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Resolving Taxonomy and Historic Distribution for Conservation of Rare Great Plains Fishes: *Hybognathus* (Teleostei: Cyprinidae) in Eastern Colorado Basins

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Similar morphology and confused historical taxonomy of *Hybognathus hankinsoni* (brassy minnow) and *Hybognathus placitus* (plains minnow) have made determination of their historic distributions and conservation status unclear in eastern Colorado basins. We developed logistic regression models from morphometric measurements to predict species identity of *Hybognathus* collections from Colorado and adjacent counties (n = 1154 specimens in 134 lots). A model based on orbit diameter, standard length, and eye position correctly predicted 98% of the specimens examined and 100% of the museum lots. *Hybognathus hankinsoni* have larger eyes centered on a horizontal line through the tip of the snout, whereas *H. placitus* have smaller eyes centered above the tip of the snout. The two species were historically sympatric in the Platte, Republican, and Smoky Hill River basins, whereas *H. placitus* was allopatric in the Arkansas River basin. The taxonomic characters defined here will allow accurate identification of future collections to determine the status of these native fishes.

NATIVE fishes of the Great Plains are well known for their tolerance of harsh physicochemical conditions, including floods, droughts, high water temperatures, and low dissolved oxygen concentrations (Matthews, 1988). Despite this tolerance, distributions of a large number of taxa have declined in recent decades. Four fish species endemic to the Great Plains region are listed as threatened or endangered under the Endangered Species Act (*Notropis girardi, Noturus topeka, Noturus placidus, Scaphirhynchus albus*), and one is a candidate for federal listing (*Etheostoma cragini*; http://endangered.fws.gov). Many other wide-ranging native fishes of the Great Plains have been extirpated or are in decline throughout much of the western part of their range (Rabeni, 1996; Fausch and Bestgen, 1997). For example, in Colorado, six of 38 native plains species are known to have been extirpated since the first fish collections were made in the late 1800s (*Anguilla rostrata, Nocomis bigutattus, Notropis heterolepis, N. girardi, Macrhybopsis tetranema, Stizostedion* spp.), and an additional 13 species are listed by the state as endangered, threatened, or of special concern (T. Nesler, Colorado Division of Wildlife, pers. comm.). Therefore, half of the native taxa have declined or been lost.

Identifying the historic distributions of these declining species is necessary to guide conservation efforts but is often hampered by two main problems. First, few early collections exist, and most of these were made after habitats were already altered, some native species were extirpated, and nonnative species were introduced. For example, fish collections are known from only 12 locations before 1900 in the Great Plains portion of eastern Colorado (Fausch and Bestgen, 1997), yet diversion of water for irrigation was well developed by the 1860s. As a result, the historic distributions of fishes described only in early reports, such as walleye or sauger (*Stizostedion* spp.) from the South Platte River and speckled chub (*Macrhybopsis tetranema*; formerly *Macrhybopsis aestivalis tetranemus*) from the Arkansas River are not fully known. Even their identity cannot be verified because no museum specimens were preserved before these species were extirpated from the state (Fausch and Bestgen, 1997; Eisenhour, 1999; Luttrell et al., 1999).

A second main problem is that identification of some taxa is difficult, resulting in inaccurate field surveys. Moreover, the historical taxonomy may be confused so that even the identity of museum specimens is unclear. Good examples of this are minnows of the genus *Hybognathus* in basins of eastern Colorado. Species in this taxon are similar morphologically, resulting in considerable taxonomic confusion during the past 150 years (Cook et al., 1992; Bestgen and Propst, 1996). At least 15 species and subspecies have been described (Bestgen and Propst, 1996) of which seven are currently recognized (Robins et al., 1991). In the western Great Plains, three forms, later recognized as *Hybognathus hankinsoni* (brassy minnow), *Hybognathus placitus* (plains minnow), and *Hybognathus argyritis* (western silvery minnow), were all originally considered variants of *Hybognathus nuchalis* (Mississippi silvery minnow; Ellis, 1914). Based on current classification, *H. argyritis* and *H. nuchalis* do not occur in Colorado (Lee et al., 1980). However, many historic museum collections from the Great Plains of eastern Colorado (e.g., Ellis, 1914) are still identified as *H. nuchalis*, because *H. hankinsoni* was not described until 1929 (Hubbs in Jordan, 1929; Bailey, 1954) and *H. placitus* was not separated from *H. nuchalis* until 1962 (Niazi and Moore, 1962; Al-Rawi and Cross, 1964).

Surveys during the past 20 yr have suggested that the two *Hybognathus* species in Colorado are in decline (Propst and Carlson, 1986; T. P. Nesler, R. VanBuren, J. Stafford, and M. Jones, Colorado Division of Wildlife, 1997, unpubl.; T. P. Nesler, C. Bennett, J. Melby, G. Dowler, and M. Jones, Colorado Division of Wildlife, 1999, unpubl.), prompting a listing of *H. hankinsoni* as threatened and *H. placitus* as endangered in Colorado in 1998. However, because of the relatively recent taxonomic clarifications, unreliable taxonomic keys, and lack of museum specimens to confirm many published records, the historic and current distributions of *H. hankinsoni* and *H. placitus* at the western extent of their range in Colorado are unclear (Fausch and Bestgen, 1997). For example, Ellis (1914) recognized small- and large-eyed forms of *H. nuchalis* throughout warm water reaches of the South Platte River, but the identity of these early collections and many other extant specimens remains undetermined. Recent field collections may also have been misidentified because existing keys are inadequate to distinguish the two species. Therefore, before natural resource managers can propose conservation measures, better taxonomic characteristics are needed to accurately identify museum specimens and determine historic and current distributions of these taxa.

Two characteristics, the shape of the basioccipital process and the number of scale radii, have often been cited as useful to distinguish among *Hybognathus* species (e.g., Beckman, 1952; Baxter and Simon, 1970; Pflieger, 1975). Unfortunately, both *H. hankinsoni* and *H. placitus* have narrow, peglike basioccipital processes (Schmidt, 1994; Bestgen and Propst, 1996). Although they differ in relative lengths, the similar shape of the basioccipital process in these two species makes it an unreliable characteristic to use. Numbers of scale radii are among the most common characters used to separate *H. hankinsoni* from *H. placitus* in keys. *Hybognathus hankinsoni* is most often described as having about 20 faint or weak scale radii of varying lengths (except 17–19 in Bailey, 1954), whereas *H. placitus* is most often reported to have 10–15 strong radii (except < 20 in Eddy and Underhill, 1969). However, no published account describes criteria to distinguish faint, weak, or strong radii, nor has the utility of this characteristic been determined.

The goal of our research was to develop a reliable technique to distinguish *H. hankinsoni* from *H. placitus* in western Great Plains watersheds where they are sympatric. Our specific objectives were first to test the utility of scale radii as a key characteristic and then to develop a quantitative model based on taxonomic characteristics to separate the two species. The model and additional qualitative characteristics were then used to identify all available museum specimens of *Hybognathus* from Colorado and adjacent counties in neighboring states, which allowed clarification of the historic distributions of *H. hankinsoni* and *H. placitus* in Colorado basins. This work, coupled with ongoing sampling and accurate identification, will help natural resource managers determine how much of their historic range in Colorado these two species currently occupy and where to focus restoration and conservation efforts.

Materials and Methods

To determine taxonomic characters that distinguish the two species, material was obtained from locations where they are allopatric (''knowns'' hereafter) in Michigan (*H. hankinsoni*, four locations, $n = 81$; see Appendix 1) and New Mexico (*H. placitus*, Canadian River, $n = 51$). A suite of characters used in earlier keys and studies (Hubbs and Lagler, 1964; Bestgen and Propst, 1996) was measured, including scale radii.

Scale radii analysis.—The number of radii on 82 scales from 60 fish, 30 of each species from the lots of knowns described above, were counted to determine the utility of this character for separating the two species. Scales were removed from the right side of the fish, posterior to the pectoral fin base and above the lateral line (DeVries and Frie, 1996). Scales were cleaned, mounted on glass slides, and viewed under a compound microscope at 30× and 100×. Distances from the focus to the scale margin were measured at three angles, 45, 90, and 135 degrees from a horizontal line through the focus. Radii were categorized as faint, incomplete, or complete by comparing them to the nearest measured distance. We defined faint radii as those less than 30% of the nearest measured distance from focus to scale margin, incomplete radii as those 31–67%, and complete radii as those greater than 67% the distance. To determine variation in scale radii counts within individuals, two scales were read from each of 22 fish, 11 of each species.

Morphometric analysis.—Nine quantitative and three qualitative characters were measured from the 132 specimens of known identity described above to develop a quantitative model to separate the two species. The lectotype of *H. hankinsoni* and four syntypes of *H. placitus* were also measured to test and verify the model. Standard length, head length, pectoral fin length, snout length, orbit diameter, body depth, body width, caudal peduncle depth, and gape width were measured following Hubbs and Lagler (1964). Qualitative characters included snout shape, dorsal fin shape, and eye position. Snout shape and dorsal fin shape were categorized as either rounded or pointed. Eye position referred to the location of the center of the eye relative to a horizontal line through the anterior most tip of the snout and was categorized as either even or above (Fig. 1). Quantitative characters were measured to the nearest 0.1 mm using digital calipers.

After developing the model based on known specimens, we then obtained all available museum collections of *Hybognathus* from Colorado and adjacent counties in neighboring states, totaling 1753 fish in 134 lots (see Appendix 1; Scheurer, 2002), for examination and reidentification. Specimens included those from the earliest collections in the state by Jordan (1891) and Juday (1904). All these lots were considered *Hybognathus* of unknown species identity (''unknowns,'' hereafter), and 1154 individuals were measured. When collections contained many individuals, a subsample of at least 30 fish of all sizes represented was measured. Damaged, deformed, or poorly preserved fish were excluded. The quantitative model was then used to predict the identity of the unknowns, and additional

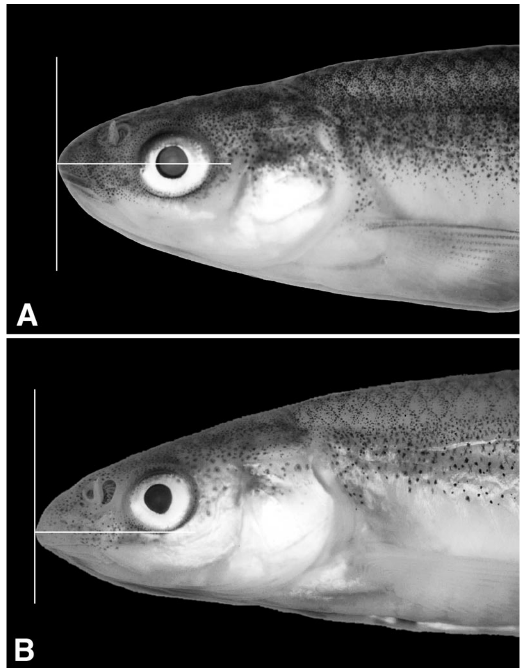

Fig. 1. Eye position characteristic for *Hybognathus hankinsoni* (A) and *Hybognathus placitus* (B). *Hybognathus hankinsoni* typically have larger eyes with the centers even with a horizontal line drawn back from the anteriormost tip of the snout. *Hybognathus placitus* have smaller eyes with the centers often above this line. Images are by R. E. Zuellig, with permission.

qualitative characters were used to verify model predictions.

Model development and testing.—Logistic regression (SAS/STAT®, PROC LOGISTIC, SAS Institute, Inc., Cary, NC, 2000, unpubl.) was used to develop a model for separating the two species, based on the morphometric independent variables measured from the known specimens. Model selection followed Burnham and Anderson (1998) using Akaike's Information Criterion (AIC) to select the top candidate models. This model selection procedure is based on an information-theoretic approach that is proposed to be superior to traditional hypothesis testing for observational data such as these because it allows comparison of more than two models at once and balances precision and bias (Burnham and Anderson, 1998; Franklin et al., 2000).

Species identity (*H. hankinsoni* or *H. placitus*) was first modeled as a function of each of the eight quantitative characters (not including standard length) and each of the three qualitative characters. Additional candidate models were developed using each quantitative character and standard length, the three variables considered a priori to be most diagnostic (orbit diameter, eye position, standard length), and these three variables with various combinations of their two-way interactions. The value of AIC_c (AIC corrected for small-sample bias), and Akaike weights were calculated and used to rank models (Burnham and Anderson, 1998). Models with the lowest AIC_c and highest Akaike weight were given the most consideration. An added criterion for selecting the best model was the percentage of known specimens that it classified as the correct species (Hosmer and Lemeshow, 2000).

Once the diagnostic model was developed using known specimens, measurements from the unknown *Hybognathus* from Colorado were entered in the model to predict their identity. These predictions were verified by the second author using supplemental characters to evaluate the efficacy of the model. With the exception of two large museum lots (UMMZ 135130 and 32241) for which only 30 specimens each could be obtained, all fish in each lot, including those not measured, were examined to ensure that no additional species were present.

The model developed from the known specimens proved only moderately useful for separating all individuals of the two species, so the two best candidate models were refit using the 1154 verified *Hybognathus* from Colorado to improve predictions. The identity of the type specimens we examined was predicted to validate each model. These models were also tested using cross-validation (Hosmer and Lemeshow, 2000:186). The data were randomly ordered and divided into 10 equal sets. Ten separate models were fit using nine of the 10 datasets, leaving out a different set each time. The data excluded were then used to test each model. The average percentage of the specimens classified correctly was used as a criterion to judge each model.

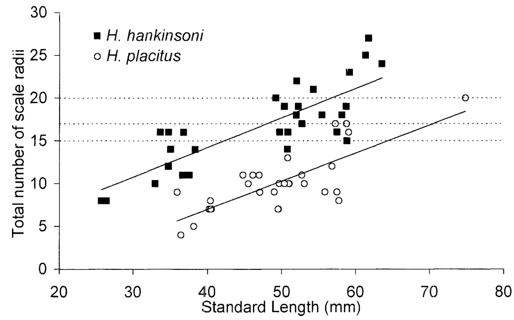

Fig. 2. Total number of scale radii (faint, incomplete, and complete) as a function of standard length for *Hybognathus hankinsoni* (filled squares) and *Hybognathus placitus* (open circles). Regression lines are shown for each species. Horizontal dashed lines indicate commonly used criteria for separating the two species. *Hybognathus hankinsoni* was reported to have 17 to 19 radii in the original full description (Bailey, 1954) and about 20 radii in subsequent keys. *Hybognathus placitus* was described as having 10 to 15 radii in most keys.

Results

Scale radii.—Scale radii were not useful for distinguishing between *H. placitus* and *H. hankinsoni.* Several sources stated that *H. hankinsoni* have about 20 weak radii, which we assumed included either complete plus incomplete radii or the total of all three categories. However, the mean number of total radii for *H. hankinsoni* from this analysis was 17 (range 8–27). More than half of the 30 fish analyzed would be misidentified as *H. placitus* based on the most conservative criterion for *H. hankinsoni* of having 17 or more total radii (Bailey, 1954), and more than 75% would be misidentified based on the most common criterion of 20 or more total radii (Fig. 2). Even higher proportions would be misidentified based on complete plus incomplete radii. In contrast, although *H. placitus* averaged 10 total radii (range 4–20), four of 30 would be misidentified as *H. hankinsoni* based on the most common criterion of 15 or fewer total radii (e.g., Baxter and Simon, 1970). In addition to often misidentifying the two species, the number of scale radii increased with standard length for both ($P < 0.001$). As a result, only *H. hankinsoni* > 60 mm and *H. placitus* < 55 mm would be consistently identified correctly.

The number of scale radii also differed between scales from the same fish, further confounding use of this characteristic. Ten of 11 *H. hankinsoni* and five of 11 *H. placitus* had different numbers of total radii on two scales. Using a criterion of 17 or more total radii for *H. hankinsoni,* only three of 11 would have been correctly identified using either scale, the rest being misidentified based on one or both scales (see Scheurer, 2002). Similarly, using a criterion of 15 or fewer total radii for *H. placitus,* only seven of 11 would have been correctly identified based on either scale.

Model based on known specimens.—Logistic regression analysis revealed that the best single quantitative variable for distinguishing between the known *H. hankinsoni* and *H. placitus* was orbit diameter. *Hybognathus hankinsoni* of a given length

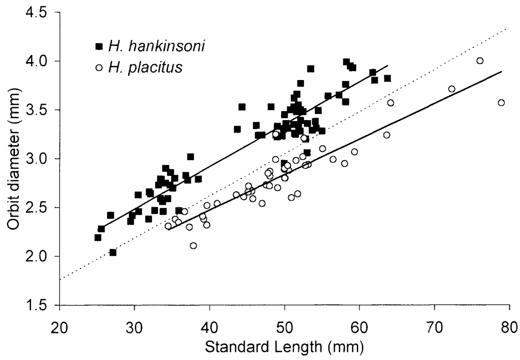

Fig. 3. Orbit diameter as a function of standard length for the 132 known specimens. Solid lines show regression lines for *Hybognathus hankinsoni* ($r^2 = 0.88$, $P < 0.001$) and *Hybognathus placitus* ($r^2 = 0.86$, $P < 0.001$). Dashed line is the 50% probability line ($y = 0.0431x + 0.8951$) predicted from logistic regression. Fish represented by points above the line are predicted to be *H. hankinsoni*, whereas those below are predicted to be *H. placitus*.

had a larger orbit diameter than *H. placitus*, and orbit diameter also increased allometrically with standard length for both species ($P < 0.001$ for both; Fig. 3). The model based on these two characteristics had the lowest AIC_c value (41.73), accounted for a high proportion of the Akaike weight of the 19 models with valid parameter estimates (0.69; weights sum to 1.0), and correctly classified 96% of the known specimens. In fact, only one other model, based on standard length, orbit diameter, and their interaction, received any support ($AIC_c = 43.35$) and accounted for nearly all the remainder of the Akaike weight (0.31). Therefore, there was no support for including any of the other variables and the model based on orbit diameter and standard length was selected as the best model for predictions (Table 1). The model was validated with the type specimens, and correctly predicted the identities of the lectotype of *H. hankinsoni* (UMMZ 84266) and four syntypes of *H. placitus* (USNM 89 [$n = 3$], MCZ 1789 [$n = 1$]).

The two-variable equation developed from the knowns for predicting species identity is

$$P(H.\ hankinsoni) = \frac{\exp(-16.8465 - 0.8123SL + 18.8391OD)}{1 + \exp(-16.8465 - 0.8123SL + 18.8391OD)},$$

where P = probability of an unknown fish being *H. hankinsoni*, SL = standard length (mm), and OD = orbit diameter (mm). If $P > 0.50$, the fish is predicted to be *H. hankinsoni*, whereas if $P < 0.50$, the fish is predicted to be *H. placitus* (Fig. 3).

Eye position was also a useful qualitative variable for distinguishing the two species. All of the known *H. hankinsoni* had the even eye position, whereas 88% of the known *H. placitus* had the above eye position (Fig. 1). However, this variable could not be included in models because there was no variation in eye position of the known *H. hankinsoni* so valid maximum likelihood parameter estimates could not be calculated.

Predictions of unknown specimens and additional models.—Of 1154 unknown fish measured and verified, 78% were predicted by the model to be the correct species. Of the 212 *H. placitus*, species identities of 210 (99%) were correctly predicted, but species identities of only 73% of the 942 *H. hankinsoni* were predicted correctly. Of the 257 incorrectly predicted specimens, 255 were *H. hankinsoni* with smaller relative orbit di-

Table 1. Maximum Likelihood Estimates of Intercept and Slope Parameters from Logistic Regression for the Best Model Based on the 132 Known Specimens and the Two Candidate Models Based on the 1154 Unknown Specimens to Predict *Hybognathus* species identity. Models predict the probability that an unknown specimen is *H. hankinsoni*. Coefficients for the eye position parameter are 0 for even and 1 for above. Standard errors of parameters are in parentheses.

Model	Intercept	Standard length	Orbit diameter	Eye position
Model based on 132 known specimens				
Standard length, orbit diameter	−16.8465	−0.8123	18.8391	—
	(4.0462)	(0.1690)	(3.8075)	—
Model based on 1154 unknown specimens				
Standard length, orbit diameter	−11.8900	−0.8222	18.9196	—
	(1.7387)	(0.0740)	(1.7511)	—
Standard length, orbit diameter, eye position	−11.3796	−0.8009	18.5823	−5.3898
	(2.0969)	(0.0839)	(2.0224)	(1.0643)

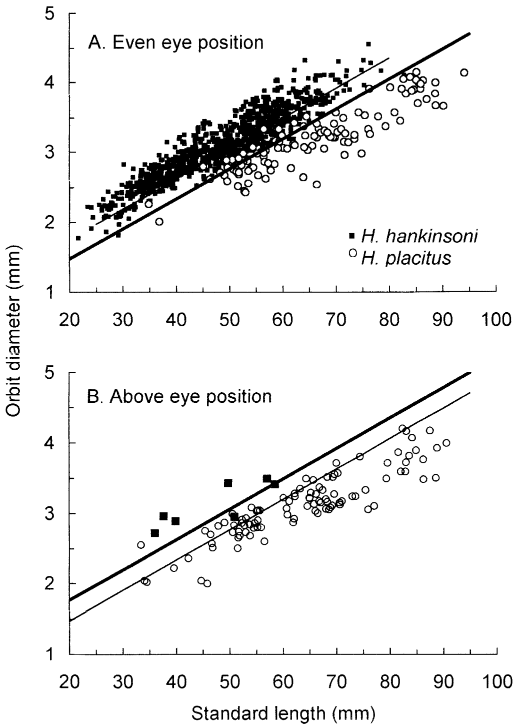

Fig. 4. Orbit diameter as a function of standard length for the 1154 unknown specimens with even eye position (A) and above eye position (B). Decision lines show where the probability of a specimen being *Hybognathus hankinsoni* is predicted to be 50%. In A, the thin line shows the prediction for the original two-variable model based on 132 known specimens (see Fig. 3), and the thick line shows the prediction for fish with the even eye position for the final three-variable model based on 1154 verified unknowns ($y = 0.0431x + 0.6119$). In B, the thick line shows the prediction from the final three-variable model for fish with the above eye position ($y = 0.0431x + 0.9019$), whereas the thin line shows the prediction for fish with the even eye position for comparison (same as thick line in Fig. 4A).

ameters than the known specimens from Michigan used to build the model (Fig. 4). This model is not useful in Colorado because it predicted that these fish were *H. placitus*, which would lead natural resource managers to overestimate the distribution and abundance of the rarer of the two species. Because of this potential bias, we elected to refit the model using all 1154 verified specimens from the region.

This new two-variable model based on orbit diameter and standard length correctly predicted the identity of 88% of the *H. placitus* and 99% of the *H. hankinsoni* verified unknowns (Table 1). This larger sample included seven *H. hankinsoni* with the above eye position, so we also fit a three-variable model including this variable. This model correctly predicted 91% of the *H. placitus* and 99% of the *H. hankinsoni* verified unknowns (Fig. 4). The parameters for intercept, standard length, and orbit diameter were similar between these two- and three-variable models, so the $P = 0.50$ decision line for predicting species identity was nearly identical for fish with the even eye position. In contrast, the added parameter for eye position allowed more accurate predictions for specimens with the above eye position. The three-variable model correctly predicted the identity of the most fish and had a lower AIC_c than the two-variable model (168.98 vs 229.92), so it was selected as the best model. For each model, cross validation resulted in identical estimates for the average percentage of fish correctly classified as those presented above because of the large sample size. Both models correctly classified all the type specimens examined.

The best predictive model, developed from the 1154 Colorado specimens, included standard length, orbit diameter, and eye position (Table 1)

$$P(H.\ hankinsoni) = [\exp(-11.3796 - 0.8009SL + 18.5823OD - 5.3898EP)] \div [1 + \exp(-11.3796 - 0.8009SL + 18.5823OD - 5.3898EP)],$$

where P = probability of an unknown fish being *H. hankinsoni*, SL = standard length (mm), OD = orbit diameter (mm), and EP = eye position ($EP = 0$ for even, $EP = 1$ for above). If $P > 0.50$, the fish is predicted to be *H. hankinsoni*, whereas if $P < 0.50$, the fish is predicted to be *H. placitus*. For example, the model predicts that a fish with standard length of 50 mm, orbit diameter of 3.2 mm, and even eye position has nearly a 100% probability of being *H. hankinsoni*, whereas a fish with an orbit diameter of 2.7 mm, the same standard length, and the above eye position has < 1% probability of being *H. hankinsoni* (Fig. 4). Therefore, the smaller-eyed fish is predicted to be *H. placitus*.

Overall, all but 25 of the 1154 unknown specimens (2%) were correctly classified by the final model. Moreover, in all lots, the majority ($\geq$ 67%) of fish were predicted to be the correct species (cf. Scheurer, 2002). Eight lots were not measured because of the poor condition of all specimens (range: 1–16 specimens each), but species identity was verified from supplemental characters. Of the 134 lots of unknown *Hybog-*

nathus, 33 (25%) were misidentified or cataloged as species that no longer exist or have been revised. Three of these were mixed lots of *H. hankinsoni* and *H. placitus*, seven lots contained *Hybognathus* mixed with other taxa, and two lots originally cataloged as *H. nuchalis* contained no *Hybognathus*.

Taxonomic characteristics of Hybognathus hankinsoni and Hybognathus placitus.—*Hybognathus hankinsoni* Hubbs was first proposed by Hubbs and Greene (1926) but was not described until Jordan (1929). Bailey (1954) elaborated on this description and designated a lectotype (UMMZ 84266) from the Dead River, a tributary of Lake Superior in Marquette County, Michigan. Earlier names used for *H. hankinsoni* and under which specimens may still be cataloged, include the following.

Hybognathus nuchalis nuchalis Agassiz (in part), 1855; Hendricks, 1950.
Hybognathus nuchalis Agassiz (in part), 1855; Jordan, 1891; Juday, 1904, 1905; Cockerell, 1908; Ellis, 1914.
Hybognathus nubilum (in part), Call, 1887; Meek, 1891; Evermann and Cox, 1896.
Hybognathus nuchale evansi (in part), Eigenmann, 1894; Evermann and Cox, 1896.

Descriptive characters.—Head: Eye large relative to *H. placitus* and about equal to snout length (Fig. 1; mean orbit diameter/snout length = 0.93, range 0.70–1.38, n = 503 Colorado fish). Horizontal line drawn through the anterior most tip of snout intersects the center of eye in most fish. Mouth terminal and slightly oblique. Head with upturned ventral profile. Basioccipital process peg shaped, shorter than in *H. placitus*. Fleshy snout not visible ventrally. Snout rounded.

Body: Average adult body size 50–70 mm total length (TL). Maximum size < 100 mm TL. Dorsal, anal, and pectoral fins rounded (Scheurer, 2002). First ray of dorsal fin shorter than second, giving rounded appearance. Prominent, dark, lateral and predorsal bands of pigment. Margins of scales outlined, especially dorsally. Scales large and prominent. Pectoral fins smaller than in *H. placitus*. Males golden during breeding season.

Hybognathus placitus Girard was first described by Girard (1856). The name *H. evansi* has line priority over *H. placitus* but the first revision by Jordan and Gilbert (1882) used the name *H. placitus* (Al-Rawi and Cross, 1964). Girard designated five syntypes (1858), now cataloged as USNM 89 (3), MCZ 1789 (1), and ANSP 5065 (1), from sluices of the Arkansas River near Dodge City, Ford County, Kansas, collected in 1853, and a sixth was recently reported (MNHN 351, Gilbert 1998). Earlier names used for *H. placitus* and under which specimens may still be cataloged, include the following.

Hybognathus nuchalis nuchalis Agassiz (in part), 1855; Hendricks, 1950.
Hybognathus nuchalis Agassiz (in part), 1855; Hay, 1887; Ellis, 1914.
Hybognathus evansi Cope, 1865; Girard, 1856, 1858 nomen nudum.
Hybognathus placita Girard; Graham, 1885; Personius and Eddy, 1955.
Hybognathus nubilum (in part), Call, 1887; Meek, 1891; Everman and Cox, 1896.
Hybognathus nuchalis placita, Jordan, 1891; Evermann, 1893.
Hybognathus nuchale, Meek, 1894.
Hybognathus nuchale evansi (in part), Eigenmann, 1894; Everman and Cox, 1896.
Hybognathus churchilli Hildebrand, 1932.
Hybognathus placitus placitus, Johnson, 1942.
Hybognathus placita placita, Beckman, 1952.

Descriptive characters.—Head: Eye smaller than in *H. hankinsoni* and notably less than snout length (Fig. 1; mean orbit diameter/snout length = 0.69, range 0.52–1.03, n = 66 Colorado fish). Horizontal line through anterior most tip of snout generally crosses below the center of the eye resulting in a decurved anterior dorsal profile. Mouth subterminal and horizontal. Head with flat ventral profile. Peg-shaped basioccipital process, longer than in *H. hankinsoni*. Fleshy snout visible ventrally. Snout relatively pointed.

Body: Average adult body size 50–90 mm TL. Maximum size 130 mm TL. Dorsal, anal, and pectoral fins pointed (Scheurer, 2002). First ray of dorsal fin usually longer than second, creating a falcate posterior fin margin. Lateral and predorsal bands of pigment present but not as prominent as in *H. hankinsoni*. Scales more embedded than in *H. hankinsoni*. Dorsal scales not darkly outlined. Pectoral fins large relative to *H. hankinsoni*.

Scott and Crossman (1973) and Becker (1983) provide more detail on descriptive characteristics for *H. hankinsoni*, and Al-Rawi and Cross (1964) and Niazi and Moore (1962) provide detailed descriptive characteristics for *H. placitus*. Researchers should refer to Eschmeyer (1998) and Gilbert (1998) for a more thorough history of the nomenclature of both species.

Historic distribution.—The correct identification of museum specimens allowed a clear determi-

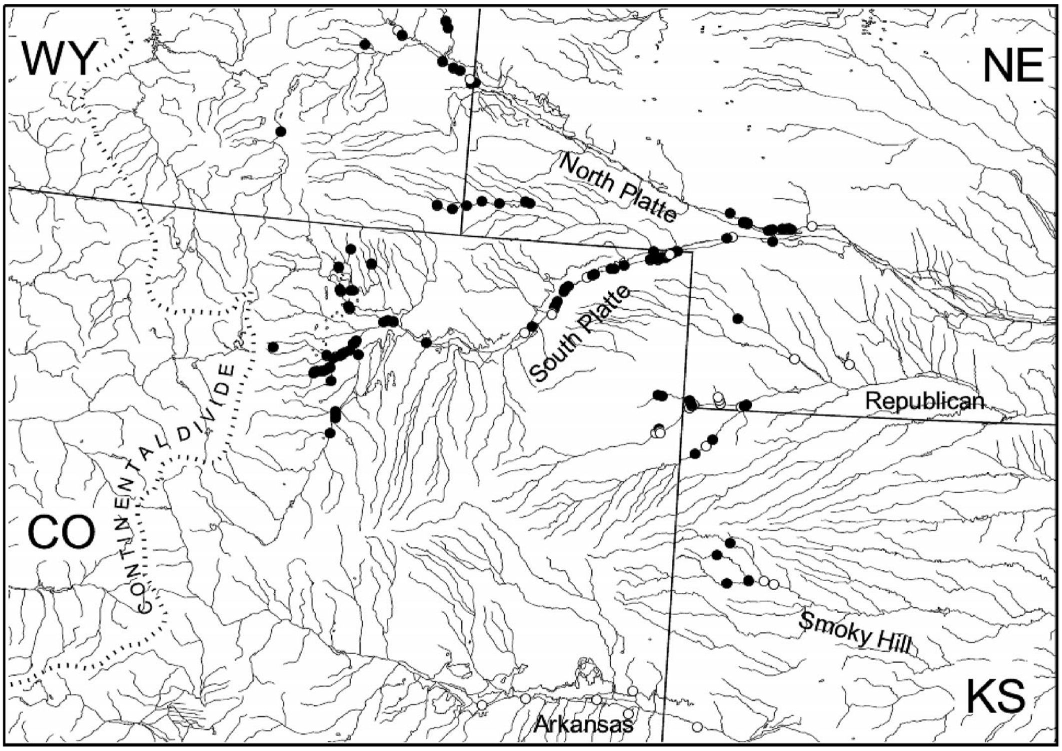

Fig. 5. Historical distribution of *Hybognathus hankinsoni* and *Hybognathus placitus* in Colorado and adjacent counties in neighboring states based on verified museum collections (Scheurer, 2002). Closed circles indicate *H. hankinsoni* and open circles *H. placitus.*

nation of the historical distribution of these two *Hybognathus* species in Colorado and surrounding states (Fig. 5). *Hybognathus hankinsoni* was distributed throughout the main stem of the South Platte River, in one of its major plains tributaries in southwestern Nebraska (Lodgepole Creek) and west to its transition-zone tributaries in the foothills of the Rocky Mountains. In the Republican River basin, its distribution extended into the headwater plains tributaries in eastern Colorado. The southwestern extent of its entire distribution was in the headwaters of the Smoky Hill River in the Kansas River basin of western Kansas. *Hybognathus hankinsoni* has never been recorded or collected from the Arkansas River basin. It was also widely distributed along the North Platte River in Nebraska and into the headwaters in Wyoming. In contrast, *H. placitus* was allopatric in the Arkansas River basin. It occurred in sympatry with *H. hankinsoni* in the Smoky Hill and Republican rivers near their headwaters and in the lower main stems of the South Platte and North Platte Rivers on the plains.

Discussion

Two characters commonly used to identify *Hybognathus* species, the shape of the basioccipital process and the number of scale radii, should not be used to separate *H. hankinsoni* and *H. placitus.* The similarity in shape of the basioccipital process between the two species makes it an unreliable characteristic to use by itself. We also do not recommend the continued use of scale radii counts because of strong overlap between the two species, the increase in number of scale radii with standard length, and variation among scales from the same fish. Measuring orbit diameter, standard length, and eye position is not only more reliable for distinguishing the two species but is faster and easier than mounting and reading scales or comparing relative lengths of the basioccipital process, which requires partial dissection.

The logistic regression model with the independent variables orbit diameter, standard length, and eye position fit to the 1154 measured and verified specimens from Colorado and adjacent counties reliably distinguished most *H. hankinsoni* from *H. placitus*. This model correctly predicted species identities for 98% of all individuals and 100% of the lots based on predictions for the majority of individuals in each lot. When this model is used in conjunction with the recommended supplemental characteristics, all but the most unusual individuals should be correctly identified. This analysis clarified the identities of all museum specimens of *Hybognathus* collected from the region known to us, 25% of which were previously misclassified. The proper identification of extant specimens allowed accurate description of the historic distributions of these species in eastern Colorado river basins.

This model will also be a useful diagnostic tool for reliably distinguishing *H. placitus* from *H. hankinsoni* in new collections and will allow their current distribution and status to be determined. The model is based on precise measurements, so proper identification will require preserving voucher specimens for laboratory analysis. It may also be helpful to compare them with other verified collections. Eventually, field biologists may become familiar with the differences between these species allowing identification without using the model. A small percentage of individuals will not fit the key because of individual or clinal variation. Extra care must be taken with small fish because measurement errors will have a proportionately larger effect on the model predictions. All model predictions should be verified using supplemental characteristics such as the shape of the snout, shape of dorsal and anal fins, mouth position, maximum body size, and coloration patterns. This is especially important when identifying fish collected from areas of sympatry and when the model yields borderline predictions (i.e., $P \approx 0.50$). This key will be especially helpful for collections from northern Kansas and Missouri northwest to Montana and North Dakota where *H. hankinsoni* and *H. placitus* are sympatric in the upper Missouri, Platte, Republican, and upper Kansas river basins (Burr, 1980; Gilbert, 1980).

Hybognathus hankinsoni in eastern Colorado basins had smaller eyes than those from near the center of their distribution in Michigan. Wells (1978) identified two morphological groups of *H. hankinsoni*, a Great Lakes form and a Missouri River form, that he believed evolved in separate glacial refugia during the Wisconsin glaciation. The Great Lakes form is described as having a larger eye than the Missouri River form, but Wells (1978) did not think taxonomic distinction of the two forms was warranted. According to his hypothesis, the original model based on fish from Michigan describes the Great Lakes form, whereas the final model based on the Colorado fish describes the Missouri River form. Overall, the final model is most useful, because it can be used to separate the two species where they are sympatric in western Great Plains basins.

The different morphologies of *H. hankinsoni* and *H. placitus* are consistent with adaptations for their preferred habitats. *Hybognathus hankinsoni* prefer small, clear streams with low velocity (Copes, 1970). These conditions favor larger eyes, more prominent scales, and a more upturned ventral profile. A preference for smaller streams may also limit the maximum body size, and low flow velocity precludes the need for large pectoral fins. In contrast, *H. placitus* prefer medium to large plains rivers (Cross, 1967). Their smaller eyes and more embedded scales may adapt them for turbid water environments. The larger pectoral fins, flatter ventral profile, and larger maximum body size of *H. placitus* also better suit them to the flow conditions encountered in larger streams. The differences in form between *H. hankinsoni* and *H. placitus* also match the patterns of form in relation to zoogeographic dispersal pathways described by Metcalf (1966). He identified a typical body form for fish of northeastern origin, such as *H. hankinsoni*, characterized by a more fusiform body outline, nearly terminal and oblique mouth, and larger eyes. *Hybognathus placitus*, on the other hand, have a southwestern origin characterized by a decurved anterior dorsal surface, flat ventral surface, inferior, horizontal mouth, and smaller eyes and scales. These patterns fit our observations about the differences between the two species.

The current distribution of these species is believed to be contracting from the western edge of their former range (e.g., T. P. Nesler, R. VanBuren, J. Stafford, and M. Jones, Colorado Division of Wildlife, 1997, unpubl.; T. P. Nesler, C. Bennett, J. Melby, G. Dowler, and M. Jones, 1999, unpubl.; Patton, 1997). However, it is impossible to verify many recent accounts because no voucher specimens exist and the species may have been misidentified in the field. We stress the importance of collecting and preserving voucher specimens for laboratory identification so that precise measurements and comparative assessments of supplemental characters can be obtained. Additionally, properly curated museum specimens are critical for ecologists attempting to determine changes in distribution (Shaffer et al., 1998) and taxonomists resolving future taxonomic conundrums of fishes like *Hybognathus* in Colorado and surrounding states.

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APPENDIX 1

Material examined.—Museum lots are listed below according to their current museum classification. Institutional abbreviations are as listed in Leviton et al. (1985). Museum lots from the Biological Surveys Division of the U.S. Department of the Interior (BSFC) are deposited in the Biological Surveys Collections in the Museum of Southwestern Biology, Albuquerque, New Mexico.

Hybognathus hankinsoni, Colorado (CO): South Platte R.; CAS 100903 (n = 5); LFL uncataloged (n = 84), KU 4795 (n = 3), KU 4668 (n = 3); UMMZ 86895 (n = 1), BSFC 906 (n = 1), BSFC 1015 (n =

1), BSFC 1125 ($n = 1$), BSFC 1923 ($n = 2$), BSFC 2176 ($n = 12$), BSFC 2311 ($n = 2$), BSFC 2420 ($n = 6$), BSFC 2888 ($n = 13$), BSFC 2897 ($n = 2$), BSFC 2903 ($n = 8$), BSFC 2919 ($n = 12$), BSFC 2929 ($n = 11$), BSFC 2940 ($n = 15$), BSFC 2959 ($n = 5$), BSFC 2986 ($n = 4$), BSFC 2999 ($n = 1$), BSFC 3009 ($n = 6$), BSFC 3032 ($n = 2$), BSFC 3086 ($n = 2$), BSFC 3106 ($n = 1$), BSFC 3256 ($n = 169$), BSFC 3292 ($n = 53$), BSFC 3400 ($n = 6$), BSFC 3705 ($n = 6$), BSFC 3707 ($n = 5$); Lodgepole Ck.; UMMZ 66155 ($n = 4$); Lonetree Ck.; BSFC 3409 ($n = 17$); Cache la Poudre R.; BSFC 955 ($n = 11$), KU 4782 ($n = 32$), KU 5565 ($n = 27$); Spottlewood Ck.; BSFC 2617 ($n = 1$); Larimer-Weld Canal; MSB 4647 ($n = 3$); MSB 4806 ($n = 33$); St. Vrain Ck.; BSFC 853 ($n = 5$), BSFC 2114 ($n = 6$), BSFC 2395 ($n = 1$), BSFC 2409 ($n = 1$), BSFC 2754 ($n = 5$), BSFC 3410 ($n = 10$); Boulder Ck.; BSFC 1072 ($n = 1$), MSB 1164 ($n = 1$); UMMZ 66159 ($n = 5$); Buffalo Ck.; MSB 1112 ($n = 1$); Republican R.; UMMZ 66144 ($n = 5$); Kansas (KS): Republican R.; KU 17283 ($n = 6$), KU 4043 ($n = 1$); Smoky Hill R.; KU 3788 ($n = 4$); USNM 38237 ($n = 2$); South Fk. Smoky Hill R.; UMMZ 160450 ($n = 1$); Turtle Ck.; UMMZ 160462 ($n = 1$); Michigan (MI): Dead R.; UMMZ 84266 ($n = 1$); Rifle R.; UMMZ 229833 ($n = 28$); Tamarack Ck.; UMMZ 232820 ($n = 15$); Carp Lake R.; UMMZ 234967 ($n = 22$); Railroad Ck.; UMMZ 234993 ($n = 16$); Nebraska (NE): North Platte R.; KU 4848 ($n = 21$); ZM 2445 ($n = 6$); ZM 2446 ($n = 4$); ZM 2503 ($n = 1$); ZM 2627 ($n = 5$); ZM 5387 ($n = 1$); ZM 5993 ($n = 1$); ZM 6108 ($n = 6$); ZM 6114 ($n = 88$); ZM 6155 ($n = 38$); ZM 6164 ($n = 1$); ZM 7015 ($n = 9$); ZM 7120 ($n = 2$); UMMZ 134430 ($n = 1$); UMMZ 134452 ($n = 4$); Lonergran Ck.; UMMZ 134398 ($n = 7$); South Platte R.; UMMZ 134390 ($n = 4$); Lodgepole Ck.; KU 2013 ($n = 153$); ZM 6438 ($n = 3$); UMMZ 132241 ($n = 30$ of 319); UMMZ 135161 ($n = 41$); Republican R.; UMMZ 134361 ($n = 7$); Arikaree R.; KU 2680 ($n = 5$); Wyoming (WY): South Platte R.; UMMZ 114653 ($n = 9$); Muddy Ck.; UMMZ 162348 ($n = 8$); North Platte R.; BSFC 1218 ($n = 10$), UMMZ 104079 ($n = 3$); UMMZ 113500 ($n = 2$); UMMZ 134811 ($n = 5$); UMMZ 169127 ($n = 16$); Rawhide Ck.; UMMZ 104069 ($n = 5$); UMMZ 115020 ($n = 4$); Laramie R.; KU 4821 ($n = 3$), KU 4808 ($n = 3$).

Hybognathus placitus, Colorado (CO): South Platte R.; BSFC 3255 ($n = 5$), BSFC 3708 ($n = 1$); Republican R.; Arikaree R.; UMMZ 66144 ($n = 2$); Purgatoire R.; KU 4744 ($n = 2$); Arkansas R.; KU 4735 ($n = 31$); UMMZ 94934 ($n = 16$); Kansas (KS): Republican R.; UMMZ 122120 ($n = 1$); Smoky Hill R.; UMMZ 122144 ($n = 1$); UMMZ 160466 ($n = 9$); Arkansas R.; USNM 89 ($n = 3$); MCZ 1789 ($n = 1$); UMMZ 160439 ($n = 8$); Nebraska (NE): South Platte R.; UMMZ 134389 ($n = 7$); Republican R.; ZM 9311 ($n = 2$); ZM 9452 ($n = 7$); ZM 9578 ($n = 2$); UMMZ 134349 ($n = 32$); UMMZ 134360 ($n = 2$); UMMZ 134370 ($n = 1$); Red Willow Ck.; UMMZ 135109 ($n = 1$); Frenchman Ck.; UMMZ 135119 ($n = 57$); North Fork Republican R.; ZM 8699 ($n = 2$); Arikaree R.; UMMZ 135130 ($n = 30$ of 374); UMMZ 145017 ($n = 37$); North Platte R.; UMMZ 135249 ($n = 1$); New Mexico (NM): Canadian R.; LFL 43030 ($n = 51$); Wyoming (WY): North Platte R.; UMMZ 104061 ($n = 3$); UMMZ 134812 ($n = 2$).

Hybognathus nuchalis, Colorado (CO): South Platte R.; UCM 342 ($n = 9$); USNM 41721 ($n = 3$); Lodgepole Ck.; UCM 343 ($n = 34$); Saint Vrain R.; UCM 17 ($n = 1$); Boulder Ck.; CAS 68226 ($n = 3$); UCM 24 ($n = 3$); UCM 344 ($n = 27$); UCM 6266 ($n = 68$); UCM 6278 ($n = 38$); UCM 6878 ($n = 1$); Rock Ck.; UCM 6324 ($n = 7$); Bear Ck.; CAS 72788 ($n = 10$); North Fork Republican R.; UCM 345 ($n = 127$); Arikaree R.; UCM 6212 ($n = 38$); Black Wolf Ck.; UCM 6224 ($n = 1$); Arkansas R.; UCM 347 ($n = 2$); USNM 41708 ($n = 1$); Kansas (KS): Republican R.; USNM 249860 ($n = 1$).

Hybognathus argyritis, Colorado (CO): South Platte R.; BSFC 3180 ($n = 2$).