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Evaluating recent taxonomic changes for alligator snapping turtles (Testudines: Chelydridae)

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The Alligator Snapping Turtle (*Macrochelys temminckii* Troost in Harlan 1835, sensu lato) has been historically treated as a single, wide-ranging species, until a recently published paper by Thomas *et al.* (2014; hereafter Thomas *et al.*) analyzed variation in morphology and mitochondrial DNA sequence data to describe two new species of *Macrochelys*: the Apalachicola Alligator Snapping Turtle (*Macrochelys apalachicola* Thomas, Granatosky, Bourque, Krysko, Moler, Gamble, Suarez, Leone & Roman 2014) and the Suwannee Alligator Snapping Turtle (*Macrochelys suwanniensis* Thomas, Granatosky, Bourque, Krysko, Moler, Gamble, Suarez, Leone & Roman 2014). The specific epithet *temminckii* was retained for populations in drainages from the Yellow River in Alabama and Florida west to the San Antonio River, Texas. Because populations of *Macrochelys* have been historically exploited by humans (Pritchard 1989) and the life-history strategies of large, long-lived turtles make them susceptible to declines from harvest (Congdon *et al.* 1994), a sound understanding of species delimitation and richness is critical for the conservation of alligator snapping turtles, especially if the acceptance of a widely distributed species disguises the presence of multiple, smaller-ranged species.

In this correspondence, we review the population phylogenetic knowledge of *Macrochelys* and evaluate the morphological and molecular data presented to reclassify *M. temminckii* (sensu lato) as three species. We argue that the morphological analyses presented by Thomas *et al.* do not provide evidence differentiating *M. apalachicola* populations from *M. temminckii* (sensu stricto) and that this newly described species is not diagnosable. This stance is supported by a recently published analysis of cranial shape variation among *Macrochelys* populations (Murray *et al.* 2014). We also note that Thomas *et al.* do not provide evidence resolving nuclear-mitochondrial discordance from Echelle *et al.* (2010) that questions the reciprocal monophyly of *M. apalachicola* and *M. temminckii* (sensu stricto). Given the arguments presented here, we conclude with recommendations for a revised taxonomy and provide a key to the species of the genus *Macrochelys*.

Macrochelys phylogenetics

Geographic variation in *Macrochelys* morphology has been described among populations (e.g., number of supramarginal scutes, skull shape; Pritchard 1989), but populations have historically been treated as comprising a single, wide-ranging species. However, because other highly aquatic organisms in Gulf Coastal drainages exhibit patterns of drainage-specific endemism (e.g., *Graptemys*; Ennen *et al.* 2010), two studies in the last 16 years have explored population genetic structure of *Macrochelys* and systematic implications of that structure.

Roman *et al.* (1999; hereafter ‘Roman *et al.*’) sequenced two partial genes of the mitochondrial genome (tRNA^{PRO}, 5’ end of the control region) and found populations to exhibit drainage-specific haplotypes; a gene tree generated from these data recovered three major clades of *Macrochelys temminckii* (sensu lato): a western clade including populations from the Trinity River to the drainages of Pensacola Bay, a central clade from the Choctawhatchee River to the Ochlockonee River, and an eastern clade restricted to the Suwannee River. In this hypothesis, the Eastern (Suwannee) population (hereafter referred to as the Eastern (Suwannee) assemblage, for consistency with literature) was basal and sister to a well-supported monophyletic group comprising populations from the central and western distribution (hereafter, central and western assemblages, respectively). However, because mtDNA is maternally inherited and fails to detect male-mediated dispersal, Echelle *et al.* (2010; hereafter Echelle *et al.*) analyzed microsatellites from the nuclear genome to further test for population genetic structure, compare phylogeographic patterns between nuclear and mtDNA, and test for past population bottlenecks. Comparison of a neighbor-joining tree summarizing microsatellite variation (F_{ST}

values) and a parsimony tree summarizing mtDNA haplotypes presented generally similar relationships, except for the drainages of Pensacola Bay, which were described by microsatellite data as being so deeply divergent as to question their membership in either the central or western clades of Roman *et al.* Both Roman *et al.* and Echelle *et al.* suggested low dispersal among drainages, high population structure among drainages, and potential for cryptic species within *Macrochelys*, but, in the absence of a thorough morphological investigation and because of nuclear-mitochondrial discordance, no taxonomic changes were made.

Morphological analyses do not differentiate all new species

Thomas *et al.* emphasized that, in an attempt to provide robust diagnoses for each species, a strong morphological analysis was needed to test the three-clade hypothesis of Roman *et al.* For their morphological analyses, the authors measured four gross morphological variables (caudal notch depth, caudal notch width, caudal notch area, and squamosal angle) and compared these measures among the three genetic assemblages from Roman *et al.* Analysis of covariance (ANCOVA) was used to test for differences in the caudal notch variables, using carapace length to adjust for differences in overall body size. For the squamosal angle, analysis of variance (ANOVA) was used to test for differences among groups, rather than ANCOVA, presumably because regression analysis did not find skull size to influence squamosal angle. For each ANCOVA and ANOVA, results indicated significant variation among groups.

While these approaches are important to test for variation among hypothesized groups, the authors also tested for morphological differences between the three hypothesized groups by comparing the mean values and standard error of the mean in post-hoc analyses. These tests, embedded in Table 1 of Thomas *et al.* (p. 148), consistently document the eastern (Suwannee) assemblage as distinct from the other two assemblages for mean values of all four variables. Indeed, a visual examination of Thomas *et al.*'s Figure 4 indicates that the statistical distribution of variables for the eastern (Suwannee) assemblage is mostly non-overlapping and distinct relative to those of the other groups (e.g., Thomas *et al.*: Figure 4B–D in particular). Thus, since all four variables differed from the other hypothesized groups, the results provide support for separation of the Suwannee population as a distinct and diagnosable taxon.

Comparison of mean values between the western and central assemblages suggested less differentiation, however. Significant differences in pairwise comparisons of mean values were only recovered for two of the four variables, and the statistical distribution of variables described considerable overlap in those comparisons (Thomas *et al.*: Figure 4). In general, this figure suggests that the uniqueness of Suwannee populations generated the significant among-group results in ANCOVA and ANOVA, and, when considered with the post-hoc analyses, the results largely suggest that the western and central assemblages overlap in morphological variation.

It is important to note that the univariate statistics are based on standard error of the mean. This statistic tests for differences in population means, but not for the confidence with which individuals measured for each variable can be correctly classified to a population. However, the authors also used a principal components analysis (PCA) “to explore whether carapace morphology could be used to distinguish individuals from three genetic assemblages” (Thomas *et al.*, p. 143). The first and second axes of the PCA (Thomas *et al.*: Figure 8) described variation in the eastern (Suwannee) assemblage to be largely distinct from the other two assemblages, but also described approximately half of morphospace overlap as shared between the central and western assemblages. While it is difficult to evaluate how interpretation of this analysis may support or falsify objectives of the paper in the absence of a *P* value, we suggest that the great overlap in variation between the central and western assemblages similarly fails to describe diagnosable differences between those groups.

While the univariate analyses and PCA are classic statistical tools for describing variation, if a goal of the analyses was to test the degree to which individuals could be correctly identified to group membership, a more appropriate multivariate approach could have been to use a discriminant function analysis. This analysis generates functions that describe variables in multidimensional space, similar to PCA, but goes beyond by quantifying the degree to which continuous variables can be used to identify individuals to hypothesized groups (Tabachnick & Fidell 2001). This approach can identify combinations of values for continuous variables that are diagnostic (by resulting in correct classification for 95% of unknown individuals) for each putative taxon, which has practical applications for systematists attempting to measure diagnosability in a taxonomic framework (e.g., Ennen *et al.* 2010) or even diagnose species in dichotomous keys.

Thomas *et al.* assert that differences in caudal notch shape among the assemblages are “easily observed in both living and preserved specimens” (p. 146). While caudal notch shape works well for diagnosing *M. suwanniensis* relative to all other specimens, the assertion of easily observable differences does not appear to be universally true for the western and central assemblages, as no dichotomous key was provided of characters diagnosing these two lineages. The authors later admit that central and western populations are difficult to diagnose by stating: “[...] *M. temminckii* and *M.*

apalachicolae have narrow, triangular or U-shaped caudal notches that, although statistically different, are more difficult to differentiate from each other” (p. 161). We conclude that data presented for the caudal notch do not support statistical differentiation between *M. apalachicolae* and *M. temminckii*, as two of three tests failed to find significant variation in that comparison. For these reasons, we suggest that the morphological analyses used to diagnose *M. apalachicolae* as a distinct taxon from *M. temminckii* (*sensu stricto*) are insufficient.

New morphological data also fail to diagnose *M. apalachicolae*

Since the publication of Thomas *et al.*, a second study (Murray *et al.* 2014) has provided new perspective on morphological variation of *Macrochelys* by analyzing cranial shape. Specifically, Murray *et al.* (2014) compiled a dataset of 81 homologous landmarks on the dorsal, lateral, and ventral surfaces of the skull, including the squamosal angle character used by Thomas *et al.*, and applied geometric morphometric methods to test whether cranial shape of Roman *et al.*'s hypothesized clades is more similar within than among clades. Their results found the eastern (Suwannee) assemblage to possess wide, robust skulls, which contrasted starkly with elongate and gracile morphologies of western populations. Central (Apalachicola) individuals were intermediate on the continuum of robust to gracile, but were characterized by greater variation that often overlapped with the other hypothesized clades. In general, however, distinctive variation in cranial shape supported the recognition of the eastern (Suwannee) assemblage as a diagnosable species but failed to distinguish between western and central assemblages, and those authors cautioned against further taxonomic revision beyond *M. suwanniensis* (Murray *et al.* 2014).

New phylogenetic analysis

Thomas *et al.* re-analyzed the sequence data from Roman *et al.* using a Bayesian-inference approach and generated a phylogeny with similar topology as that of Roman *et al.*; this was not surprising, given that the data sets were identical. What is surprising is that the authors did not obtain sequence data from more mitochondrial and/or nuclear loci to confirm the topology of their gene tree. It is well known that single gene trees can describe misleading relationships among populations or species, particularly for mtDNA (e.g., turtles, Wiens *et al.* 2010), and, in the present case, more loci would have served to strengthen the test of Roman *et al.*'s hypothesis by decreasing the probability of type I error.

What is also surprising was a failure to acknowledge the conflicting results between mitochondrial and nuclear data documented by Echelle *et al.* That study raises questions about the content of the western clade because it recovered specimens from Pensacola Bay drainages as being so divergent to question their membership with either central or other western populations. For this reason, we suggest that the nuclear data question the allocation of *Macrochelys* from the Ochlockonee River westward into two lineages demonstrating reciprocal monophyly. Reciprocal monophyly is often an important criterion when using genetic data to delimit species (Wiens & Penkrot 2002), but Thomas *et al.* do not convincingly resolve the problematic history of the western clade identified by Echelle *et al.* Therefore, for these reasons, we suggest the phylogenetic analysis by Thomas *et al.* does not represent inspiring new evidence describing Roman *et al.*'s western and central assemblages as distinct monophyletic lineages.

Conclusions and recommendations

In the technical process of species description, the diagnosis is a critical component because it illustrates unique characters that distinguish species from closely related taxa. We suggest that the primary diagnostic characters quantified and illustrated by Thomas *et al.* succeeded in separating *M. suwanniensis* as being morphologically and genetically distinct from all other members of the genus. Of the remaining specimens, we argue that these authors failed to diagnose the central assemblage as being morphologically distinct from the western assemblage and failed to explain away nuclear data from Echelle *et al.* that question the content of the western lineage. Instead, accumulating morphological and genetic data are most consistent with a pattern of drainage-specific divergence for the central and western clades (Echelle *et al.*, Murray *et al.* 2014). Although the genetic data are consistent in describing limited gene flow between adjacent drainages, they do not necessarily eliminate the possibility of rare dispersal events. Observations of barnacles growing on shells of *Macrochelys* in coastal regions (Mount 1975) have been used to imply sufficient salt tolerance to make such dispersal possible, and microsatellite data suggested recent gene flow from the Pensacola to Apalachicola (Echelle *et al.*). Such dispersal and gene flow would serve to maintain species cohesion between central and western populations, while the geographic isolation of *M. suwanniensis* would limit dispersal and promote divergence (Thomas *et al.*).

In conclusion, until data are presented to address the concerns presented here, we recommend that two species of

alligator snapping turtles should be recognized: the Alligator Snapping Turtle (*Macrochelys temminckii* Troost in Harlan 1835), a broadly-distributed taxon in Gulf Coastal drainages from the San Antonio River east to the Ochlockonee River, and the Suwannee Alligator Snapping Turtle (*Macrochelys suwanniensis* Thomas *et al.* 2014), a narrow-ranged endemic taxon in the Suwannee River. Given the perspective presented here, we provide a key to the species of *Macrochelys*:

Key to the species of the genus *Macrochelys*

- 1a. Distance between distal tips of right and left 12th marginal scutes wider than distance between distal tips of 11th and 12th marginal scutes on either side *Macrochelys suwanniensis*
- 1b. Distance between distal tips of right and left 12th marginal scutes equal to or narrower than distance between distal tips of 11th and 12th marginal scutes on either side *Macrochelys temminckii*

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