Cranchiids of the South Atlantic Mid-Oceanic Ridge: results from the first southern MAR-ECO expedition

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Cranchiids were the most diverse squid family collected during the first southern MAR-ECO expedition in late 2009, with nine taxa identified to species. A total of 45 young specimens were collected (mantle length 7.4–59.2 mm), allowing a survey of early ontogenetic tentacular morphology in cranchiids using scanning electron micrographs. Paralarval tentacular sucker morphology appeared similar among species within the same subfamily: in the Cranchiinae, the paralarval suckers possess relatively large, narrowly polygonal or ovoid-faced pegs in the innermost ring around the aperture, and the infundibular ring lacks the dentition observed in most taoniin genera. Hook development in \textit{Galiteuthis armata} appears to vary widely among small individuals. Tissue samples were also collected from five genera (\textit{Cranchia}, \textit{Galiteuthis}, \textit{Helicocranchia}, \textit{Leachia} and \textit{Teuthowenia}); phylogenetic trees (maximum-likelihood and Bayesian methods) built using these cytochrome oxidase subunit I sequences and others available from GenBank show some support for the subfamilies Cranchiinae and Taoniinae, and that within the latter, the hooked taxa group together. It is hoped that reporting this opportunistic systematic and genetic information may be of eventual assistance in helping to resolve this most problematic of squid families.

\textbf{Keywords:} Cranchiidae; ontogeny; sucker ultrastructure; South Atlantic

\section*{Introduction}

Research expeditions under the MAR-ECO initiative (a field project of the Census of Marine Life) have been investigating biodiversity on the Mid-Atlantic Ridge (MAR) since 2003. This research focused on the north Atlantic until late 2009, when a first exploratory cruise began sampling the southern MAR between the Canary Islands and Cape Town (see Figure 1), a relatively poorly known area of the deep sea (Perez et al. 2012). Collections from this voyage included over 7000 fish specimens (Kobyliansky et al. 2010), plus benthic and pelagic invertebrates; the latter included 262 paralarval and juvenile cephalopods. Of these, the squid family Cranchiidae was represented by the greatest diversity of taxa, as was found during the northern MAR expeditions (Vecchione et al. 2010), although more and slightly different species were encountered herein.
Figure 1. Locations sampled with the Isaacs–Kidd Midwater Trawl during the South Atlantic MAR-ECO cruise, 25 October to 29 November 2009. Stations with and without catches of cranchiid squids are represented by different symbols. Boxes indicate three sectors targeted by the R/V Akademik Ioffe in the South Atlantic: South Equatorial Mid-Atlantic Ridge Sector (upper box), Tropical Mid-Atlantic Ridge Sector (middle box) and Walvis Ridge Sector (lower box).
Cranchiids are a diverse family in general, with more than 60 species believed to exist, many undescribed (Voss et al. 1992). Early ontogenetic stages of most genera, which occur in tropical to sub-polar epipelagic waters, are often characterized by stalked eyes and arm crowns, and bear little resemblance to adults. Although the morphology of some young cranchiid species appears unmistakeable (e.g. Mesonychoteuthis hamiltoni), others are more difficult to identify, especially given the large number of undescribed taxa. Some genera, such as Liocranchia and Leachia, go through considerable morphological changes during ontogeny. Therefore, it is hoped that providing extra morphological information on a subset of cranchiid taxa, from a relatively poorly studied region, may assist in the eventual, badly needed systematic revision of the family.

Overall tentacular club morphology is useful for identifying squid throughout ontogeny, and detailed examination of specific tentacular hook/sucker morphology has proven these structures’ particular value in differentiating taxa in some families (e.g. Sepiolidae, see Okutani and Horita 1987; Okutani and Takayama 1991; Mastigoteuthidae, see Salcedo-Vargas 1995), including paralarvae and juveniles (Onychoteuthidae, see Bolstad 2010). To date, sucker microstructure in young cranchiids remains largely unreported, although Pfeffer (1900) and Chun (1910) both illustrated overall tentacle morphology for early ontogenetic stages of several genera. Aldred (1974) briefly described and illustrated tentacular suckers for Bathothauma lyromma, while Dilly and Nixon (1976) and Nixon and Dilly (1977) included scanning electron micrographs (SEMs) of Teuthowenia megalops arm and tentacular suckers. Since young specimens from nine of the 13 known cranchiid genera were collected during the recent South Atlantic MAR-ECO cruise (Table 1), an opportunity was recognized to examine their tentacle clubs using SEM and provide a first

Table 1. Cranchiid specimens collected on the first South Atlantic MAR-ECO cruise, November, 2009.

| Species                        | Specimens | Mantle length (mm) | Geographic range | Stations collected |
|-------------------------------|-----------|--------------------|------------------|--------------------|
| Bathothauma cf. lyromma       | 1         | 10.0               | CTST*            | 1004               |
| Cranchia scabra               | 8         | 6.7–15.3           | CTST             | 1003, 1018         |
| Galiteuthis armata            | 3         | 13.4–37.2          | ATT(N)           | 1004, 1011         |
| Helicocranchia cf. pfefferi   | 9         | 8.2–29.0           | ATT(N)**         | 1004, 1005, 1011   |
| Leachia atlantica             | 2         | 14.6, 28.8         | AST(N)           | 1018               |
| Liguriella podophthalma       | 2         | 16.6, 26.6         | CTST             | 1044               |
| Liocranchia reinhardtii       | 15        | 7.7–41.1           | CTST             | 1003, 1004, 1009, 1015 |
| Megalocranchia sp.            | 1         | 59.2               | CTST             | 1026               |
| Teuthowenia pellucida         | 3         | 16.1–28.9          | CSC              | 1047               |
| Cranchiidae n.i. (Taoniinae)  | 1         | 14.9               |                  | 1001               |
| **Total**                     | **45**    |                    |                  |                    |

Notes: *and into temperate waters of the northeastern Atlantic; ** for Atlantic species. Geographic ranges: AST(N), north-Atlantic subtropical; ATT(N), Atlantic tropical to northern temperate; CSC, circumglobal southern subtropical convergence; CTST, cosmopolitan tropical/subtropical.
survey of paralarval and juvenile tentacular sucker morphology in the family. Tissue samples were also collected from five taxa for cytochrome oxidase subunit I (COI) sequencing, molecular analysis and submission to GenBank.

Material and methods
Specimens were collected by the R/V Akademik Ioffe (Shirshov Institute, Academy of Sciences, Russia), beginning 25 October 2009 in Las Palmas (Gran Canaria, Spain) and ending 29 November 2009 in Cape Town (South Africa). Four target areas along the MAR were sampled (Figure 1; Table 2): the Romanche Fracture Zone (two stations, ~0°35′ N, 17° W), the “south equatorial” MAR sector (two stations, 4–5° S, 12° W), the “tropical” MAR sector (two stations, ~18° S, 13° W) and the Walvis Ridge (four stations, 29°30′–33°40′ S, 01°10′–02°50′ E). Opportunistic samples were also collected between these study areas. Most cephalopod specimens were collected with an Isaacs–Kidd midwater trawl, as modified by Samyshev-Aseev (Kobyliansky et al. 2010): 25 m long, made of knotless netting, with 5-mm mesh and a cod-end insert of kapron sieve 15; opening area 6 m². Oblique step tows were conducted at ~3 knots. Two tows were conducted in each station, one to depths as close as possible to the summit of the ridge, and another to the Sound Scattering Layer depth as detected acoustically.

Specimens were photographed fresh, fixed in 4% buffered formalin, and transferred to 75% ethanol after 24 hours. Where material permitted, tissue samples for genetic analysis were preserved in 96% ethanol before fixing the remainder of the specimen. Preliminary on-board identifications were confirmed or revised following more in-depth examination of specimens on shore, following Voss et al. (1992). Mantle length (ML) was measured according to Roper and Voss (1983) using digital callipers and recorded to the nearest 0.1 mm. All specimens including genetic vouchers will be acquisitioned into the Natural History Museum of the University of Parana, Brazil.

Specimens examined using environmental scanning electron microscopy (ESEM) were critical-point dried, sputter-coated in gold–palladium, and imaged at the University of Auckland. Terminology for sucker morphology follows Nixon and Dilly (1977) and Salcedo-Vargas (1995), with size of the pegs (e.g. “large pegs”) indicating the diameter of the peg relative to the polygonal process from which it protrudes.

Tissue snips were collected from representatives of five genera (Cranchia, Galiteuthis, Helicocranchia, Leachia and Teuthowenia), and from four additional cranchiid specimens (three Galiteuthis phyllura and one Taonius borealis) opportunistically sampled in the Monterey Canyon (northeast Pacific) in March, 2013, and stored in 96% ethanol for molecular work (Table 1). The mitochondrial gene COI was targeted. DNA extraction, polymerase chain reaction (PCR) and sequencing of each of the MAR-ECO samples were carried out at the barcoding facility at Guelph, Canada (details in Allcock et al. 2011). Sequence data are available from GenBank (accession numbers KF369197–KF369201 and KF309244–KF309247, see Table 3). DNA extraction and sequencing of these five samples were carried out using the high-salt method (Sambrook et al. 1989). The 25-µl PCR contained 12.5 µl MyTaq Red Mix (Bioline, London, UK), 20 µM of each universal primer (Folmer et al. 1994) and ~100 ng of template. PCR conditions included a denaturation step of 94°C for 2 min,
Table 2. Sampling tows using an Isaacs–Kidd Midwater Trawl during the SA MAR-ECO cruise, November, 2009.

| Station | Date       | Time (GMT) | Latitude      | Longitude     | Sector | Bottom depth | Depth stratum |
|---------|------------|------------|---------------|---------------|--------|--------------|---------------|
| 1001    | 03/11/2009 | 02:02      | 00°34.48’N    | 17°14.35’W    | SEMS   | 3223         | 1000–0        |
| 1003    | 03/11/2009 | 06:11      | 00°38.88’N    | 17°9.916’W    | SEMS   | 3773         | 75–0          |
| 1004    | 03/11/2009 | 09:29      | 00°26.31’N    | 17°5.683’W    | SEMS   | 886          | 800–0         |
| 1005    | 03/11/2009 | 12:25      | 00°25.91’N    | 17°14.43’W    | SEMS   | 1358         | 300–0         |
| 1009    | 06/11/2009 | 21:05      | 03°3.983’S    | 13°18’W       | SEMS   | 3555         | 300–0         |
| 1011    | 07/11/2009 | 10:14      | 03°7.266’S    | 12°25.1’W     | SEMS   | 3200         | 1250–0        |
| 1015    | 08/11/2009 | 01:09      | 03°48.63’S    | 12°15.75’W    | SEMS   | 1789         | 300–0         |
| 1018    | 10/11/2009 | 01:30      | 11°2.433’S    | 12°45.8’W     | TMS    | 2487         | 200–0         |
| 1026    | 18/11/2009 | 00:14      | 25°43.5’S     | 02°21.1’W     | TMS    | 1533         | 250–0         |
| 1044    | 22/11/2009 | 00:28      | 33°40.43’S    | 02°35.33’E    | WRS    | 4740         | 1500–0        |
| 1047    | 26/11/2009 | 00:50      | 25°55.41’S    | 07°18.51’W    | WRS    | 2518         | 550–0         |

 Depths are in metres. Sector abbreviations: SEMS, South Equatorial MAR Sector; TMS, Tropical MAR Sector; WRS, Walvis Ridge Sector.
followed by 35 cycles of 94°C for 40 s, 50°C for 40 s and 72°C for 90 s. A final extension step of 72°C for 10 min completed each PCR. The PCR purification and sequencing were completed by Macrogen (Seoul, Korea).

Additional cranchiid COI sequences were obtained from GenBank (Leachia lemur EU735389, Leachia atlantica AY557530, Leachia sp. GU145067, Megalocranchia sp. EU735382, Liocranchia valdiviae AF000050, Cranchia scabra AF000035, Helicocranchia sp. GU145061, Helicocranchia pfefferi AF075412, Teuthowenia megalops AY617064, Mesonychoteuthis hamiltoni EU73597, Taonius pavo EU735381, Taonius borealis AY075415, Helicocranchia pfefferi GU145078, Galiteuthis armata EU735396, and Leachia pacifica KC020189).

Sequences were aligned using Geneious 6.0.5 using the default settings within the “Geneious alignment” option. The sequence KC020189 (“Leachia pacifica”) contained several indels (insertions/deletions) when compared with all remaining sequences suggesting that it may be a pseudogene. In addition, this sequence showed the highest maximum identity to sepiolid sequences on GenBank rather than any cranchiid sequence and is suspected to have been misidentified. For these reasons this sequence was excluded from further analyses. All remaining sequences aligned without indels. The alignment comprised 685 base pairs.

The molecular dataset was analysed using the GTR + \( \Gamma \) (general time reversible + gamma) model in a maximum likelihood framework implemented within raxmlGUI 1.2 (Silvestro and Michalak 2011), a graphical user interface for RAxML (Stamatakis 2006). One thousand thorough bootstrap replicates were used to assess node support.

In addition, the data set was analysed using the GTR + \( \Gamma \) model within a Bayesian framework using MrBayes (Huelsenbeck and Ronquist 2001) implemented within Geneious 6.0.5. The default Monte Carlo Markov Chain settings were used. The posterior output of the run was visualized within Geneious 6.0.5 to ensure that the analysis had reached stationarity and that the default burn-in was adequate.
Trees were rooted using *Cranchia* (and as a result also *Liocranchia*) following Lindgren et al. (2012).

Due to the existence of many undescribed cranchiid taxa (*fide* Voss et al. 1992), specimens reported herein are only attributed to species in presently monotypic genera (e.g. *Cranchia*) or where species characters within the genus appear unambiguous at present (e.g. *Liocranchia*). Where specimens resemble known species (according to physical characters and/or geographically restricted distributions), but the genus is reported to contain additional undescribed species (according to Voss et al. 1992), the provisional species ID is that of the most likely known species, preceded by “cf.”.

**Results**

In all, 45 cranchiid specimens were collected during the southern MAR-ECO cruise, representing nine genera (Table 1) and comprising 23% of the total cephalopod species caught. These records have been submitted to OBIS (IOC-UNESCO). Morphological descriptions of the tentacle clubs from each taxon follow, divided into subfamilies.

**Cranchiinae**

*Cranchia scabra* (Figure 2A) was represented in cruise collections by eight small specimens (ML 6.7–15.3 mm). The manus was unexpanded (Figure 3A), with ~16 rows of four small suckers, of equal size across each transverse row and all of similar morphology along the manus and dactylus. Each sucker possessed a single row of elongate-polygonal-faced pegs proximally and two rows of smaller circular-faced pegs distally. The margin of the infundibular ring was smooth proximally and distally, or distally produced into several low, rounded teeth.

*Liocranchia reinhardti* (Figure 2B, C) was the most numerous cranchiid collected (*n* = 15), and represented the greatest size range (ML 7.7–41.1 mm). The overall morphology of these specimens appeared appropriately intermediate between the smaller and larger specimens figured by Voss (1980: fig. 2). At ML 7.7 mm (Figure 3B), the tentacle clubs were stubby, with ~13 transverse rows of four suckers each, following a carpal region of four to six suckers (the latter being present in all specimens examined). The carpal region suckers appeared similar to those of the manus, and manus and dactylus were poorly differentiated, their suckers all appearing similar in size and morphology: the infundibular ring possessed three variably sized, rounded processes distally; a single ring of ovoid to oblong-faced pegs surrounded the aperture; and two to five additional, round-faced pegs were present in a second row distally. At ML ~40 mm, the carpal suckers were distinct in morphology (orally flattened, with many low, irregular pegs), and the manus remained only slightly expanded (Figure 3D), with ~10 rows of four suckers,
increasing in size through about the seventh row (with those of the medial series slightly larger than those of the marginal series), thereafter quickly decreasing in size. The dactylus, now well differentiated, comprised a further ~15 rows of suckers, their morphology distinct from those of the manus. Those of the manus possessed one or two rows of small, widely spaced pegs proximally; two or three rows of small, widely spaced pegs distally; and approximately five narrow, pointed teeth around the distal margin of the infundibular ring, with the innermost row of pegs distally also resembling elongate teeth in some suckers. The dactylic suckers possessed smooth inner margins of the infundibular ring, and numerous, densely set pegs, in: two rows proximally, with oral faces of inner row pegs largest, and being elongate polygonal in outline (as in smaller specimens); and four to five rows distally, with circular to ovate oral faces.

Two specimens of *Leachia cf. atlantica* (ML 14.6, 28.8 mm) were collected. The clubs (Figure 4A) bore ~14 rows of (generally) four suckers, preceded proximally by two rows of two suckers each, forming a carpal region. The next eight or nine rows comprised the manus, and the final seven comprised the dactylus. The medial manus suckers were greatly enlarged relative to the marginals, and bore two or three proximal rows of pegs, with the innermost largest and ovoid- to polygonal-faced, and two or three distal rows of smaller, approximately circular-faced pegs. The infundibular ring of each medial sucker was produced beyond the oral peg-bearing surface of the sucker, and bore low, slender teeth (about 20 in total) around its entire perimeter. The marginal and dactylic suckers were similar to each other, in having two rows of large, ovoid-faced pegs proximally (with those of the inner row larger);
Figure 3. Tentacle clubs of cranchiin species. (A) Cranchia scabra, ME 38828, mantle length (ML) 10 mm. (B–D) Liocranchia reinhardtii: (B) ME 39432, ML 7.7 mm; (C) ME 39772, ML 11.2 mm; (D) ME 38838, ML 41.1 mm. Scale bars = 500 µm.
Figure 4. Tentacle clubs of cranchiin and taoniin species. (A) *Leachia cf. atlantica*, ME 38942, mantle length (ML) 28.8 mm. (B) *Bathothauma cf. lyromma* sucker, ME 38494, ML 10.0 mm. (C, D) *Galiteuthis armata*: (C) ME 38550, ML 16.0 mm; (D) ME 38534, ML 37.1 mm. Scale bars = (A, C, D) 1 mm, (B) 20 µm.
approximately three rows of densely set, circular-faced pegs distally; and the inner margin of the sucker ring often produced beyond the oral peg-bearing surface of the sucker, but generally smooth (without teeth).

**Taoniinae**

One *Bathothauma cf. lyromma* specimen of ML 10.0 mm was taken. The specimen was not in good condition and the tentacle clubs were damaged, but more than 30 rows of four suckers appeared to have been present. Several sucker rings in the distal portion of the club remained (Figure 4B); these possessed two rows of small, concave and approximately circular-faced pegs proximally and distally, and about four teeth distally on the infundibular ring.

*Galiteuthis armata* (Figure 5A) was represented by three specimens (ML 13.4, 16.0, 37.2 mm). The tentacle clubs were unexpanded. Those of the smallest specimen bore ~14 rows of suckers: four rows of two in the carpal region, then about 10 rows of four. In the median two series, the proximal-most sucker had not yet begun to transform into a hook, but the subsequent three suckers had; the remaining suckers were all of similar morphology: on the inner margin of the infundibular ring, four to six low teeth with concave oral faces were present distally, and a single low, wide process was present proximally (as in the larger specimens). In the ML 16.0-mm specimen, the club bore ~16 rows of suckers (Figure 4C): four rows of two in the carpal region, interspersed with low fleshy bumps, then about 12 rows of four. The proximal-most sucker in each median series had enlarged to about half again the diameter of the marginal sucker flanking it, and had begun transforming into a hook; the subsequent three suckers distally and four ventrally were all fully formed hooks. The remaining suckers in all distal rows and the marginal suckers flanking the hooks were all of similar morphology, with two to three rows of densely set, circular-to-ovoid-faced pegs proximally, and four to five rows of similar but smaller pegs distally, as well as approximately three cylindrical teeth around the distal margin of the infundibular ring. The proximal portion of the infundibular ring was produced into a single low, wide, irregular process or tooth across the sucker ring aperture. The club of the ML 37.2-mm specimen (Figure 4D), although similar in counts and distribution of manus suckers, and slightly larger in absolute size, actually appeared to be at an earlier stage of hook development than the ML 16-mm specimen: the largest hooks were less completely transformed, and the distal-most hook in the ventral row was little more than a large sucker with the hook cusp beginning to emerge from the distal portion of the infundibular ring. The distal suckers and marginal suckers flanking the hooks were similar in morphology to those of the ML 16-mm specimen, with three or four cylindrical teeth distally and the characteristic flat process proximally.

*Helicocranchia cf. pfefferi* (Figure 5B) was the second-most numerous cranchiid encountered, represented by nine specimens (ML 8.2–29.0 mm). The minute tentacle clubs (Figure 6A) were not well preserved but appeared to have a short carpal region followed by ~11 transverse rows of four suckers, all of similar size across each row, with the diameter of suckers in the third to sixth rows slightly larger than those in the more proximal and distal rows. Sucker morphology appeared consistent overall across rows and along series, with one or two rows of densely set, small circular-to-oval faced pegs proximally, and three to four rows distally, with those of the inner
row largest; up to six teeth were also observed distally on the infundibular ring. On several suckers, one or more pegs in the inner row appeared abruptly enlarged, and in one instance, several of the distal polygonal platelets and their pegs appeared fused together, while a number of pegs in the outer-most distal row appeared intermediate in morphology between “normal” oval-faced pegs and the elongated, rectangular plates of the outer sucker rim.

One specimen of *Liguriella podophtalma* (Figure 5C) was collected, but the tentacles were in poor condition. The clubs were expanded proximally, narrowing rapidly, and appeared to have a distinct carpal region of about five suckers, followed by ~15 transverse rows of four. No sucker rings remained apart from a distal cluster of about eight; these possessed two rows of large, low, circular to oval flat-faced pegs proximally and approximately five similar rows distally, with those of the inner-most row largest, and about six broad, rounded teeth along the distal margin of the infundibular ring.

The single *Megalocranchia* sp. (Figure 5D) collected was the largest cranchiid specimen, at ML 59.2 mm. Its tentacle club (Figure 6B) bore ~30 transverse rows of four suckers. Carpus, manus and dactylus each bore suckers with distinct morphologies, but the transitions between regions were not distinct, i.e. several suckers of intermediate morphology were present between “typical” manus and “typical” dactylus suckers. A clear terminal pad region was also present. Sucker size was approximately subequal across each transverse row; those in rows 11–15 were largest on the club, and possessed one or two rows of very small, narrow pegs around the aperture, and approximately six very large, conical teeth produced from the distal inner margin of the infundibular ring. As sucker size reduced distally, the size of the teeth also reduced (and their distribution occasionally included the entire inner margin of the sucker ring), while the relative size of the pegs increased. Dactylic suckers were thus characterized by five to seven low, conical teeth on the inner margin of the infundibular ring, with one or two rows of low, round-faced pegs surrounding the aperture proximally and two or three rows distally.

*Teuthowenia pellucida* was represented by three specimens (ML 10.4–28.9 mm, Figure 5E, F). The tentacle club (Figure 6C) was slightly expanded but not clearly differentiated from the stalk, which bore the characteristic transverse rows of four suckers. From the point in the carpal region at which the dorsal and ventral membranes originated, the club bore ~20 transverse rows of suckers, gradually increasing in size through rows 5–8 and largest in the ventral two series. The sucker rings bore two rows of small, round- and concave-faced pegs proximally and two to three rows distally, with six to eight longer teeth produced from the distal inner margin of the infundibular ring. In some suckers, particularly on the distal portion of the club, the teeth continued proximally to a total of about 16 teeth spaced around the entire inner margin; in the more proximal suckers, these teeth were often reduced and sometimes merged into low triangular processes.

Figure 5. Taoniin specimens collected by SA MAR-ECO. (A) *Galiteuthis armata*, ME 38534, mantle length (ML) 37.2 mm; (B) *Helicocranchia* cf. *pfefferi*, ME 38898, ML 20.1 mm; (C) *Liguriella podophtalma*, ME 39753, ML 16.6 mm; (D) *Megalocranchia* sp., ME 39935, ML 59.2 mm; (E, F) *Teuthowenia pellucida*, ME 39697: E, ML 16.1 mm; F, ML 28.9. Scale bars = (A–D, F) 10 mm, (E) 1 mm.
COI sequences were available for 10 cranchiid genera: five of those sampled in this study (*Cranchia*, *Galiteuthis*, *Helicocranchia*, *Leachia* and *Teuthowenia*; Table 3), plus

**Genetics**

COI sequences were available for 10 cranchiid genera: five of those sampled in this study (*Cranchia*, *Galiteuthis*, *Helicocranchia*, *Leachia* and *Teuthowenia*; Table 3), plus
four additional genera whose sequences were available in GenBank (Liocranchia, Megalocranchia, Mesonychoteuthis and Taonius) and a likely fifth labelled “Helicocranchia pfefferi” (GU145078). Four additional COI sequences from recently collected specimens of Galiteuthis phyllura and Taonius borealis from the Monterey Canyon were also included (Table 3).

Maximum-likelihood phylogenetic analysis and Bayesian inference strongly supported the monophyly of each genus investigated in this study, with the exception of a sequence obtained from GenBank purported to be Helicocranchia pfefferi (GU145078), which did not group with other Helicocranchia individuals (Figure 7). A sister-taxon relationship was supported between this sequence (GU145078) and all Galiteuthis species [posterior probability (pp) = 0.93, Bootstrap (BS) = 58]. Together this clade was the sister taxon to Mesonychoteuthis but this relationship received low posterior probability support only (pp = 0.73). Taonius, Mesonychoteuthis, Galiteuthis – the three taxa bearing hooks (Galiteuthis and Mesonychoteuthis) or

![Figure 7. Bayesian topology of cranchiid relationships obtained using the GTR + Γ (general time reversible + gamma) model. The tree was constructed using nine new cranchiid cytochrome oxidase subunit I sequences [Table 3] in addition to 14 cranchiid sequences obtained from GenBank. Bayesian posterior probabilities are presented above the nodes and maximum-likelihood bootstraps below the nodes. * indicates a posterior probability of 1.0.](image-url)
hook-like suckers (*Taonius*) – and the sequence identified as “*Heliocranchia pfefferi*” (GU145078) together formed a monophyletic group (pp = 0.95). *Liocranchia* and *Cranchia* were highly supported sister taxa (pp = 100, BS = 88). Although the genera comprising the subfamilies Cranchiinae and Taoniinae did group together, support was low for this division, and *Megalocranchia* grouped with the Cranchiinae in spite of its morphological alignment with Taoniinae.

The sequences obtained for this study have been submitted to GenBank (Table 3), raising the number of available cranchiid COI sequences from 14 (not counting the herein excluded “*Leachia pacifica*” sequence KC020189) to 23.

**Discussion**

In general, tentacular morphology in cranchiid paralarvae and small juveniles is characterized by long, slender tentacles with unexpanded manus and carpus regions, and a poorly differentiated dactylus comprising of several small suckers. However, as in adults, the tentacle clubs of the paralarval and juvenile specimens observed herein demonstrate considerable morphological diversity. For some of the nine taxa examined, observations were consistent with the findings of earlier authors; both Pfeffer (1900) and Chun (1910) reported the morphology of a number of young cranchiids, with some attention to details of the tentacle club, and several additional studies have reported on individual species. Many of these treatments report the number/relative size of the suckers, presence of the pegs, and presence/absence of teeth on the infundibular ring, but few present the structure of the suckers in detail. Pfeffer’s (1900) general observations of young *Leachia* (his *Pyrgopsis* spp., fide Nesis 1987), *Cranchia*, *Megalocranchia*, *Helicocranchia*, *Liocranchia* and *Galiteuthis* (this last under the generic names *Taonidium*, *Crystalloteuthis* and *Phasmatoteuthion*, fide Voss 1980) were largely in agreement with the present findings, as were Chun’s (1910) for most of the same genera, although he reported that the smallest pegs (“denticles”) were observed proximally on the sucker rings of *Cranchia*, whereas the opposite was observed in the present material. He additionally mentioned that paralarvae (or small specimens) of *Cranchia* could not be distinguished from *Liocranchia* at ML 4–5 mm, but the slightly larger specimens examined herein (ML 6–8 mm) were in fact clearly separable, with the mantle of the former visibly covered in its characteristic tubercles.

Curiously, greater discrepancies were found between the more recent, more detailed reports of individual species’ juvenile tentacular morphology and the present findings. Aldred (1974) described the growth and ontogeny of *Bathothauma lyromma*, illustrating one tentacular sucker (his fig. 2B) with 10 strong, conical teeth distally, which appears quite dissimilar to the sole sucker found in good condition on the *B. cf. lyromma* specimen examined herein (Figure 4B), but surprisingly similar to those observed on *Megalocranchia* sp. (Figure 6B). Aldred reported that this dentition was “fairly constant” among *B. lyromma* specimens of ML 30 mm and greater, whereas smaller specimens possessed fewer teeth; the dissimilarity could therefore result from the size difference of the parent specimens. The distal position of the single whole *Bathothauma* sucker observed here may also account for the differences, because it is not clear from what portion of the club Aldred’s sucker was illustrated. The resemblance in dentition between Aldred’s illustration and those of *Megalocranchia* remains peculiar, however, given that these two taxa belong to different phylogenetic
subgroups according to Voss and Voss (1983, fig. 8). Large, conical teeth on the tentacular suckers have also been observed in the genera Taonius and Galiteuthis (see Young et al. 2003a, 2003b), and to a slightly lesser extent in Teuthowenia (see Dilly and Nixon 1976; Voss and Voss 1983; Voss 1985), all of which group reasonably closely together according to COI data (Figure 7) and are believed to have shared a common ancestor with Megalocranchia (Voss and Voss 1983), but these have not been reported in any of the other genera in Bathothauma’s theorized subgroup.

Nixon and Dilly (1977) noted morphological similarities (primarily pore-bearing pegs) between the suckers of several widely disparate squid taxa that all feed on crustaceans during some life stage, and suggested that the form of the suckers may be an indication of diet, in which case resemblances between confamilial genera may be phylogenetically meaningless. However, in the Onychoteuthidae, another oegopsid family in which paralarval tentacular sucker structure has been reported in detail for multiple taxa (Bolstad 2010), sucker morphology was markedly similar among congeneric species compared with non-congeners, suggesting that in some groups, such similarities may be relevant beyond dietary indications. Herein, some morphological similarities in tentacular suckers were noted between the groupings suggested by Voss and Voss (1983); for example, in the cranchiin genera Cranchia, Liocranchia and Leachia, the paralarval suckers possess relatively large, narrowly polygonal or ovoid-faced pegs in the innermost ring around the aperture, and the infundibular ring tends to lack the dentition observed in most taoniin genera, at least in the more distal suckers (the largest suckers of the largest Liocranchia specimen did possess about five teeth in the distal region). The faces of the pegs observed in the taoniin genera appeared more rounded and of more consistent size across the sucker ring’s surface, and more often possessed pronounced teeth on the distal margin of the infundibular ring.

The development of these distal teeth is a matter of some interest. It is perhaps noteworthy that the dentition of tentacular suckers observed on Teuthowenia pellucida herein differed markedly from those illustrated for T. megalops by Dilly and Nixon (1976), and Nixon and Dilly (1977). These studies reported sharp, conical infundibular teeth on the large manus suckers of a specimen of ML 180 mm, which could well represent later ontogenetic stages of the cylindrical, concave-faced teeth observed on the ML 28.9-mm specimen in Figure 6C; however, the T. megalops sucker in their fig. 31 (Nixon & Dilly 1977), also differs markedly from those illustrated here for T. pellucida in Figure 6C, in having larger-furred, shorter and more densely set pegs over most of its surface, including the distal inner margin of the infundibular ring, where the presently examined specimen has long, narrow teeth extending well into the aperture. It is not clear from what portion of the tentacle their T. megalops sucker was sourced, but differences were consistent between it and most of the suckers examined on the T. pellucida club herein (although the ML 4-mm T. megalops sucker illustrated in their fig. 33 does resemble those observed in the carpal region for T. pellucida here). Given that Voss (1985) illustrated toothed suckers for T. pellucida similar to those of T. megalops in Nixon and Dilly (1977), it may be that the ontogenetic development of these teeth (perhaps earlier in T. pellucida and later in T. megalops) has taxonomic value, as in the Onychoteuthidae, where transitions in tentacular morphology (e.g. development of hooks, loss of marginal suckers) can be used to assist in identifying the paralarvae of some species (Bolstad 2007). Certainly, the teeth observed in the large manus suckers of the largest Liocranchia reinhardtii
specimen herein (Figure 3D) can be seen developing in the suckers of a smaller specimen (ML ~10 mm, Figure 3C), but are not at all apparent in the smallest specimen (ML 7.7 mm, Figure 3B). Many small paralarval cranchiids in particular undergo ontogenetic changes that can be traced back through ontogeny when growth series of specimens are available, but that complicate specimen IDs when only very small individuals are encountered. For this reason, complete and detailed descriptions of these very early stages, such as those presented for T. pellucida (Evans and Bolstad this issue), should be compiled where growth series of material in good condition permits.

In at least one taxon examined herein, the rate of development in tentacle club armature appears widely variable; the hooks in a specimen of Galiteuthis armata at ML 37.2 mm (Figure 4D) appeared less developed than those in a specimen of less than half its size (ML 16 mm, Figure 4C). Individual growth rates in squid can be highly variable (Boyle and Boletzky 1996); laboratory experiments have also shown that same-age squid paralarvae reared at the same temperature displayed considerable growth plasticity in response to food availability within a remarkably short time period (10 days, Vidal et al. 2006). Additionally, paralarvae exposed to higher temperatures have higher growth rates, developing more rapidly than those exposed to lower temperatures (Vidal et al. 2002). However, the three specimens sampled here were all taken in equatorial waters (0–4° S), at similar times of day (late morning), under the influence of an 800-m-deep permanent thermocline with temperatures ranging from 25°C at the surface to 4°C at the bottom (Perez, J.A.A., unpublished). The trawls in which they were collected differed in maximum depth (Table 2; the smaller specimen was collected in a tow that reached 1250 m, whereas the tow with the larger specimen only reached 800 m), but since the nets were open for the entire trawl duration, it is impossible to know the actual depth and temperature where the specimens were taken. It is likely, however, that the hooks develop rapidly in Galiteuthis paralarvae up to a certain (somewhat variable) size, then slow down considerably; the hooks in both the ML 16-mm and 37-mm specimens appear developed enough to be functional (although certainly smaller relative to the overall club size than those seen in adults of G. armata), so perhaps rapid additional growth/development of these structures is not critical to the animal. Young Galiteuthis may also go through a series of distinct ontogenetic stages characterized by certain morphological developments, as has been recently found for Teuthowenia pellucida (Evans and Bolstad, this issue), in which case the two specimens in question here could represent the smaller and larger ends of the spectrum in a certain stage.

While representative cranchiid species have been included in several molecular phylogenetic studies to date (Carlini and Graves 1999; Anderson 2000; Lindgren et al. 2004, 2010; Bucklin et al. 2010), these sequences have not previously been combined into a single tree to investigate relationships within the family. At present, COI data are available for at least nine of the 13 cranchiid genera, and a preliminary Bayesian tree (Figure 7) reveals several groupings of interest. The division between subfamilies Cranchiinae and Taoniinae is present but not strongly supported, and the three genera known to bear hooks (Galiteuthis, Mesonychoteuthis) or hook-like tentacular suckers (Taonius) form a monophyletic group together. The sequence GU145078 (“Helicocranchia pfefferi”), which is strongly supported as a sister group to Galiteuthis (posterior probability 0.93), appears anomalous within this hooked group and, although submitted to GenBank by Bucklin as part of a larger project,
was not actually published in the parent study (Bucklin et al. 2010). Based on morphology and geography (Sargasso Sea) it is possible that this individual could have been a mis-identified specimen of *Sandalops* or perhaps *Liguriella*, both of which superficially resemble *Helicocranchia* at early ontogenetic stages (large funnel; eyes on short stalks with small projections; elongate mantle with small, paddle-shaped terminal fins – see Voss 1985). However, none of these three taxa is known to have hooked tentacular armature, so the position of this individual within the tree remains mysterious; it would be useful to examine a voucher for this sequence if possible, especially given that the *Helicocranchia* sequence that was published (GU145061) by Bucklin et al. (2010) does group together with two other *Helicocranchia* sequences.

The confusion and lack of phylogenetic support at deeper nodes in the tree highlight the need for further systematic work on the Cranchiidae, ideally combining detailed descriptions of early ontogenetic series and genetic data from additional taxa and for a variety of loci. As the body of published research on teuthoid systematics, characters and genetics continues to expand, it is hoped that this information may be of some eventual use in resolving this most problematic of squid families. Little information has been published on any cephalopods collected from the southern Mid-Atlantic Ridge, so perhaps these findings may also further understanding of the faunal composition of this underreported region. As Nixon and Dilly (1977) recommended, additional studies on the infundibular ring’s growth and relationship with underlying epithelial cells would also be quite useful in understanding its development, and perhaps in assessing the taxonomic value (if any) of its varied structures and their rates of development.

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