

LARVAL DEVELOPMENT OF THE CUATRO CIÉNEGAS CICHLID,
CICHLASOMA MINCKLEYI

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ABSTRACT—Ontogeny of *Cichlasoma minckleyi* was investigated with emphasis on development of the trophic polymorphism involving the pharyngeal apparatus. At about 6 days post fertilization (5.3 mm SL; standard length) the first skull elements that stained as ossified in cleared and stained specimens were isolated, pointed pharyngeal teeth; also at this time jaw teeth were developing on the premaxilla and dentary. By 10 days after fertilization (6.2 mm SL) ca. 27 (estimated 11 top and 16.5 bottom) finger-like, pointed papilliform pharyngeal teeth were ossified in all specimens as the quadrate, angular, dentary, coracoid, parasphenoid, premaxilla, and maxilla were also ossifying. The skull roof and surrounding structures remained unossified. All specimens younger than 100 days (30 mm SL) had only papilliform pharyngeal teeth. Molariform pharyngeal teeth were observed only in older, larger (30 to 65 mm SL) specimens. Offspring of a molariform x molariform cross included molariform, papilliform, and intermediate, pharyngeal morphologies.

RESUMEN—Se describe la ontogenia de *Cichlasoma minckleyi*, un cíclido con un polimorfismo trófico de los dientes y aparato faríngeo, con énfasis en los dientes faríngeos. Aproximadamente 6 días después de la fertilización (Longitud Patrón 5.3 mm), los dientes faríngeos y sus precursores están aparentes en especímenes aclarados y acolorados como dientes aislados y afilados; y son entre los primeros elementos del cráneo a osificarse. Al mismo tiempo los dientes mandibulares aparecen en la maxila y la dentaria. A los 10 días después de la fertilización (LP 6.2 mm), aproximadamente 27 (11 superiores, 16.5 inferiores) dientes alargados y puntiados papiliformes están osificados en el arco faríngeo mientras se osifican también los huesos quadrate, angular, dentary, coracoid, parasphenoid, premaxila, y maxila. El techo del cráneo y estructuras alrededores de el quedan sin osificar. En los especímenes con menos de tres meses de edad (LP 30 mm) se observaron únicamente dientes faríngeales papiliformes. Dientes faríngeales molariformes se observaron solo en especímenes más grandes (LP 30–65 mm) y de mayor edad. Entre el progenie de parientes que eran ambos con dientes faríngeales de tipo molariforme encontramos individuos con dientes molariformes, papiliformes e intermedios.

Cichlasoma minckleyi, endemic to the Cuatro Ciénegas Basin, Coahuila, México, is an extreme example of the plasticity exhibited by many Neotropical cichlids. The species is polymorphic, exhibiting 3 distinct feeding morphologies: a detritivore with small, relatively weak, papilliform pharyngeal teeth; a molluscivore with hypertrophied molariform pharyngeal teeth; and a rare and little-studied piscivore (LaBounty, 1974; Kornfield and Taylor, 1983). The piscivore has an elongate, slender body and head compared to the deeper bodies of the other two, which are externally indistinguishable and may have either molariform or papilliform pharyngeal teeth (La Bounty,

1974; M. J. Stephens and D. A. Hendrickson, pers. observ.; Arizona State University [ASU] 2313, 2314, 2340, 2262, 2321, 3689, 3705, 3658). The detritivore is further characterized by having a longer intestine than the molluscivore (Kornfield and Taylor, 1983). Morphometric, genetic, breeding, and other studies substantiate the hypothesis that this is a polymorphic species and that observed variation is not interspecific (Kornfield et al., 1982). These authors considered their finding that 57% of wild breeding pairs were heteromorphic to be inconsistent with the hypothesis that the different morphs were reproductively isolated species. Polymorphism of this species also

could not be ruled out by allozymic studies (Kornfield and Taylor, 1983). In addition to trophic polymorphism, all morphs appear to share polymorphisms in sexual dichromatism (Konings, 1994).

The three morphotypes of *C. minckleyi* are similar in form to well-studied African cichlids hypothesized to have evolved morphologically to exploit different trophic niches (Fryer and Iles, 1972). Given that African Rift Valley cichlids with morphologies as divergent as those of *C. minckleyi* typically are placed in different genera, it is interesting that *C. minckleyi* apparently has achieved similar levels of morphological specialization to several trophic niches without speciating. This complex variation has long impeded phylogenetic studies, and relationships of *C. minckleyi* remain speculative (Kornfield and Taylor, 1983), as is the mechanism by which morphologies are determined.

Ontogenetic traits could provide clues to maintenance of polymorphism or prove useful in resolving the phylogeny of *Cichlasoma*. Ontogenetic origins often are used to determine homology of characters. Alberch (1980) stressed the importance of relating development to systematics, and ontogenetic description may differentiate between actual and artifactual differences in characters (Mabee, 1993). Phylogenies based on ontogenetic characters have corroborated phylogenetic hypotheses based on different character sets (Strauss, 1990). We provide a description of an ontogenetic series of the species as a contribution toward eventual resolution of systematic relations of North American cichlids.

MATERIALS AND METHODS—In 1995, we obtained 16 specimens (12 papilliform, 3 molariform, and 1 intermediate) that were first generation, aquarium-produced offspring of individuals of unknown pharyngeal morphology which had been collected in 1993 (Konings, 1994) from Poza de la Becerra, a habitat described by Minckley (1969). These 16 specimens were housed in 214 to 525 l aquaria filled with deionized municipal water and maintained to eventually become the broodstock which produced the specimens reported on in this study. Submersible heaters maintained all tanks at 25.5 to 30°C, photoperiod was kept at 12L:12D, and eroded limestone provided refuge. We offered a diversity of food items; sinking brine shrimp pellets and Tetramin flake food initially were not accepted, but eventually became the staple diet.

Specimens examined were from 4 clutches of offspring. Aggressive behavior of this species generally did not permit forced pairing of individuals, which would have left no doubt regarding parentage of offspring. Successful breeding of this species in captivity requires large tanks housing numerous individuals in a community setting, mimicking their natural conditions. Two pairs spawned in the community tanks (9 July 1996 and 16 January 1997), and we were thus unable to determine parentage. We were able to isolate 1 pair (molluscivore \times molluscivore), spawn them (16 November 1995 and 22 April 1996), and raise their offspring in a separate tank for unambiguous knowledge of parentage.

Breeding adults laid eggs on surfaces that they cleaned such as rocks or flower pots. Eggs were removed as soon as possible to 39 l incubation tanks with aeration, sponge filtration, and methylene blue (2 to 3 drops per 3 l) antifungal treatment, without which fungal infections resulted in >90% egg mortality. Larvae were fed newly hatched, live brine shrimp during the first 10 to 15 days post-hatching and then Tetramin flakes and sinking brine shrimp pellets.

Following fertilization, specimens were preserved at varied time intervals in 3 to 10% formalin (depending on size and age) for 1 week, then specimens were moved to 50% ethanol for 1 day, then 70% ethanol for storage. Through periodic preservation of 3 to 10 specimens at a time from different broods we were able to obtain sequences of fish representing every or every other day of life for the first 60 days of post-fertilization development, and at least one sample every five to 10 days thereafter. Few fish survived to ages greater than 100 days due to mortalities caused by aggressive behavior. The oldest specimens reared and examined were 100 to 200 days. One to 3 specimens from each sample were cleared and stained (Potthoff, 1984). It was sometimes necessary to re-stain some individuals that did not absorb alizarin red well, or to adjust the amount of time in trypsin digestion. M. J. Stephens drew illustrations of specimens while observing them under binocular dissecting and compound microscopes at 37.5 to 2000 \times . Polarized light was used for myomere counts. Pharyngeal teeth were often difficult to count. To estimate teeth numbers, multiple counts were made (which differed less than 30%). All ages are expressed as days after fertilization and size at each age as mean standard length (SL) in mm.

RESULTS—A total of 468 specimens (Texas Natural History Collection [TNHC] 25547–25603) were examined (72 cleared and stained; 396 formalin-fixed). An overview of character states based on 57 specimens of various ages and sizes representative of the first

TABLE 1—Summary of ontogenetic events over time (days post-fertilization) in *Cichlasoma minckleyi*. Variation in growth rates among individuals and among cohorts, as well as sampling effects, contribute to apparent uneven growth. Only selected representative specimens are included. AN = angular, BR = branchiostegal rays, C = coracoid, CL = cleithrum, D = dentary, F = frontal, GA = gill arches, M = maxilla, PM = premaxilla, POS = pre-opercular-symplectic, PS = parasphenoid, Q = quadrate, SC = supraoccipital crest.

Days post fertilization	Mean SL (mm)	n	Fins	Pigmentation	Pharyngeal teeth	Skull
4	5.2	4	Pectoral fins present since hatch	Present on yolk sac and cephalic areas posterior to eye		
5	5.2	4	Caudal fin first appears in some individuals	More appearing dorsally and on caudal fin		
6	5.3	3			Arches and apophysis appearing. Three papilliform teeth visible in lateral view	Conical jaw teeth forming on D and M; PS, SC, BR begin ossifying
7	5.8	2	Anal and dorsal fins first appear in some individuals			Adhesive head glands start to disappear
10	6.2	3		More pigment present on dorsal body surface, head, caudal fin, and ventrally on body	Slender papilliform teeth present and clearly ossified before other surrounding structures. Estimated count; top = 11, bottom = 16.5	Q, AN, D, C, M, PM begin ossifying. Processes of F forming around the orbit
11	6.0	4		Continuous from operculum to caudal peduncle		Jaw teeth fully formed
12	6.1	7	Caudal fin apparent in all specimens; pelvic fins first appear in some individuals		Tooth count increasing estimated top = 15, bottom = 25–30	
15	5.5	3	Dorsal and anal fins apparent in all specimens			
16	6.1	3			Estimated tooth counts: top = 20 to 30, bottom = hard to estimate, but apparently > earlier	PS, BR, C, Q, D, AN, and POS ossified except for edges
17	6.2	3	Caudal fin rays ossified, epurals and plurals ossified except anterior tips			

TABLE 1—Continued.

Days post fertilization	Mean SL (mm)	n	Fins	Pigmentation	Pharyngeal teeth	Skull
27	10.3	6	Anal and dorsal fin pterygiophores becoming prominent, spines and rays distinguishable	First appearance of juvenile pigmentation consisting of a dark triangle is obvious on caudal peduncle, and overall grey pigmentation with dark bars	Numerous hooked papilliform teeth present, bottom pharyngeal occlusal surface ca. 1.1 mm wide	PM is more prominent. All bones appear ossified, with only hints of cartilage remaining at articulations. Posterior point of SC more pointed than in younger specimens (though less than in adults)
29	9.9	3	Pelvic fins well formed in all specimens			
>100	30–50	12	All fins with developed rays	Juvenile pattern	Papilliform ($n = 4$), molariform ($n = 4$) and intermediate ($n = 1$) developed from spawn of known homomorphic parentage	All elements ossified. SC pointed as in adults

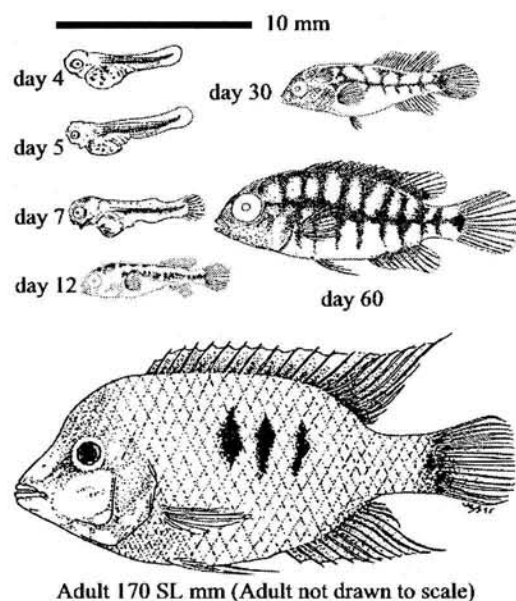


FIG. 1—*Cichlasoma minckleyi*. Ontogeny of external anatomy and pigmentation from 4 days through adult.

appearance of ontogenetic characters is presented in Table 1. Hatching occurred ca. 94 h after fertilization at 30°C, and larvae were free swimming by 6 to 7 days.

Fins developed in the following sequence: pectorals (present at hatching), caudal, dorsal and anal, and pelvic. Pelvics formed only after all other fins had rays. The mean number of myomeres present at hatching and throughout development was 30.2 ($n = 21$; $SD = 1.47$; range 28 to 33).

Larval pigmentation (Fig. 1) consisted of asymmetrical blotches that darkened and increased in number until the juvenile pattern was attained. The pre-hatch embryo was pigmented; upon hatching the yolk sac was speckled with melanophores, and there was pigment on the top of the head posterior to the eye. Pigmentation later appeared dorsally on the body and caudal fin, followed by an overall increase of pigment density on dorsal body surfaces, the head, caudal fin, and ventral body areas. By 11 days (6.0 mm) body pigmentation was more or less continuous from the opercu-

lum to caudal peduncle. Characteristic juvenile pigmentation, consisting of a distinct black triangle on the caudal peduncle and an overall gray background body color with dark vertical bars, was first apparent at 27 days (10.3 mm). Sexually dimorphic coloration became obvious at 9 to 10 months.

Papilliform pharyngeal teeth and the fifth ceratobranchials were recognizable in cleared and stained specimens at 6 days (5.3 mm—Table 1; Fig. 2A). Pharyngeals ossified before surrounding skull bones, and became prominent by 10 days (6.2 mm—Table 1). Examination of pharyngeal teeth in specimens younger than 100 days (SL <30 mm) almost always revealed only pointed papilliform teeth (Fig. 2C), which were long, slightly hooked, and darker at the tips. Of all specimens <100 days examined, only 2 had slightly wider, medial teeth with more blunted occlusal surfaces surrounded by more slender, clearly papilliform teeth (Fig. 2D). Pharyngeal teeth unambiguously classifiable as molariform were observed only in some of the 100 to 200 days (30 to 65 mm) specimens (Fig. 2E). Among specimens of known parentage (molariform \times molariform cross), we raised molariform ($n = 4$, 50 to 65 mm), papilliform ($n = 4$, 35 to 55 mm), and intermediate ($n = 1$, 60 mm) individuals.

Conical jaw teeth were forming on the premaxilla and dentary when the first pharyngeal teeth were noticeable at 6 days (5.3 mm). The supraoccipital crest, branchiostegal rays, and parasphenoid were ossifying at this same time. Shortly thereafter, the head glands that secreted the adhesive filaments which attached the larvae to surrounding surfaces disappeared, and larvae became free swimming and started feeding. By 10 days (6.2 mm) the quadrate, angular, dentary, coracoid, premaxilla, and maxilla were ossifying. At the same time, frontals were ossifying toward each other around the orbit. Shortly after this, jaw teeth were fully formed on the premaxilla and dentary. By 27 days (10.3 mm) the premaxilla became more prominent, and all skull bones appeared ossified except for hints of cartilaginous articulations. Also at this time, the anterior dorsal ridge of the supraoccipital crest was becoming more pointed, although it was still not fully formed. General adult head morphology was attained by ages >100 days (30 to 65 mm).

DISCUSSION—All pharyngeal teeth observed in specimens <100 days (30 mm) were clearly papilliform, with molariform dentition first appearing at 30 to 65 mm. This is approximately the same size at which Smith (1982) reported a change in field microhabitat preferences from littoral to more open areas. Perhaps a change in trophic morphology allows young fish to forage on prey more commonly found in more open areas.

Unlike previous studies (Sage and Selander, 1975) where parentage of broods with >1 pharyngeal morphology was questionable, parentage in this study was known for two matings. Siblings reared on a soft diet and known to be progeny of molariform parents developed molariform, papilliform, and intermediate pharyngeal dentitions. Our samples were of small specimens (SL 30 to 65 mm), and it is not known what the final adult dentition would have been. Molariform dentition might still have developed at larger sizes in some individuals classified at preservation as intermediate or papilliform, but our sample included papilliform specimens of greater age and size than most of those classified as molariform. It seems highly unlikely that individuals with molariform teeth in our samples would have reverted to papilliform dentition later, but additional studies are needed to determine at what age and size pharyngeal morphology is fixed, and to verify that morphotype is not plastic after that point.

The mosaic of trophic adaptations within the Cichlidae has allowed the clade to radiate impressively (Leim and Osse, 1975). Because *C. minckleyi* exhibits multiple phenotypes, this species offers an opportunity to study evolution of the pharyngeal apparatus. The numerous isolated and semi-isolated pools and waterways that are the natural habitat of this species might be considered to constitute a natural microevolutionary experiment in progress. To date, little is known of the spatial and temporal dynamics of this polymorphism or interactions among individuals of each morphotype, factors that affect niche specialization and fitness of individuals that differ morphologically.

Our future studies will attempt to increase sample size at all developmental stages, especially during time of morphological divergence of pharyngeal dentition, and to rear large samples to sizes well beyond the size at which mor-

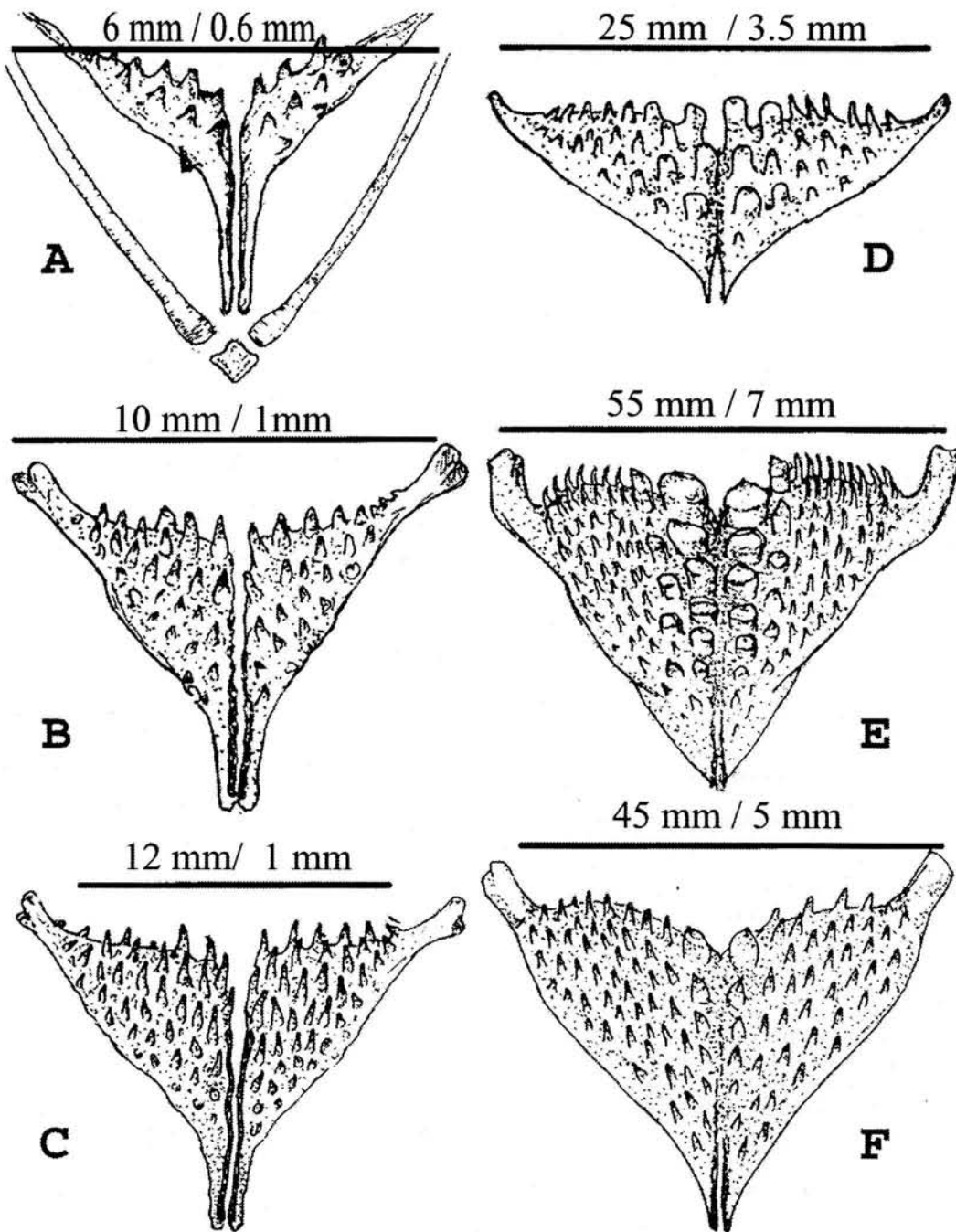


FIG. 2—*Cichlasoma minckleyi*: Ontogeny of the fifth ceratobranchial (lower surface) showing development of papilliform and molariform morphologies. Numbers above bars indicate SL of the specimen from which ceratobranchial was taken, followed by the dimension the bar represents in magnified illustration. A. 7 days; B. 30 days; C. 40 days; D. 60 days intermediate individual in which medial teeth were more stout than papilliform, but not as wide as molariform; E. 120 days juvenile with presumed developing molariform dentition; F. 120 day juvenile with papilliform morphology.

phological divergence appears to occur in order to more rigorously test the hypothesis that homomorphic matings produce heteromorphic progeny. We also hope to compare frequencies of morphotypes among localities within the Cuatro Ciénegas Basin, and compare current frequencies to those found in past collections from the same localities.

Analyses of similar ontogenetic sequences of potentially related or geographically close species such as *Cichlasoma carpintis*, *Cichlasoma labridens*, and *Cichlasoma beani* could be useful in phylogenetic analyses within the genus *Cichlasoma*. Investigation of the relation of growth rate to ultimate trophic morphology also would be useful because it remains possible that ultimate morphology may be partly determined by growth rates.

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