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Coryphopterus kuna, a new goby (Perciformes: Gobiidae: Gobiinae) from the western Caribbean, with the identification of the late larval stage and an estimate of the pelagic larval duration

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Abstract

A new goby, *Coryphopterus kuna*, is described from the Atlantic coasts of Panama and Mexico. The species is distinguished from other *Coryphopterus spp*. by the low median fin and pectoral fin ray counts and the morphology of the pelvic fin. The pelvic fins are fully joined with a rounded outline and have branched and longer innermost pelvic fin rays. There is no frenum connecting the two pelvic fin spines and the fin is heavily speckled with black spots in the male holotype. The late larval stage of *C. kuna* is identified by DNA sequence matching and is morphologically similar to other larval *Coryphopterus spp*. but has a distinct melanophore pattern. Examination of the otolith microstructure reveals a relatively long pelagic larval duration of 63 days with a narrowing of the later daily increments suggesting delayed metamorphosis. The species is the first vertebrate to include gene sequence barcoding under the Barcode of Life Data System (BOLD) in the species description.

Key words: Gobiidae, Goby, New Species, BARCODE, BOLD, Fish, Informatics, Larvae, Larval Identification, DNA, Larval Stage, Pelagic Larval Duration, Otolith, Panama, Mexico, Caribbean, Western Atlantic

Introduction

Although a number of gobioid species have been recently described from both coasts of the Americas, the genus Coryphopterus in the New World has seen few changes since the original treatment by Bohlke and Robins in 1960 and 1962. They listed nine Atlantic species and two eastern Pacific species. Since their treatise, Coryphopterus venezuelae (Cervigón) has been described from Venezuela (Cervigón 1966, 1994) and Coryphopterus tortugae (Jordan) has been redescribed and is widespread in the Caribbean (Garzon-Ferreira and Acero 1990, Greenfield & Johnson 1999). A number of new Indo-Pacific Coryphopterus spp. have been described by Randall (2001) who provided a key to the species for that region. However, the validity of including the Indo-Pacific species in this genus has been questioned recently by Thacker and Cole (2002) and they suggest that those species be returned to *Fusigobius spp*. Furthermore, they found the one temperate species (from the eastern Pacific) to be unrelated and returned it to Rhinogobiops nicholsii (Bean). In this paper I describe a new Atlantic species from the western Caribbean, Coryphopterus kuna. Individuals of this species have been found as an adult in Panama and as larvae northward to Banco Chinchorro, off of the coast of Yucatan, Mexico. The new species is remarkable for having the lowest fin ray counts of the benthic sandperching group and a united, rounded, and darkly-pigmented pelvic fin without a frenum. Despite the morphological similarities, the mtDNA sequence (COI) for the new species is more than 25% divergent from other Coryphopterus spp.

This species is the first vertebrate to include in the description the DNA barcode developed for the informatics system of the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007, Hanner, pers. com.). The DNA sequence used for barcoding (COI) was obtained from both the holotype as well as the laval stage and the close match confirms the larval identification. This new species description is also notable in that the late larval stage is identified along with the adult. I include a comparison to other regional larval *Coryphopterus spp.* as well as to other gobioid larvae with similar meristics (Victor 2006, 2007). Furthermore, the daily increments in the otolith microstructure of the transitional stage permits a direct estimation of the pelagic larval duration, which is 63 days, relatively long for the large group of gobies that settle at a small size of less than 10 mm SL (Victor 2006, 2007). The pattern of increasingly narrow increments during the latter part of the pelagic larval phase is similar to that found to represent delayed metamorphosis in the larvae of some other reef fishes (Victor 1986; Cowen 1991; McCormick 1999).

Materials and methods

Counts, measurements, and techniques follow Randall (2001). All fish lengths are standard length (SL). SIO is the institutional abbreviation for the Marine Vertebrate Collection of the Scripps Institution of Oceanography. Otoliths were extracted, cleaned, dried, and placed in immersion oil and examined under a compound microscope with transmitted light and polarizing filters. Digital photographs of the otolith increment sequences were taken at 400X magnification. The mitochondrial DNA sequences were obtained, processed, and archived following the BOLD procedure outlined in Ratnasingham and Hebert (2007).

Coryphopterus kuna, new species Fig. 1

Holotype. SIO-07-5, 17.1 mm SL, male, Panama, San Blas Islands, South side of Taintupo reef (9°32'44"N 78°57'26"W), 15 meter depth, sand, dipnet, 30 December 1982, B. Victor. Genbank Accession No. EF550211.

Diagnosis. Dorsal elements VI, I,8; anal fin elements I,8; pectoral fin rays 15; longitudinal scale series 25; head naked except for scales on the side of the nape reaching near the level of the preopercular margin. Pelvic fins fully joined medially by membrane, the innermost rays are branched and the longest and there is no frenum between the two pelvic fin spines. The pelvic fin is notably darkly-pigmented in the male holotype (the only known adult specimen). Translucent with moderate black speckling on the head and body, two lines of black spots along the lowest branchiostegal membranes forming an X across the isthmus, heavy black speckling over the pelvic fin membranes, a faint black spot smaller than the pupil on the proximal upper pectoral fin rays (not on the base of the fin), a black stripe across the mid-spinous dorsal fin, and prominent black spots in two rows across the top of the eyeball. Apparently a small species, despite the small size of the holotype it is a mature male since it has a long pointed urogenital papilla.

Description. Dorsal elements VI + I,8; anal elements I,8; all dorsal and anal soft rays branched, the last to base; pectoral rays 15, the upper and lowermost unbranched; pelvic elements I,5 with the rays all branched, united as a disk with a rounded edge, the innermost ray clearly longest, no frenum, a small fold of skin from each pelvic spine to the body not connected to the fold on the other side; branched caudal rays 12, upper unbranched caudal rays 9, posterior 3 segmented; lower unbranched caudal rays 8, posterior 2 segmented; longitudinal scale series 25; transverse scale series 7; circumpeduncular scales 12; gill rakers 2+6; branchiostegal rays 5; vertebrae 10+16; spinous dorsal-fin pterygiophore formula 3-22110.







FIGURE 1A–C. Holotype of *Coryphopterus kuna*, 17.1 mm SL male (SIO-07-5)(A); head and fin markings (B); pelvic fins united along full-length (C).



FIGURE 1D, E. Holotype of *Coryphopterus kuna*, pelvic fin frenum absent (D); prominent melanophores over eyeball (E).



FIGURE 2. Transitional larva of Coryphopterus kuna, 7.3 mm SL (SIO-07-55).

Body elongate, depth 6.13 in SL, and compressed, width 1.12 in depth; ventral part of head and chest broad and nearly flat; head triangular when viewed from above, its length 3.8 in SL, no head crest; snout pointed and short, its length 4.14 in head; orbit diameter 3.05 in head, the eye extending above dorsal profile

of head; interorbital space extremely narrow, 33.5 in head; caudal-peduncle depth 2.42 in head; caudal-peduncle long, its length 1.31 in head. Mouth large, the maxilla ending below anterior 1/3 of pupil, the upper-jaw length 2.23 in head; lower jaw projecting; mouth oblique, the gape forming an angle of 35° to horizontal axis of head and body; upper jaw and lower jaws with multiple irregular rows of well spaced-apart slender curved conical teeth. Tongue truncate with rounded corners.

Gill opening extending forward to below middle of opercle. Gill rakers nubs in holotype. Head naked except for scales on side of nape extending forward nearly to eye; no scales on fins except a few on base of caudal fin that are smaller than largest scales on body. Scales ctenoid except those on side of nape, thorax, prepectoral area, and a few just above base of pelvic fins that are cycloid. Anterior nostril a short membranous tube at level of middle of eye. Head pores prominent, as follows: a nasal pore, an anterior and a posterior interorbital pore, a postorbital pore, an infraorbital pore below the postorbital, a pore at each end of a lateral sensory canal; a short posterior lateral canal with a pore at each end, and 3 preopercular pores (i.e. Birdsong's B', C, D, E, F, G, H', K', L', M', N, O'). Head papillae in rows vertically along the lower opercle and along the lower rim of the preopercle extending forward along the line of the lower jaw.

Origin of dorsal fin behind upper base of pectoral fin, predorsal distance 2.79 in SL; spines of fins slender and flexible; 1st dorsal fin lower than 2nd; 1st dorsal spine through the fifth about equal in length, 2.2 in head, sixth spine shorter; spine of 2nd dorsal fin 4.33 in head; 1st dorsal soft ray longest, 1.93 in head; origin of anal fin below base of 1st soft ray of 2nd dorsal fin, preanal distance 1.84 in SL; anal spine 4.0 in head; caudal fin moderately rounded, 3.58 in SL; pectoral fins pointed, the middle rays longest, 3.45 in SL; origin of pelvic fins directly beneath base of pectoral fins, prepelvic distance 3.39 in SL; pelvic fins fully joined by membrane; pelvic frenum absent; pelvic spine 5.27 in head; 5th pelvic soft ray longest, nearly reaching origin of anal fin, its length 4.22 in SL (1.29 in head); 4th pelvic soft ray about 94% length of 5th ray. Length of genital papilla of male holotype almost equal to pupil diameter and narrow, the length-to-width ratio is 3.1.

Barcode sequence. A 652-nucleotide sequence of the section of COI gene used for barcoding by the BOLD informatics database (Ratnasingham and Hebert 2007) was obtained for both the holotype and the larval stage specimen (Genbank accession numbers EF550211 and EF550210 and protein sequences ABQ22956 and ABQ22955 respectively). The two sequences were a close match with 0.62% sequence divergence. Following the database management recommendation of the BOLD the 652 bp sequence (5' - 3') of the holotype is presented here as well:

Color of holotype in alcohol. Pale yellowish with sparse black speckling and white and iridescent spotting. Black spots on the body small and few; in irregular rows along the dorsal aspect below the base of the dorsal fins, along the dorsal midline, lateral midline, and along the posteriormost portion of the ventral midline of the tail. White spots, mostly made up of clusters of tiny single leukophores, sparse over the body and along the lateral midline and speckling the operculum and jaws and a broad iridescent stripe across the operculum onto the pectoral fin. No distinct spot at the base of the caudal fin but a thin line of melanophores at the base of the first six ventral caudal fin rays. A small patch of melanophores on the proximal portion of the pectoral fin rays between the 2nd and 6th rays (not on the fin base), a few melanophores on the inner side of the pectoral fin base (the axil), and a white patch along the proximal portions of the 5th through 15th pectoral fin rays. Sparse black spots speckling the top of the head and an indistinct black triangle directly below the orbital rim down to the end of the maxilla. Prominent black spots over the dorsal aspect of the eyeball in two irregular rows: two spots in the inner row, about seven in the outer row. Black spots line the lowest branchiostegal rays in an X shape across the isthmus and then in a patch just forward of the pelvic fin. Pelvic fin is extensively speckled with black spots concentrated along the fin membranes. Spinous dorsal fin has a distinct black spot at the base of the first interspinous membrane and an additional black stripe along the mid-portion of the fin across all of the spines and membranes and a white edging to the fin membrane tips; second dorsal fin is covered in fine white spotting as are the caudal and anal fins. Anal fin has an additional dusting of fine black spots.

Distribution. Known from the Western Caribbean at the San Blas Islands of Panama (the adult) and Banco Chinchorro, Mexico (larval specimen).

Etymology. Named for the Kuna indigenous people of the Kuna Yala, the region of Atlantic Panama in which the holotype was collected, in recognition of their cooperation in marine biological research. Although *Coryphopterus* is masculine, kuna is a noun in apposition and the "a" ending is thus appropriate.

Comparisons. The regional congenerics that share the low median and pectoral fin ray counts are distinctly different: the masked goby *Coryphopterus personatus* (Jordan and Thompson) and the glass goby *Coryphopterus hyalinus* (Bohlke and Robins) are hovering, not benthic, species with divided and unmarked pelvic fins, dark masks from the snout through the eye, black rings around the anus, and orange, not brown or black, body markings (Bohlke and Robins 1960, 1962; Randall 1996).

Among the benthic *Coryphopterus spp.*, all other species have more than 15 pectoral fin rays: 16 or 17 in *Coryphopterus alloides* (Bohlke and Robins), 16 to 18 in *Coryphopterus lipernes* (Bohlke and Robins), and 17 to 20 in *Coryphopterus dicrus* (Bohlke and Robins), *Coryphopterus eidolon* (Bohlke and Robins), *C. glaucofraenum*, (along with *C. tortugae* and *C. venezuelae*), *Coryphopterus punctipectophorus* (Springer) and *Coryphopterus thrix* (Bohlke and Robins) (Bohlke and Robins 1960, 1962; Springer 1960). Similarly, all other *Coryphopterus* species have more than nine second dorsal fin elements: 10 or 11 (except a rare 9 recorded for *C. glaucofraenum*, *C. alloides* and *C. thrix*). The 9 anal fin elements are shared only with *C. alloides* and rare specimen of *C. eidolon* (Bohlke and Robins 1960).

Other than fin ray counts, the pelvic fin morphology is distinctive for *Coryphopterus kuna*, i.e. a united rounded dark pelvic fin with the innermost rays longer than the next ray and no frenum connecting the pelvic fin spines into a sucking disk. *C. alloides*, the species that shares nine anal fin elements with *C. kuna* (and has only one or two more pectoral fin rays) has a distinctively divided pelvic fin morphology (and the innermost ray unbranched) (Bohlke and Chaplin 1993). *C. lipernes* also has divided pelvic fins (Bohlke and Robins 1962). The remaining united-pelvic-fin group comprises *C. dicrus* with the innermost pelvic fin rays markedly shorter and no frenum, *C. glaucofraenum* (and both *C. tortugae* and *C. venezuelae*) with the innermost pelvic fin rays about equal to the next ray and with a frenum, and *C. eidolon, C. thrix*, and *C. punctipectophorus* with a distinct frenum and the innermost pelvic fin rays somewhat shorter than the next ray (Bohlke and Robins 1960; Springer 1960; Bohlke and Chaplin 1993).

In markings *Coryphopterus kuna* most resembles the benthic species without prominent stripes behind the eye. It shares the dorsal fin stripe with *C. alloides*, but the latter species does not have fused pelvic fins. It is distinguished from *C. thrix* by having the stripe across the spinous dorsal fin and the absence of the pectoral fin base spot and extended second dorsal fin spine (but this spot is often indistinct and the fin spine is not extended in *C. thrix* less than 20 mm SL). Males of *Coryphopterus spp.* usually do share the dusky pelvic fins of the holotype of *C. kuna. C. eidolon* is a pale goby without dark markings and has orange head stripes sometimes outlined in thin black lines. The prominent black spots on the dorsal aspect of the eyeball is shared with

several other *Coryphopterus spp.*, however, in most other species the spots are few (usually three or four) and large and not in two distinct rows.

Remarks. The holotype was perching on the bottom on a fine sandy plain in relatively deep (15-20m) water and was collected with a dipnet. Along with the holotype, several other sandbed fishes were collected: *Chaenopsis spp., Diplogrammus pauciradiatus* (Gill), and *Achirus lineatus* (Linnaeus). Although *Coryphopterus spp.* are ubiquitous in Caribbean fish collections and abundant in the region, this was the only specimen of this species collected by me in the San Blas Islands of Panama. Explanations for why this species is so elusive may rest on the fact that it occupies an obscure habitat: it was collected in deeper water than is usually surveyed, was far from reef substrate, and the area is subject to some siltation from the Panamanian mainland. In addition, the species is not particularly different-looking from the usual sand-bed gobies and thus would not attract attention. Nonetheless, one would have expected its occurrence in trawling samples elsewhere and its meristics would have immediately distinguished the specimen. Its occurrence only in the Western Caribbean also assures that it was not discovered in the extensive collecting focused on the Bahamas, the Antilles, and in US waters.

DNA Sequence. The COI sequences of the adult and larval specimens matched closely (0.62% divergence) and confirm the identification of the larval specimen. The DNA sequence for *Coryphopterus kuna* is quite divergent from other *Coryphopterus spp.* and *Fusigobius spp.*, despite the morphological similarities. Percent sequence divergence from other regional *Coryphopterus spp.* is 25.2% from *C. dicrus*, 27.8% from *C. glaucofraenum*, 25.9% from *C. lipernes*, 25.4% from *C. personatus*, 26.1% from *C. thrix*, 25.8% from *C. tortugae* and 26.5% from the eastern Pacific species *C. urospilus*. The percent sequence divergence is similar from the Indo-Pacific species: 26.1% from *Fusigobius signipinnis* (Hoese and Obika) and 24.9% from *Fusigobius neophytus* (Gunther) (equivalent to Randall's (2001) *Coryphopterus signipinnis* and *Coryphopterus neophytus*). This marked divergence raises the question of the status of the genus *Coryphopterus* and the relatedness of the many species that have resided within the genus at one time or another (Randall 2001; Thacker and Cole 2002). Thus the position of this species in the genus is provisional and elucidating the relationships of these species would await a more complete molecular phylogenetic survey of the numerous other species and related genera in the Gobiidae.

Early life history. A larval Coryphopterus kuna collected from a light trap at the Banco Chinchorro, off of Yucatan, in Mexico is illustrated in Fig. 2. The specimen was collected by Dave Jones on March 29, 2006 (SIO-07-55). The larvae of C. kuna are recognized by the fin ray count of D-VI,9 A-9 pectoral 15 and the identification is confirmed by the almost identical DNA sequence to the adult holotype. The body is relatively thin, long and narrow with a large eye and a terminal mouth. The pectoral fins are long, reaching to the level of the vent. The pelvic fins are also long, reaching to the vent, apparently united and with no visible frenum. The dorsal and anal fin bases are relatively short, the caudal peduncle long and narrowing rapidly and there are 5 to 7 procurrent caudal fin rays. Larval C. kuna are lightly marked, mostly along the lower body: melanophores are on the ventral midline at the isthmus and the pelvic fin insertion and then in a row along the anal fin base, paired and one per side between the third and ninth element, then after a space, there is a row of melanophores extending along the ventral midline of the caudal peduncle ending near the start of the lower procurrent caudal fin rays. Internal melanophores are present around the sacculus, at the dorsal surface of the swim bladder, and around the gut near the vent. Transitional larval C. kuna develop a pattern of large discrete melanophores on the dorsal aspect of the body, most notably three or four large melanophores over the eyeball, a triangle of three with the vertex forward at the anterior midline between the eyes, several around the back of the braincase, and then a row of sometimes paired melanophores along the dorsal midline of the body at the base of the soft dorsal fin rays. A large stripe of iridophores extends backward on the head from the upper eye. Melanophores develop along the first dorsal spine and at the base and tip of the second dorsal fin spine. There are no melanophores at the angle of the jaw or on the caudal fin rays.

Larval *Coryphopterus kuna* have similar proportions and morphology to larval *C. dicrus, C. glaucofraenum,* and *C. tortugae* (the latter species are visibly indistinguishable as larvae and are identified by mtDNA sequences only) and larval *C. personatus/hyalinus* (Victor 2006, 2007). However, these similar larvae do not share the low fin ray counts of the *C. kuna* larval type. Larval *C. kuna* differ from larval *C. dicrus, C. glaucofraenum,* and *C. tortugae* in missing the lower caudal fin melanophores and the melanophores at the angle of the jaw and behind the last dorsal fin ray. At transition, larval *C. kuna* have a pattern of a few discrete large melanophores on top of the head vs. stripes going back from the eye of transitional *C. dicrus, C. glaucofraenum,* and *C. tortugae* larvae and a patch of tiny melanophores on top of the head and a distinctive patch around the vent in transitional *C. personatus/hyalinus* larvae.

The melanophores over the dorsal aspect of the eyeball of larval *C. kuna* are more prominent than is observed in most other larval *Coryphopterus spp.* and break up into two stripes of relatively smaller melanophores on the eyeballs of adult *C. kuna*. This description of larval *C. kuna* agrees with most of the characters enumerated for an unknown larval type (*Coryphopterus* B of Baldwin and Smith (2003)), except one of the larvae of that type that was raised in captivity for a few days had apparently divided pelvic fins and *C. kuna* has fused pelvic fins. This is not likely an ontogenetic change, since divided pelvic fins in *Coryphopterus spp.* are a derived character and at least two of the species with divided pelvic fins, *C. personatus* and *C. lipernes*, have fused pelvic fins as larvae and at settlement (Victor 2007). Since the collection site was Belize, included within the range of *C. kuna* (Yucatan to Panama), further collections should resolve whether there are more cryptic species of *Coryphopterus* in the region.

Other gobioid larvae that may rarely overlap the fin ray counts for larval *Coryphopterus kuna* comprise the gobies *Lythrypnus spp.* and *Bathgobius curacao* (Metzelaar), as well as the eleotrid *Eleotris amblyopsis* (Cope). The larvae of these potentially confounding species have been identified and characterized in Victor (2006, 2007) and they are quite different from the *C. kuna* larval type. Larval *Lythrypnus spp.* are shorter and wider than larval *Coryphopterus spp.* and have a conspicuous melanophore at the angle of the jaw, not present on the larval *C. kuna*. Furthermore, *Lythrypnus spp.* larvae have no dorsal melanophores before other distinctive transitional melanophore patterns develop on the head. The transitional larvae of *Lythrypnus spp.* have a characteristic Òradiating spokesÓ pattern of melanophores around the eye that develops before other melanophores along the dorsal aspect (but occasionally a single melanophore at the rear edge of the dorsal fin develops early).

Larval *Bathgobius curacao* are very different and have rows of melanophores along the dorsal midline, the ventral midline, and internally along the spine and are smaller at the same stage of development than larval *Coryphopterus kuna*. Larval *Eleotris amblyopsis* have a completely different appearance: they are larger with a stout thick body and the eleotrid larval melanophore pattern of long streaks or rows of melanophores along the ventral midline along with a conspicuous patch of melanophores covering the caudal peduncle. In addition, most *Eleotris amblyopsis* larvae have narrowed eyes and distinctive melanophores covering the surface of the iris (Victor 2006, 2007).

Otolith microstructure. The sagittal otoliths of the transitional stage of larval *Coryphopterus kuna* reveal a clear increment array (Fig. 3). These arrays have been interpreted as daily in numerous otolith studies for reef fishes and experimentally validated for a variety of fishes, although not for this species in particular (Thorrold and Hare 2002). At the center of the core there is a rod-like or oblong primordium about 7 microns long (typical of gobiid otoliths). The core is an area with no distinct increments extending about 20 microns from the center and outlined by an oval ring that can be brightly demarkated at some focal planes. This core region is formed before hatching in gobies. Surrounding the core there is an increment array of relatively narrow but clearly delineated dark and light lines easily visible in all quadrants of the otolith when illuminated with transmitted light from the lateral aspect of the otolith. The optimal array for counting and measuring increments is along the longest axis of the sagitta. The lapillus shows a similar increment pattern, but since this pair of otoliths in gobies is often much smaller than the sagittae, the array is compressed and the narrower

bands merge together. The asterisci, the third pair of otoliths in bony fishes, usually have indistinct increments and are not used in otolith aging.

The count of 63 days from the edge of the core region to the edge of the otolith indicates a pelagic larval life of 63 days. In species with broadcast eggs, some number of days need to be added to the increment count for the hatching and embryonic developmental stages (Victor 1991). However, these gobies have brooded eggs that hatch with the core already developed and the hatchlings begin their pelagic life at that point. The capture of transitionally-marked larvae at light traps off the reef edge indicate that the larvae were about to settle, probably that same night, onto the reef. Thus otolith counts from transitional larvae caught at the reef edge are one of the most direct estimates of pelagic larval duration. In later life stages, a settlement mark needs to be interpreted and validated, and the counts subdivided to infer a pelagic larval duration (Victor 1991; Thorrold and Hare 2002).

The array of increment widths on the otoliths of larval *Coryphopterus kuna* show a distinct narrowing at day 30-35 from increments as wide as 5 microns down to increments as narrow as one micron that continue over the next 25 days. Near the very edge of the otolith there are a few indistinct slightly wider increments that could represent faster growth associated with approach to shore waters or transitional changes in head morphology commonly found in transitional larvae of reef fishes. A similar pattern of narrowing otolith increments during the latter portion of the pelagic larval life has been shown to correlate with slower growth in another reef fish (Victor 1986) and indicates delayed metamorphosis, a phenomenon common in marine invertebrate larvae (Pechenik 1990), and perhaps an important part of the pelagic stage of many reef fishes.



FIGURE 3. Sagittal otolith of transitional larva of *Coryphopterus kuna*, lateral view, photographed at 400x. The array of daily otolith increments extends along the longest radius of the sagitta from the center of the otolith (at left) to the edge of the otolith (at right). The primordium is the horizontal black oblong at the left edge of the figure and is about 7 microns long, the core (pre-hatching) is the area without clear increments about 20 microns out from the center.

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