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## SUBSPECIFIC IDENTITY OF THE AMARGOSA PUFFISH, *CYPRINODON NEVADENSIS*, FROM CRYSTAL SPRING, ASH MEADOWS, NEVADA

Jack E. Williams<sup>1</sup> and James E. Deacon<sup>2</sup>

**ABSTRACT.**—Samples of pupfish from Crystal, Marsh, and Point of Rocks springs, Ash Meadows, Nevada, were examined to determine the subspecific identity of *Cyprinodon nevadensis* presently inhabiting Crystal Spring. Meristic and morphometric analyses indicate that Crystal Spring is inhabited by *C. n. mionectes*. The presence of this subspecies is most likely explained by their precarious survival in the spring's outflow after they were eliminated by transplanted largemouth bass in the spring pool, and their subsequent reestablishment throughout the spring system after the extirpation of the bass.

Crystal Spring (= Big Spring of Miller 1948) is the type locality for the Ash Meadows pupfish, *Cyprinodon nevadensis mionectes* Miller. Crystal Spring was chosen by Miller (1948) as the type locality because its pupfish population "has characters which very closely approach the average for the subspecies as determined by an analysis of all populations." In recent years, however, the subspecific identity of the pupfish in Crystal Spring has been questioned.

On 1 January 1966, J. E. Deacon, C. L. Hubbs, and R. R. Miller searched Crystal Spring for pupfish and found none (J. E. Deacon, field notes; Miller 1969). However, at least 10 transplanted largemouth bass, *Micropterus salmoides*, were seen in the main spring pool. The pupfish population "reappeared" by early February 1975 (Liu and Soltz 1983) and was later described as in "fine shape" with a population of approximately 1,500 pupfish (Hardy 1980).

Two subspecies of *Cyprinodon nevadensis* occur in Ash Meadows. In addition to its presence in Crystal Spring, *C. n. mionectes* occurs in a variety of lower-elevation springs (Miller 1948, Soltz and Naiman 1978). Among other springs, *Cyprinodon n. mionectes* occurs in Jack Rabbit, Point of Rocks, the Bradford Springs, and springs at the northern end of Ash Meadows that discharge water into the formerly vast Carson Slough area. Several of these springs, and an introduced population of *C. n. mionectes* at Collins Ranch (Baugh et

al., in press), are within 3 km of Crystal Spring. *Cyprinodon n. pectoralis* also occurs in nearby springs, such as Indian, Marsh, School, and Scruggs. The population of *C. n. pectoralis* from Indian Springs was particularly suspect as a source for the Crystal Spring population because that spring's outflow frequently discharges into the outflow of Crystal Spring. Both subspecies of *Cyprinodon nevadensis* are listed as endangered by the U. S. Fish and Wildlife Service.

The potential for surface water connection among the various springs is compounded by periodic flash floods, which may distribute pupfish some distance from their usual habitat, and by the formation of Crystal Spring Reservoir, which is fed by outflow water from Crystal Spring.

Thus, at least three hypotheses can be employed to explain the recurrence of pupfish in Crystal Spring:

1. pupfish from another spring reached Crystal Spring by surface water connection,
2. pupfish from another spring were introduced into Crystal Spring by man, or
3. the pupfish in Crystal Spring were not eliminated by the largemouth bass but only reduced to such low numbers that they appeared to be extirpated.

Because of the geographic proximity of other springs, either of the first two hypotheses could explain the presence of either *C. n.*

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*mionectes* or *C. n. pectoralis* in Crystal Spring. The latter hypothesis, of course, would only be appropriate for explaining the presence of *C. n. mionectes*. The purposes of this report are to determine the subspecific identity of *C. nevadensis* presently inhabiting Crystal Spring and to explain their occurrence.

#### MATERIALS AND METHODS

Meristic and morphological characters were utilized to compare the unknown *C. nevadensis* from Crystal Spring to populations of *C. n. mionectes* from Point of Rocks Springs and *C. n. pectoralis* from Marsh Spring. The two previous taxonomic studies of *C. nevadensis* from Ash Meadows have documented that the number of pectoral fin rays and number of preopercular pores in the cephalic lateral-line system are diagnostic in separating the two subspecies (Miller 1948, Miller and Deacon 1973). These two characters plus the structure of the preopercular canal were used to determine the identity of the Crystal Spring population.

Thirty specimens longer than 25 mm SL were analyzed from each of the following three collections of *C. nevadensis* made 17 January 1985 by T. M. Baugh and J. W. Pedretti:

1. UMMZ 213444. 59 *Cyprinodon nevadensis* ssp. (26.6–37.9 mm SL) from Crystal Spring.
2. UMMZ 213445. 60 *Cyprinodon nevadensis mionectes* (22.8–35.6 mm SL) from Point of Rocks Springs.
3. UMMZ 213446. 60 *Cyprinodon nevadensis pectoralis* (21.2–39.3 mm SL) from Marsh Spring.

Samples from Point of Rocks and Marsh Spring were chosen because of their geographic proximity to Crystal Spring. Also, Marsh Spring is adjacent to Indian Springs and should closely represent any *C. n. pectoralis* that may have entered Crystal. No meristic data are available for *C. n. pectoralis* from Indian Springs. The "Indian Spring" referred to in Miller and Deacon (1973: Fig. 1 and text) clearly is Marsh Spring. The two spring systems are in close proximity. Marsh Spring is located more northerly and its outflow is partially impounded by a small reservoir.

The methods of Miller (1948) were employed with the exception that only the left pectoral fin rays were counted. Counts of the left and right preopercular pores were separated from some analyses so that a count of 7-6, for example, refers to a specimen with 7 preopercular pores on the left side of the head and 6 preopercular pores on the right. Otherwise, counts on the left and right sides were combined.

#### RESULTS AND DISCUSSION

After all counts were completed, results from known samples of *Cyprinodon n. mionectes* and *C. n. pectoralis* were compared to previous results of Miller (1948) and Miller and Deacon (1973). Results from all studies were expected to be very similar because the methodology for performing the counts was nearly identical.

Counts of the diagnostic meristic characters of the Point of Rocks Springs population of *C. n. mionectes* were very similar to those given by Miller (1948) (Tables 1 and 2). Pectoral fin rays were modal at 16 in both studies and averaged 15.68 for Miller (1948) and 15.73 (present study). Counts of preopercular pores were modal at 12 for both studies but averaged slightly higher in Miller (1948) than in this study (12.54 v. 12.17, respectively).

Counts of the diagnostic meristic characters of the Marsh Spring population of *C. n. pectoralis* also were very similar to the earlier studies (Tables 1 and 2). The number of pectoral fin rays were modal at 17 and averaged 16.61 (Miller and Deacon 1973) and 16.50 (present study). Although the presence of 17 v. 16 pectoral fin rays is the primary character that separates *pectoralis* from *mionectes*, the Marsh Spring population of *C. n. pectoralis* has a higher frequency of individuals with 16 pectoral fin rays than other populations within the subspecies (Miller and Deacon 1973). Previous data on preopercular pore counts for the Marsh Spring population were lacking, so data from this study were compared to preopercular pore counts of the typical form of the subspecies from School Spring. Preopercular pore counts averaged 13.36 in the School Spring sample (= Lovell's Spring of Miller 1948) and 13.30 in the Marsh Spring sample (present study).

TABLE 1. Comparison of pectoral fin-ray counts of *Cyprinodon nevadensis* from three springs in Ash Meadows, Nye County, Nevada.

		13	14	15	16	17	18	$\bar{x}$
Crystal Spring	Miller 1948: Table 16, 1942		8	102	112	11		15.54
	present study, 1985		1	10	18	1		15.63
Point of Rocks Springs	Miller 1948: Table 16, 1942	1	3	65	112	12		15.68
	present study, 1985		2	8	16	4		15.73
Marsh Spring	Miller and Deacon 1973:137				24	30	2	16.61
	present study, 1985			1	13	16		16.50

TABLE 2. Comparison of preopercular pore counts of *Cyprinodon nevadensis* from four springs in Ash Meadows, Nye County, Nevada.

		10	11	12	13	14	15	16	$\bar{x}$
Crystal Spring	Miller 1948: Table 26, 1942		1	39	18	8			12.50
	present study, 1985		3	18	8	1			12.23
Point of Rocks Springs	Miller 1948: Table 26, 1942	1	2	55	20	18			12.54
	present study, 1985		3	21	5	0	1		12.17
School Spring	Miller 1948: Table 26, 1939		1	24	18	54	1	2	13.36
Marsh Spring	present study, 1985		1	7	10	8	2	2	13.30

A comparison of pectoral fin-ray numbers in the three 1985 collections indicates that the Crystal Spring population is *C. n. mionectes* (Table 1). A similar conclusion is reached when comparing the number and frequency of preopercular pore counts (Table 2). The Crystal Spring sample of *C. nevadensis* averaged 15.63 pectoral fin rays and 12.23 preopercular pores. Both values are within the results expected for *C. n. mionectes*.

The Crystal Spring sample of *C. nevadensis* was modal at 16 pectoral fin rays but included a relatively large percentage (33%) of individuals with 15 pectoral fin rays. This contrasts sharply with the Marsh Spring sample of *C. n. pectoralis*, which was modal at 17 pectoral fin rays and contained a relatively large percentage (43%) of individuals with 16 rays and only one fish with 15.

The Crystal Spring sample of *C. nevadensis* averaged 12.23 preopercular pores. This value is very similar to the average number of preopercular pores in the Point of Rocks Springs sample (12.17) but is quite distinct from the average in the Marsh Spring sample (13.30). The modal number of preopercular pores in the Crystal Spring sample is 12. This agrees with the value from the Point of Rocks sample but again contrasts with results from Marsh Spring, where those pores are modal at 13 (Table 2). Preopercular pore counts, with frequency of counts given parenthetically, are as follows for Point of Rocks: 5-6 (1), 6-5 (2),

6-6 (21), 6-7 (3), 7-6 (2), 8-7 (1); Crystal: 5-6 (2), 6-5 (1), 6-6 (18), 6-7 (2), 7-6 (6), 8-6 (1); and Marsh: 5-6 (1), 5-7 (1), 6-6 (6), 6-7 (5), 7-6 (5), 7-7 (8), 8-7 (2), and 8-8 (2).

The structure of the preopercular canal also varied significantly between the Point of Rocks Springs and Crystal Spring samples and the *C. n. pectoralis* from Marsh Spring. In the Marsh Spring sample, 13% of the fish possessed at least one interrupted preopercular canal. Both the left and right canals were interrupted in one specimen. Although interruption of the canal results in a higher pore count, the primary causal factor in the higher pore counts in the Marsh Spring population was the presence of a seventh pore on the anterior end of the preopercle. Most *C. n. mionectes* contain only 6 pores on each side. No preopercular canal interruption was noted in *C. n. mionectes* from Point of Rocks or Crystal Springs, although one specimen from Crystal Spring possessed an open canal on one side.

Delays in completion of the cephalic canal system until adulthood have been noted for certain cyprinodonts (Hubbs and Miller 1965) and could possibly explain some of the above differences noted between *C. n. mionectes* and *C. n. pectoralis*. Differences in standard length among the subsamples examined, however, are slight. Ranges and means of standard length for the subsamples examined are: Point of Rocks Springs, 26.8–35.6 mm ( $\bar{x}$

= 30.98); Crystal Spring, 28.3–37.9 (33.26); and Marsh Spring, 25.3–39.3 (29.48).

Finally, the meristic characters of the Crystal Spring sample were compared to data from the original study of the Crystal Spring population by Miller (1948). Very close agreement is seen in the average number of pectoral fin rays observed in the Crystal Spring sample taken in 1942 (Miller 1948) and in 1985 (present study) (Table 1). The 1942 sample averaged 15.54 rays, whereas the 1985 sample averaged 15.63. A similar agreement is seen in the number of preopercular pores from samples collected in 1942 and 1985 (Table 2). The 1942 sample included 59% of individuals with 12 preopercular pores and 27% with 13. The 1985 sample contained 60% of individuals with 12 preopercular pores and 27% with 13.

Based on the results of this study, the subspecific identity of the pupfish from Crystal Spring is *C. n. mionectes*. It appears that the present *C. n. mionectes* population is descended from those individuals who withstood the pressure from largemouth bass. The question as to whether the present Crystal Spring population persists by natural occurrence, was introduced, or made its way by surface water connection from another *C. n. mionectes* population may be answered as follows. Although the predaceous largemouth bass apparently eliminated *C. n. mionectes* from the spring pool at Crystal, it may be that some pupfish survived in the spring outflow. According to field notes of one of the authors (JED 66-1), only 100 yards of the outlet ditch were searched. Robert R. Miller (10 October 1985 in litt. to JEW) supported the hypothesis that *C. n. mionectes* may not have been eliminated from the entire spring system and stated, in part:

to say unequivocally that *Cyprinodon* was gone from this system [Crystal] would be awfully hard to defend. In frigid midwater [ice on ground, 1 January 1966] you might not expect to see pupfish, especially when they had been impacted for X-years by that dastardly predator *Micropterus salmoides*. Granted that the pupfish could certainly have been wiped out at the main spring pool. I think it is unjustified to assume they were eliminated throughout the long outlet ditch as well.

The causes of disappearance of largemouth bass from Crystal Spring are uncertain as well.

The warm water (30.9 C) of the spring pool may have inhibited reproduction, though such would not be the case throughout the cooler outlet ditch and reservoir. This, coupled with constant angling pressure by the workers in Ash Meadows, may have eliminated bass from the spring system. Regardless of the causes, it is quite reassuring to know that the type locality of *Cyprinodon nevadensis mionectes* still harbors that unique form of pupfish and that the spring has rid itself of the introduced bass.

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