyi* were obtained. 1, Choj-Ha; 2, Nohoch Nach Chich; 3, Álamo; 4, Bang; 5, Chan-Hol; 6, Muknal; 7, Odyssey; 8, Nahach Wennen Ha.

DNA extraction and COI sequencing

The barcode region of the COI gene was sequenced for two larvae and two adult individuals of *C. morleyi* and compared to other palaemonids to confirm the specific identity of the larvae. The tissue was digested with 1.25 ml of Proteinase K solution at 56 °C overnight. Genomic DNA was obtained from gill tissue from adult *C. morleyi* and from whole larvae using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's directions. We used a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to test the DNA quantity and quality. The barcode region of the COI gene was amplified from the obtained DNA using the primers LCO1940 and HCO2198 (Folmer *et al.*, 1994). PCR reactions were performed with a medium consisting of: 50–90 ng μl^{-1} of total DNA, 9.475 µl double-distilled water, 1.25 µl PCR buffer 10×, 0.5 µl $MgCl_2$, 0.5 µl BSA, 0.25 µl dNTPs, 0.2 µl (10mM) of each primer, and 0.125 µl Taq Platinum®. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 40 cycles at 94 °C for 40 sec, annealing at 55 °C for 45 sec, and an extension at 72 °C for 1 min, final extension at 72 °C for 10 min. Amplification was confirmed through 1% agarose gel electrophoresis with RedGel and loading buffer as nucleic acid markers in a TBE buffer at 125 mV for 25 min.

The PCR products were purified using Amicon® ultra-0.5 ml centrifugal filters (Sigma-Aldrich, St. Louis, Mo, USA). For the sequencing reactions we used the BigDye® Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific) following the manufacturer´s directions. The final products were purified in Sephadex CentriSep spin columns (Princeton Separations, Adelphia, NJ, USA) and sequenced in an Applied Biosystems 3500XL genetic analyzer (Thermo Fisher Scientific). The obtained sequences were edited in Chromas Lite v.2.01 (Technelysium, South Brisbane, QLD, Australia) and aligned with MAFFT 7.0 (Katoh *et al.*, 2019. Mesquite v.2.75 (Maddison & Maddison, 2011) was used to analyze the alignment of sequences and the presence of stop codons in the sequences. All sequences were deposited in GenBank (Supplementary material Table S1).

Data analysis

Two phylogenetic reconstruction analyses were used: maximum likelihood (ML) with RAxML v.8.2.12 (randomized axelerated maximum likelihood) (Stamatakis, 2014) and Bayesian inference (BI) via Markov chain Montecarlo (MCMC) methods with MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Data partition was used to analyze substitution variation among sites, each one following its own model and parameters as suggested by jModelTest. We added to the analysis the sequences of two other palaemonid shrimps: *Macrobrachium rosenbergii* (de Man, 1879) and *Palaemon suttkusi* (Smalley, 1964) obtained from the GenBank with accession numbers MF563572 and KJ769068, respectively (Supplementary material Table S1). The sequence of *Alpheus peasei* (Armstrong, 1940) (Alpheidae) (MK757254) was included as the external group.

We used jModelTest 2.1.7 to determine the appropriate nucleotide substitution model (Darriba *et al.*, 2012) for the ML reconstruction; the analysis was run in RAxML v.8.2.12 as implemented in (cyberinfrastructure for phylogenetic research (CIPRES) (Miller *et al.*, 2010). For the BI analysis a MCMC program was run for 30 million generations with tree samplings every 10,000 generations with four parallel chains, starting with a random tree. Substitution models used were those selected by JModelTest with the gene partitioned in the same run. We used a burn-in of 10%, observed with Tracer v.1.5.0 (Rambaut & Drummond, 2009); the posterior probabilities (PP) were calculated with the remaining trees and the 50% consensus tree obtained. The obtained trees were visualized with FigTree c.1.3.1 (Rambaut, 2008).

RESULTS

Abundance and distribution of larvae

A total of 41 larvae and two juveniles were collected in eight of the 16 field surveys. Larvae were found at depths of 11 to 15 m in all caves and always above the halocline. The salinity in the water mass above the halocline ranged from 2 to 10 psu. No seasonality was observed in the presence of larvae as they were found in February and December 2013, June 2014, January and June 2015, and March, August and October 2016. The first three larval stages were the most abundant, with 9 to 15 individuals in each stage, whereas stages IV to VI and the juvenile were represented by one to three individuals each (Fig. 2). The total length (tip of the rostrum to tip of the telson) of the smallest larva was 4.2 mm and the largest juvenile 12.5 mm (Fig. 2).

Genetic analysis

Four COI sequences were obtained, two from adult *C. morleyi* and two from the larvae being described (Supplementary material Table S1). The obtained sequences were compared to those of *Palaemon suttkusi*, *M. rosenbergii*, and *A. peasei* (Fig. 3). Because the trees obtained with ML and BI were almost identical, the latter one was chosen as the final result. The tree shows that the larvae and the adult *C. morleyi* are identical (Fig. 3), which was the objective of the comparison.

Description of larval stages

Stage I. Total length mean 4.7 mm ($N = 15$, 4.2–5.1 mm) (Fig. 4A). Rostrum not developed (Supplementary material Fig. S2A); carapace globose, approximately as long as high in lateral view, devoid of spines, translucid, with abundant vitellum (Fig. 4A). Abdomen smooth, first somite shortest, somites 2–5 subequal in length, sixth somite longest (Fig. 4A). Telson subtriangular, 1.55 times as wide as long, posterior margin with 20 long, simple setae; median indentation; uropods absent (Supplementary material Fig. S2B). Eyes sessile, unpigmented, in dorsal view as long as wide (Supplementary material Fig. S2A). Antennule, peduncle not segmented; inner flagellum short with single apical seta, external flagellum twice as long as inner one, with three plumose setae on apex, short subdistal spine (Supplementary material Fig. S2C). Antenna biramous, protopodite 2-segmented; basis simple, devoid of spines; scaphocerite overreaching antennular peduncle by one third of its length, internal, distal borders with 14 long, plumose setae, 2 short simple setae along external margin proximal half devoid of setae. Endopod digitiform, unsegmented (Supplementary material Fig. S2D). Mandible without palp, molar and incisor processes poorly developed, primordium of molar process with 4–5 minute denticles, area of incisor process smooth (Supplementary material Fig. S2E). Maxillule rudimentary, endites starting to develop, each with 2 spines; palp indicated by spine on small indentation (Supplementary material Fig. S2F). Maxilla with endites appearing as small rounded projections; palp well developed, with 4 apical setae. Scaphognathite oval, anterior portion prominent, with 8 setae along margin, posterior portion not developed with large, thick seta (Supplementary material Fig. S2G). Maxilliped 1 with simple protopodite; endopodite with subapical single seta, apex with 3 setae. Exopodite unsegmented, about 3 times as long as endopodite, with 5–7 apical plumose setae (Supplementary material Fig. S2H). Maxilliped 2 with simple protopodite; endopodite 4-segmented, with 2 small spines near basal portion, single apical spine; exopodite 2.5 times length of endopodite, apical tuft of long setae (Supplementary material Fig. S2I). Maxilliped 3 with simple protopodite; endopodite 5-segmented, with apical tuft of short setae; exopodite longer than endopodite, with 6 apical plumose setae (Supplementary material Fig. S2J). Pereopod 1 with simple endopodite, not chelate, 4-segmented; exopodite twice

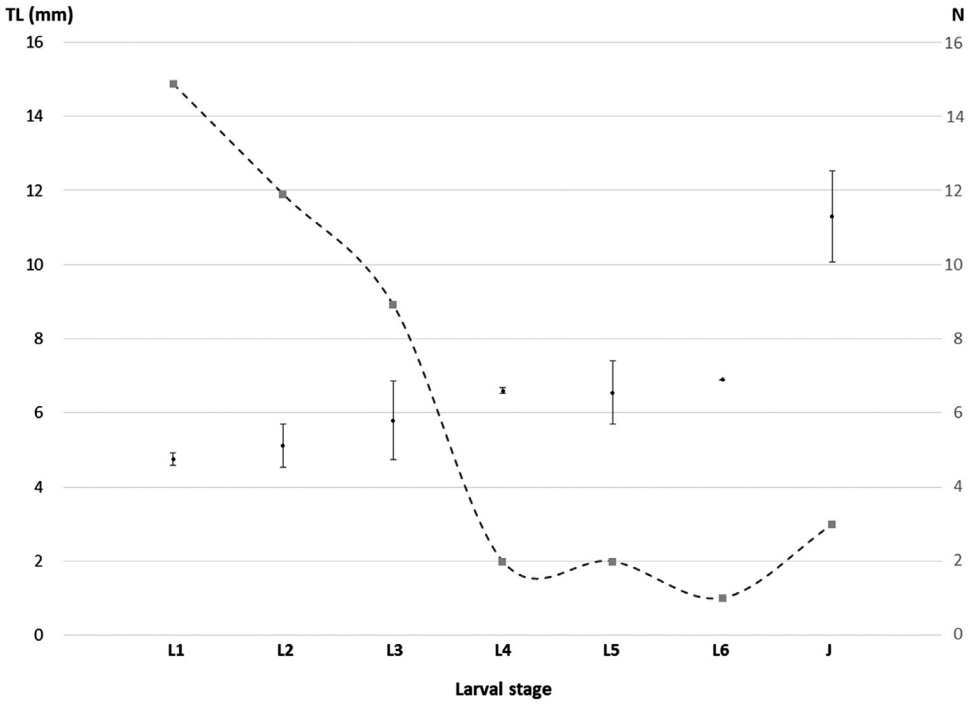

Figure 2. Plot of number and size of each larval stage of *Creaseria morleyi* examined. Grey squares represent number of larvae and the black dots the average size (mm) of each larval stage and the juvenile.

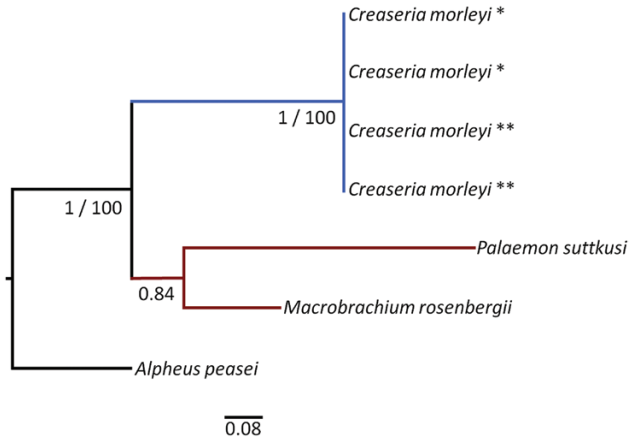

Figure 3. Tree based on COI partial sequences using Bayesian inference of *Creaseria morleyi* adults and larvae and two other palaemonids, *Palaemon suttkusi* and *Macrobrachium rosenbergii*. The outgroup is the alpheid shrimp *Alpheus peasei*.

length of endopodite, unsegmented, with 5 short, apical setae (Supplementary material Fig. S2K). Pereopods 2, 3 with simple endopodite, not chelate, 4-segmented; exopodite 0.3 longer than endopodite, with short apical setae (Supplementary material Fig. S2L, M). Pereopod 4, protopodite unsegmented, smooth; endopodite 4-segmented; exopodite 0.5 longer than endopodite (Supplementary material Fig. S2N). Pereopod 5with undeveloped exopodite, endopodite 4-segmented, next to distal, partially divided article (Supplementary material Fig. S2O). Pleopods not developed (Supplementary material Fig. S2A).

Stage II. Total length mean 5.11 mm (N = 12, 3.3–6.8 mm) (Fig. 4B). Rostrum short, triangular in dorsal view (Supplementary material Fig. S3A); carapace globose as in stage I, 1.5 times as long as high in lateral view, vitellum granules visible through dorsal portion (Fig. 4B). Abdomen smooth, relative length of somites as in stage I. Uropods not developed, their primordia discernible within telson (Supplementary material Fig. S3B). Telson subtriangular, same length:width proportion as in stage I, posterior margin bilobed with 20 long, simple setae (Supplementary material Fig. S3B). Eyes subrectangular in dorsal view, 1.4 times longer than wide (Supplementary material Fig. S3A). Antennule with inner flagellum larger than external flagellum in comparison with stage I (Supplementary material Fig. S3C), with scattered setae on third peduncular article. Antenna with flagellum 1.5 times length of scaphocerite (Supplementary material Fig. S3D). External margin of scaphocerite straight, without setae; internal margin with about 17 long plumose setae. Mandible with molar process as conical tooth, incisor process rounded, with minute denticles (Supplementary material Fig. S3E). Maxillule with endites and palp differentiated; posterior endite subtriangular, with minute denticles; anterior endite broadly rounded with scattered minute marginal teeth; palp as a simple acute projection (Supplementary material Fig. S3F). Maxilla with endites as small irregular projections, palp distinct, scaphognathite bordered by 13 plumose setae (Supplementary material Fig. S3G). Maxilliped 1 similar to that of stage 1 differing in exopodite length, 2.5 times length of endopodite (Supplementary material Fig. S3H). Maxillipeds 2, 3 similar to those in stage I (Supplementary material Fig. S3I, J). Pereopods 1–3 simple, not chelate, endopodite 4-segmented; exopodites with apical tuft of setae, longer than endopodites (Supplementary material Fig. S3K–N). Pereopod 4 with endopodite and exopodite subequal in length, exopodite with apical tuft of long setae (Supplementary material Fig. S3N). Pereopod 5 incomplete, exopodite absent (Supplementary material Fig. S3O). Pleopods not developed (Fig. 4B).

Stage III. Total length mean 5.8 mm (N = 9, 3–7.9 mm) (Fig. 4C). Rostrum triangular in dorsal view, almost reaching distal margin of eyes (Supplementary material Fig. S4A); carapace globose, 1.8 times as long as high in lateral view, vitellum granules visible

Figure 4. Lateral view of the six larval stages and the juvenile of *Creaseria morleyi*.

through dorsal portion (Fig. 4C). Abdomen smooth, relative length of somites as in stage II. Uropods rudimentary, endopod finger-like, slender, short projection; exopod oval with 7 marginal setae (Supplementary material Fig. S4B). Telson subtriangular, lateral angles rounded, posterior margin bordered with setae, with shallow medial indentation (Supplementary material Fig. S4B). Eyes subrectangular in lateral view (Fig. 4C), trapezoidal in dorsal view (Supplementary material Fig. S4A). Antennule and antenna similar to those of previous stage (Supplementary material Fig. S4C, D). Antennule with longer inner flagellum relative to stage II (Supplementary material Fig. S4C). Mandible similar to those in stage II (Supplementary material Fig. S4E). Maxillule differing from that in stage II by rounded posterior endite, palp approximately rectangular, more developed (Supplementary material Fig. S4F). Maxilla with endites as rounded small projections with short setae; palp as in stage II; scaphognathite elongated, suboval, bordered with long plumose setae; inferior lobe twice as long as that of stage II (Supplementary material Fig. S4G). Maxillipeds 1, 2 similar to those of stage II, exopodite length 2–2.5 times length of endopodite (Supplementary material Fig. S4H, I). Maxilliped 3 with endopodite and exopodite subequal in length (Supplementary material Fig. S4J). Pereopods simple, not chelate; endopodites 5-segmented. Exopodites of all pereopods 1.2–1.5 times length of endopodites (Supplementary material Fig. S4K~N). Pleopods appearing as short digitiform, undivided buds (Fig. 4C).

Stage IV. Total length mean 6.6 mm ($N = 2$, 6.5–6.7 mm) (Fig. 4D). Rostrum triangular in dorsal view, reaching distal margin of eyes (Supplementary material Fig. S5A); carapace globose, in 1.9 times as long as high in lateral view, vitellum granules visible through dorsal portion (Fig. 4D). Abdomen smooth, relative length of somites as in stage III. Uropods as in stage III, endopod clearly separated (Supplementary material Fig. S5B). Telson similar to that of stage III but narrower (Supplementary material Fig. S5B). Eyes subquadrate in lateral view (Fig. 4D), trapezoidal in dorsal view (Supplementary material Fig. S5A). Antennule similar to that of stage III (Supplementary material Fig. S5C). Antenna with external margin of scaphocerite straight, inner and distal margins with long plumose setae; flagellum 1.5 times longer than scale (Supplementary material Fig. S5D). Mandible with molar process as thick tooth, incisor process with rounded surface, minute denticles (Supplementary material Fig. S5E). Maxillule with endites of similar size, palp rectangular (Supplementary material Fig. SF). Maxilla as in stage III (Supplementary material Fig. S5G). Maxillipeds without significant differences from those of stage III (Supplementary material Fig. S5H–J). Pereopods 1–4 with endopods 5-segmented, endopodites as long or slightly longer than exopodites (Supplementary material Fig. S5K–N). Pereopod 5 missing. Pleopods longer than in stage III, separation of the branches discernible (Fig. 4D).

Stage V. Total length mean 6.55 mm ($N = 2$, 5.5–7.6 mm) (Fig. 4E). Rostrum with 2 dorsal teeth posteriorly, with 3 swellings anteriorly that have not developed into teeth, reaching distal margin of eyes (Supplementary material Fig. S6A); carapace globose, 1.7 times as long as high in lateral view, vitellum granules visible through anterior portion (Supplementary material Fig. S6A). Abdomen smooth, relative length of somites as in stage IV. Uropods with well-developed endopod, longer than telson, bordered with long setae; exopod wider, longer than endopod (Supplementary material Fig. S6B). Telson notably narrower than in stage IV, posterior margin bilobed, of approximately the same width throughout its length (Supplementary material Fig. S6B). Eyes as in stage IV (Supplementary material Fig. S6A). Antennule with inner flagellum more slender, longer than in stage IV; outer flagellum divided into two, with short accessory flagellum on inner side (Supplementary material Fig. S6C). Antenna similar to that in stage IV, flagellum longer (Supplementary material Fig. S6D). Mandible as in stage IV (Supplementary material Fig. S6E). Maxillule with palp with long simple seta (Supplementary material Fig. S6F). Maxilla with well-developed endites, palp simple, devoid of setae, scaphognathite suboval with setose margin (Supplementary material Fig. S6G). Maxilliped 1, endopodite with few apical setae; exopodite with 7 apical, long, plumose setae; external margin of base of exopodite setose (Supplementary material Fig. S6H). Maxilliped 2, exopodite more than twice length of endopodite (Supplementary material Fig. S6I). Maxilliped 3 similar to that of stage IV (Supplementary material Fig. S6J). Pereopod 1 chelate, ischium, merus, carpus subequal in length; cutting edges of fingers simple, palm shorter than fingers (Supplementary material Fig. S6K). Pereopod 2 chelate, ischium, carpus, merus subequal in length; chela longer than previous articles, fingers twice length of palm (Supplementary material Fig. S6L). Pereopods 3–5 simple, exopodites less than half length of endopodites (Supplementary material Fig. S6M–O). Pleopods 2-segmented, distal article with 2 branches, endopods about 2/3 length of exopods (Supplementary material Fig. S6A).

Stage VI. Total length mean 6.9 mm (N = 1) (Fig. 4F). Rostrum reaching beyond distal margin of eyes (Supplementary material Fig. S7A); dorsal surface of carapace slightly inflated, 1.7 times as long as high in lateral view, few vitellum granules visible through anterior portion (Supplementary material Fig. S7A). Abdomen without significant changes. Uropods as in stage V (Supplementary material Fig. S7B). Telson becoming narrower posteriorly, posterior margin slightly bilobed, pair of large spines on distolateral border, 8 short setae along posterior margin (Supplementary material Fig. S7B). Eyes as in stage V (Supplementary material Fig. S7A). Antennule, antenna similar as in stage V, antennule more setose (Supplementary material Fig. S7C, D). Mandible and maxillule as in previous stage (Supplementary material Fig. S7E). Maxilla with scaphognathite densely bordered with plumose setae, palp with long apical seta, endites separated by deep cleft (Supplementary material Fig. S7F). Maxilliped 1 as in stage V (Supplementary material Fig. S7G). Maxilliped 2 endopodite thicker, more than half length of exopodite (Supplementary material Fig. S7H). Maxilliped 3, endopodite longer than exopodite (Supplementary material Fig. S7I). All pereopods similar to those of stage V (Supplementary material Fig. S7J–N). Pleopods completely developed (Fig. 4F).

Juvenile. Total length mean 11.6 mm (N = 3, 10–12.5 mm) (Fig. 4G). Rostrum with 6–8 dorsal, one ventral teeth, clearly reaching beyond distal margin of eyes, reaching third article of antennular peduncle in dorsal view (Supplementary material Fig. S8A); dorsal surface of carapace straight, smooth, no vitellum granules visible (Fig. 4G). Abdomen with length of somites similar to adults, sixth somite the longest; fifth, fourth subequal in length, longer than first three (Fig. 4G). Uropods longer than telson, endopod oval, exopod with external margin straight, strong posterolateral spine. Telson subtriangular, anterior margin 3 times wider than posterior margin, dorsal surface with 2 pairs of spines on posterior third; posterior margin with 2 pairs of lateral spines, inner pair longer (Supplementary material Fig. S8B). Eyes oval shaped in dorsal, lateral views (Fig. 4G, Supplementary material Fig. S8A). Antennule with proximal peduncular article armed with 2 small, sharp spines on lateral margin; inner flagellum thin, long; external flagellum divided into 2 branches, external branch thick, long, internal branch short, with 10 articles bearing 2 aesthetascs in every joint (Supplementary material Fig. S8A, C). Antenna similar to that of previous stage (Supplementary material Fig. S8D). Mandible with incisor process formed by three sharp teeth, molar process with two sharp margins forming concavity in between, primordium of palp emerging between processes (Supplementary material Fig. S8E). Maxillule with subrectangular palp with apical digitiform process, anterior margin sinuous with 2 spiniform setae; anterior endite oval-shaped with 7 marginal spiniform setae; posterior endite smaller than anterior endite, distal portion rounded with 8 marginal spiniform setae (Supplementary material Fig. S8F). Maxilla with scaphognathite bordered with plumose setae, palp subrectangular, endites longer than palp, with setose tips (Supplementary material Fig. S8G). Maxilliped 1 with long exopodite with apical tuft of seta; endites divided by shallow cleft, with setae along gnathal border, anterior endite larger than posterior endite; caridean lobe discernible, with marginal setae on apex (Supplementary material Fig. S8H). Maxilliped 2 endopodite 5-segmented, gnathal border of last 2 articles with short, thick setae; epipodite, long, slender, with apical tuft of setae (Supplementary material Fig. S8I). Maxilliped 3 protopodite with bilobed epipodite; endopodite 3-segmented, first 2 articles with scattered setae, distal article with dense row of marginal setae on gnathal border; exopodite with apical tuft of setae, as long as first endopodite article (Supplementary material Fig. S8J). Pereopod 1 slender, 0.57 length of pereopod 2; ischium longest article, fingers 0.6 length of chela, cutting edges smooth (Supplementary material Fig. S8K). Pereopod 2 with merus the longest article, chela 0.45 length of whole appendage, fingers 0.6 length of chela; cutting edges smooth, tip of fingers hook-like (Supplementary material Fig. S8L). Pereopods 3–5 increasing in size posteriorly, propodi with 3–5 very fine spines along posterior margin, dactyli ending in sharp tips. Pleopods unchanged from stage VI.

DISCUSSION

We identified six larval stages and the first juvenile of *C. morleyi*. Because it was not possible to follow one cohort of larvae through time and through successive molts, but rather different individuals at various stages of development that were taken to construct a sequence, there is some variation in how particular structures change. Further, samples were collected in eight different caves over a two-year period. It is remarkable that with many potential sources of variation, all the recovered larvae fit well within the described developmental sequence.

While the presence of chromatophores has been noted in a number of decapod larvae, it is noteworthy to note that none were seen in any of the studied larvae. When alive, the larvae are transparent, but can be easily seen because the vitellum granules are bright yellow to orange.

Stage I larvae show a lower degree of overall development compared to the first larval stages of palaemonids with partially abbreviated development (e.g., Alvarez *et al.*, 2002; Mejía-Ortíz *et al.*, 2010). The segmentation of the pereopods is difficult to determine, or it is incomplete, particularly in the endopods, while there is a large quantity of vitellum and the mandibles are non-functional, blunt structures. This condition resembles the first zoea of *P. cummingi* in the subtriangular telson and the absence of pleopods, but differs in that the mandibles are more developed in *P. cummingi* (Dobkin, 1971). The first zoea of *P. antrorum* is also similar to that of *C. morleyi* in that the carapace is globose and it contains vitellum, but it is not as rounded as in *C. morleyi*, being 1.3 times as long as high. Another conspicuous difference is the presence of pleopodal buds in the first zoea of *P. antrorum*, which are absent in *C. morleyi* (see Strenth *et al.*, 1988) (Table 1). The development of pleopods starts in the third larval stage of *C. morleyi*, later than in the two stygobitic species of *Palaemon* (Table 1), but they may not be functional until the fifth larval stage, when they appear long enough to be able to propel the shrimp (Fig. 4E).

Table 1. Comparison of selected characters of the larval development among stygobitic palaemonid species.

	Creaseria morleyi	*Palaemon cummingi*	*Palaemon antrorum*
Number of larval stages	6	3	3
Emergence of pleopods	3	2	1
Size of first larval stage (mm)	4.2–5.1	4.0	4.0–5.2
Size of juvenile (mm)	11.6		7.0–8.0
Larval stages with vitellum	6	1	3
Stage at which mandibles develop	juvenile	third larval stage	first larval stage

Although adult *C. morleyi* have a two-segmented mandibular palp, it is not present in the six larval stages described; it starts to develop in the juvenile stage, appearing as a small bud between the incisor and molar processes (Supplementary material Fig. S8E). The same pattern appears in other palaemonid species whose larval development has been described: the mandibular palp is absent in the larval stages and starts to develop in the juvenile stage (e.g., *P. cummingi* (Dobkin, 1971); *M. nattereri* (Heller, 1862) (Magalhães, 1989); *M. tuxtlaense* Villalobos & Alvarez, 1999 (Alvarez *et al.*, 2002); *M. totonacum* Mejía-Ortíz, Alvarez & Hartnoll, 2003 (Mejía-Ortíz *et al.*, 2010)). The slow development of the mandible, as well as the presence of vitellum in all larval stages, suggest that the larvae do not feed until they reach the juvenile stage, when well-developed molar and incisor processes appear.

The first stages of *C. morleyi*, *P. antrorum*, and *P. cummingi* are very similar in size, ranging from 4.0 to 5.2 mm in total length (Table 1). They are, however, very different in the amount of vitellum they carry and in the size of the eyes (Dobkin, 1971; Strenth *et al.*, 1988). There is very little vitellum in the first zoea and the eyes are pigmented in *P. cummingi*; in *P. antrorum* the amount of vitellum resembles that of the stages IV–V of *C. morleyi* and the eyes are broadly rounded in contrast to the subrectangular eyes of *C. morleyi* (Dobkin, 1971; Strenth *et al.*, 1988). The three species are similar in that the first larval stage has simple, not chelate, pereopods 1, 2 (Table 2). The first larval stages of the strictly freshwater but epigean *Macrobrachium tuxtlaense* (6.7–7.7 mm; Alvarez *et al.*, 2002) and *M. totonacum* (5.5–6.45 mm; Mejía-Ortíz *et al.*, 2010) hatch from eggs slightly bigger and more developed.

The gradual reduction in the amount of vitellum can be seen in stages II–VI. In contrast to *P. antrorum* and *P. cummingi*, all larval stages of *C. morleyi* have vitellum (Table 1). The presence of vitellum in all larval stages can be an adaptation to the oligotrophic condition of cave waters (Pohlman, 2011). Since *C. morleyi* occurs only in the freshwater layer of anchialine caves, it cannot make use of any zooplankton that might enter the caves in the lower seawater layer.

The larval sequence shows a size increment of only 2.2 mm, from 4.7 mm at Stage I to 6.9 mm at Stage VI; however, there is a gap of 5 mm with the juvenile stage (Fig. 2). It is possible that we might be missing some larval stages between stages I and VI, and it is almost certain that there are one or two additional stages between our Stage VI and the juvenile. Although speculative, it is relevant to consider that the full development of *C. morleyi* could

Table 2. Number of larval stages reported for 32 species of palaemonid shrimps inhabiting three habitats: strictly freshwater, anfidromous, and estuarine. *C.*, *Creaseria*; *E.*, *Euryrhynchus*; *M.*, *Macrobrachium*; *P.*, *Palaemon; Ps.*, *Pseudopaleaemon*. Roman numerals in parenthesis denotes the larval stage at which the larval stage develop chelae on the first two pereopodse. [1]Magalhães, 1988a; [2]Magalhães, 1988b; [3]Magalhães, 2000; [4]Román *et al.*, 2000; [5]Mejía-Ortiz *et al.*, 2010; [6]Bueno & Rodrigues, 1995; [7]Magalhães, 1989; [8]Strenth *et al.*, 1988; [9]Dobkin, 1971; [10]Rodríguez-Almaraz *et al.*, 2010; [11]Magalhães, 1986a; [12]Muñiz-Martínez, 2012; [13]Dobkin, 1963; [14]Rodríguez-Almaraz *et al.*, 1997; [15]Magalhães, 1986b; [16]Oliphant *et al.*, 2013; [17]this study; [18]Hubschman & Broad, 1974; [19]Broad & Hubschman, 1963; [20]Broad, 1957; [21]Sandifer, 1973; [22]Alvarez *et al.*, 2002; [23]Jackson, 1992; [24]Gamba, 1998; [25]Knowlton & Vargo 2004; [26]Magalhães, 1985; [27]Menu-Marque, 1973; [28]Choudhury, 1970; [29]Dugger & Dobkin, 1975; [30]Monaco, 1975; [31]Uno & Kwon, 1969.

Number of Larval stages	Strictly freshwater	Anphidromous	Estuarine
1	*E. amazoniensis* (I)[1]		
	E. burchelli (I)[1]		
	E. wrzesniowskii (I)[1]		
	P. mercedae (I)[2]		
2	*M. jelskii* (I)[3]		
3	*M. vicconi* (I?)[4]		
	M. totonacum (I)[5]		
	M. iheringi (I)[6]		
	M. nattereri (I)[7]		
	P. antrorum (III)[8]		
	P. cummingi (III)[9]		
	P. hobbsi (I)[10]		
	P. ivonicus (I)[11]		
	P. lindsayi (I)[12]		
	P. mexicanus (I)[10]		
	P. paludosus (I)[13]		
	P. suttkusi (I)[14]		
	Ps. chryseus (I)[15]		
4			
5			*P. varians* (III)[16]
6	***C. morleyi*** (III)[17]		*P. intermedius* (V)[18]
	P. kadiakensis (IV)[19]		*P. pugio* (IX)[20]
			P. vulgaris (V)[21]
7	*M. tuxtlaense* (I)[22]	*P. pandaliformis* (VIII)[24]	*P. northropi* (I?)[23]
8	*P. floridanus* (VIII)[25]		
9	*P. argentinus* (I?)[27]	*M. amazonicum* (VIII)[26]	
10		*M. acanthurus* (VII)[28]	
		M. olfersii (XI)[29]	
11+		*M. americanum* (I?)[30]	
		M. carcinus (X)[28]	
		M. rosenbergii (I?)[31]	

consist of at least eight stages, situating this species among the group of *Macrobrachium* species with extended development, rather than with the strictly freshwater palaemonids (Table 2).

Botello & Alvarez (2013) examined the phylogenetic relationships among the strictly freshwater palaemonid genera distributed in Mexico based on partial sequences of the 16S mtDNA gene. Their results showed that *C. morleyi* and *Neopalaemon nahuatlus* Hobbs, 1973, another monotypic stygobitic shrimp from Oaxaca, share a branch independent of *Macrobrachium*, *Palaemon*, *Cryphiops*, and *Troglomexicanus* (see Botello & Alvarez, 2013). In this case, the described similarities in larval morphology among genera might be traced to the ancestral freshwater palaemonid stock that gave rise to these genera, and may further suggest an independent invasion of the freshwater habitat by the ancestor of *C. morleyi*. In such case, the similar type of larval development among different palaemonid lineages should be viewed as an evolutionary convergence.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. List of species, catalogue number (CNCR) or source, locality, and GenBank accession number for the COI partial DNA sequences used in the analysis.

S2 Figure. *Creaseria morleyi* stage I larva.

S3 Figure. *Creaseria morleyi* stage II larva.

S4 Figure. *Creaseria morleyi* stage III larva.

S5 Figure. *Creaseria morleyi* stage IV larva.

S6 Figure. *Creaseria morleyi* stage V larva.

S7 Figure. *Creaseria morleyi* stage VI larva.

S8 Figure. *Creaseria morleyi* juvenile.

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