SALAMANDRA	41	1/2	21-26	Rheinbach, 20 May 2005	ISSN 0036-3375

On the hybridisation between two distantly related Asian turtles (Testudines: *Sacalia* × *Mauremys*)

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Abstract. This is the first report of a hybridisation between *Sacalia* and *Mauremys* (Bataguridae). New data and a review of the literature show that 19 batagurid hybridisations are documented. Many more undoubtedly exist, but have not been documented and reported. Most hybrids are members of the *Cuora* + *Mauremys* clade and 17 of 19 reported hybrids have at least one member from this clade. The *Sacalia* × *Mauremys* hybridisation reported here is only the third hybridisation between a species of the *Cuora* + *Mauremys* clade and a species outside of that clade.

Key words. Bataguridae: *Mauremys*, *Sacalia*; hybrids.

Introduction

Hybrids of batagurid (Bataguridae = Geoemydidae; see JOYCE et al. 2004) turtles were first reported from Japan where researchers discovered the propensity for distantly related species to breed in the wild and in captivity (AOKI 1990, YASUKAWA et al. 1992, OTANI 1995a, b). Since that time, several other batagurid hybrids have been identified (FRITZ 1995, WINK et al. 2001, PARHAM & SHI 2001, PARHAM et al. 2001, SHI & PARHAM 2001, FRITZ & MENDAU 2002, GALGON & FRITZ 2002, SCHILDE et al. 2004, SPINKS et al. 2004), some of which match species described from the Hong Kong pet trade (WINK et al. 2001, PARHAM et al. 2001, SPINKS et al. 2004). New hypotheses of batagurid evolutionary relationships (BARTH et al. 2004, SPINKS et al. 2004) allow us to compare these hybridisation events in a phylogenetic context. Most hybrids occur within or between the genera *Cuora* and *Mauremys*. We use the latter genus to include *Chinemys* and *Ocadia* because multiple groups of workers have discovered that *Chinemys* and *Ocadia* are nested within traditional *Mauremys* (FELDMAN & PARHAM 2004, BARTH et al. 2004, SPINKS et al. 2004). BARTH et al. (2004) correctly point out that two possible solutions (lumping or splitting) can provide a monophyletic taxonomy. We support and use the "lumping" solution following the arguments of FELDMAN & PARHAM (2002, 2004), PARHAM & FELDMAN (2002), and SPINKS et al. (2004). The hybrids reported here are between two distantly related batagurid species, *Sacalia quadriocellata* and *Mauremys reevesii*. We take this opportunity to review all documented batagurid hybrids.

Materials and Methods

The hybrid turtles reported here were hatched by a private breeder in California, USA, in August of 2000. Of approximately 300 terrestrial, semi-aquatic, and aquatic turtles kept by this breeder, the majority live indoors in large aquaria, cattle troughs, or custom-made enclosures. Among the latter are several water-tight plexiglass enclosures measuring 60 × 60 × 240 cm. The hybrids were created in one of these custom enclosures filled with water to a depth of approximately 15 cm. In the spring of 2000, this enclosure included the following turtles (all of unknown origin, purchased via the pet trade): six *Mauremys reevesii* (four females),

five *Sacalia quadriocellata* (three females), and four *Mauremys caspica caspica* (two females). Prior to 2000, only the *M. reevesii* had successfully reproduced under the breeder's care. The breeder assumed genetic incompatibility among the species and no aggressive behaviour was noted. However, the breeder noticed that the male *S. quadriocellata* exhibited rather indiscriminate sexual activity.

For nesting purposes, as well as for aerial basking, a rubberised plastic tub measuring about 60 × 45 × 12 cm was kept filled with sand and mulch, reached by a ramp from the water below. Concrete blocks at the opposite end provided additional basking areas. The breeder did not witness oviposition by any turtle during the spring of 2000 but discovered two clutches, consisting of four and five elongated eggs, in June. These eggs resembled those of *M. reevesii* yet were more laterally compressed and slightly less oval.

The eggs hatched after incubating in moist vermiculite (1.5 parts water to 1 part vermiculite) at a temperature of 28 °C after 70-75 days. The hatchlings did not resemble any neonate turtles previously seen by the breeder nor JRB. Because the neonates had head spots as in *Sacalia*, but did not match *Sacalia* in other features, they were recognised as hybrids with either *M. caspica* or *M. reevesii*.

The nine hybrids have since been dispersed to several collections. Three died of natural causes and were preserved in the Museum of Vertebrate Zoology (MVZ), Berkeley, California, USA. Their specimen numbers are MVZ 241500, 241501, and 241502. In Autumn 2003, one of the remaining six specimens escaped from a private collection and is apparently lost forever. At the time of this writing, the remaining five are still alive in private collections in the following United States cities: Conyers, Georgia (JODY KARLIN, n=2); Oakland, California (JRB, n=1); Berkeley, California (JFP, n=1); Davis, California (PHIL SPINKS, n=1).

In order to determine the maternal parentage of the clutches, we sequenced a 700 bp fragment of the mitochondrial gene COI and a 900 bp fragment of the mtDNA gene ND4 and linked tRNAs from specimen MVZ 241502 and compared it to the larger data set that includes every valid species of *Mauremys* (FELDMAN & PARHAM 2004; see also for sequencing methods).

Results

Molecular data

The sequence data of MVZ 241502 (Genbank AY562183; AY562185) are almost identical (< 0.6 % sequence divergence) to those from a pet trade specimen of *Mauremys reevesii* (MVZ 230533; Genbank AF 348263, AF348288), but are distinct from all other *Mauremys* (5.6 % different from *M. caspica*). Thus, the DNA data confirm that one (or more) of the female *Mauremys reevesii* in the enclosure, not the *Mauremys caspica*, laid the nine eggs. The presence of "eye spots" on the back of the head (Fig. 1) and other *Sacalia* features (see below) implicate that the father was one of the aforementioned *Sacalia quadriocellata* that exhibited indiscriminant sexual activity.

Description of hybrids

The light brown carapace of the hybrids is heavily marked with tiny black dots and the periphery is highlighted by a pale streak on the outermost edges of most or all marginal scutes. The plastron has a symmetrical or subsymmetrical, cloudy, dark, central figure, the peripheral third of the plastron scutes are whitish (Fig. 1). The overall shape of the neonates is oval, not subcircular as seen in many hatchling turtles. The soft parts are predominantly grey, especially the head. The flexor surfaces of the limbs are pale, and the chin is heavily streaked. Some of the forelimb scales as well as tiny tubercles on the thighs are partly or completely whitish.

Behind the eye is a prominent yellow spot, or sometimes paired spots, the smaller

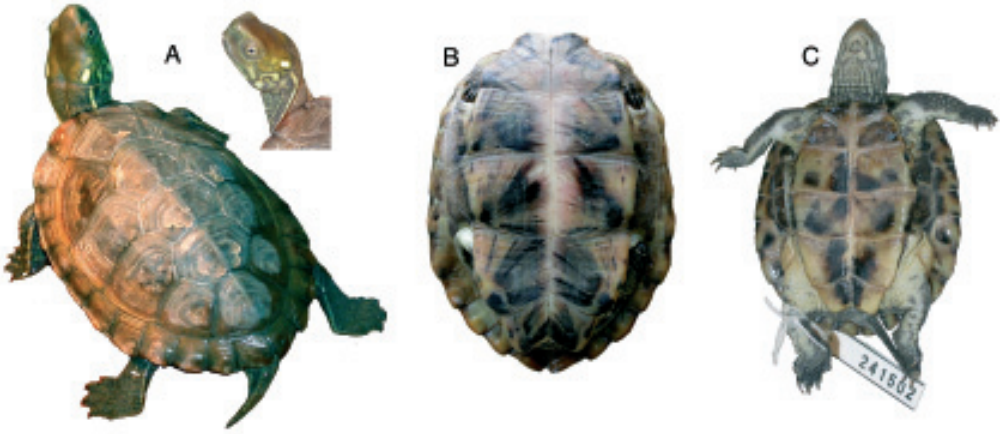

Fig. 1. *Sacalia quadriocellata* × *Mauremys reevesii* hybrids. A) Living specimen in the care of JFP showing head coloration including detail of "eye spots" and malar region; B) same specimen in ventral view; C) The specimen sequenced in this study (MVZ 241502) in ventral view.

lying between the eye itself and the larger temporal spot (Fig. 1). These "eye spots" are a diagnostic feature of *Sacalia*. On the top of the head a yellow stripe extends from the occiput toward the nuchal region, flanked by a similar stripe on each of the temporal neck folds which did not quite reach the larger temporal spot itself. Beneath these, a poorly defined reticulate assemblage of yellowish squiggles, some edged in black, adorn the malar region.

The "eye spots" on the back of the head clearly indicate that one of the parents must have been a *Sacalia*. Although the tricarinate carapace, long tail, and pale iris are reminiscent of neonate *M. reevesii* (Fig. 1), the overall shape of the neonates is less robust and more flattened. In this respect, the hybrids match the *Sacalia* neonates (Fig. 1). The predominantly greyish soft parts of the hybrids, particularly the head, are highly distinct from those of a neonate *M. reevesii*. Similarly, the chin of the hybrids is heavily rather than sparsely streaked as in *M. reevesii*. Furthermore, the highly variable malar pattern in neonate *M. reevesii* is never as prominent or "garish" as in the hybrids.

Review of batagurid hybridisations

Nineteen hybridisation events are documented for batagurid turtles, most from captivity. The authors have seen others in private collections (see Discussion) or in Chinese turtle farms. However, in some cases the parental species can only be inferred. Our list is restricted to previously reported hybrids from the scientific literature and our study. The hybrids reported by Aoki (1990), Fritz (1995), Fritz & Mendau (2002), Fritz & Wischuf (1997), Galgon & Fritz (2002), Otani (1995a, b), and Stemmler (1973) are not verified by genetic data. Where known, the paternal species is listed first, maternal species second. In some cases, we do not know the specific identity of the maternal versus paternal lineages. We mark these hybridisations with an asterisk (*).

a) Within *Cuora* (n=2)

Cuora mouhotii × *Cuora bourreti* ("*Cuora serrata*" – Parham et al. 2001, Stuart & Parham 2004). Origin: unknown, but probably wild.

Cuora mouhotii × *Cuora galbinifrons* ("*Cuora serrata*" – PARHAM et al. 2001, STUART & PARHAM 2004). Origin: unknown, but probably wild (see SHI et al. in press).

b) Within *Mauremys* (n=7)
Mauremys caspica × *Mauremys rivulata** (STEMMLER 1973, FRITZ & WISCHUF 1997).
Mauremys japonica × *Mauremys reevesii** (OTANI 1995a). Origin: captivity.
Mauremys japonica × *Mauremys sinensis** (OTANI 1995a). Origin: captivity.
Mauremys reevesii × *Mauremys japonica** (AOKI 1990; presented in greater detail by YASUKAWA et al. 1992). Origin: captivity.
Mauremys reevesii × *Mauremys mutica* ("*Mauremys pritchardi*" – WINK et al. 2001). Origin: unknown.
Mauremys reevesii × *Mauremys sinensis** (OTANI 1995a). Origin: captivity.
Mauremys sinensis × *Mauremys annamensis* ("*Ocadia glyphistoma*" – SPINKS et al. 2004). Origin: unknown.

c) Between *Cuora* and *Mauremys* (n=5)
Cuora amboinensis × *Mauremys annamensis** (FRITZ & MENDAU 2002). Origin: captivity.
Cuora trifasciata × *Mauremys mutica* (cf. "*Mauremys iversoni*" – PARHAM et al. 2001). Origin: captivity.
Mauremys japonica × *Cuora flavomarginata** (OTANI 1995a). Origin: captivity.
Mauremys mutica × *Cuora trifasciata* ("*Mauremys iversoni*" – WINK et al. 2001). Origin: unknown.
Mauremys reevesii × *Cuora amboinensis* (GALGON & FRITZ 2002). Origin: captivity.

d) One parental species outside of *Cuora* + *Mauremys* clade (n=3)
Cuora flavomarginata × *Geoemyda japonica* (OTANI 1995a, b). Origin: wild and captivity.
Cyclemys shanensis × *Mauremys sinensis* (SCHILDE et al. 2004). Origin: captivity.
Sacalia quadriocellata × *Mauremys reevesii* (this study). Origin: captivity.

e) Both parental species outside of *Cuora* + *Mauremys* clade (n=2)
Rhinoclemmys punctularia × *Rhinoclemmys diademata** (FRITZ 1995). Origin: captivity.
Rhinoclemmys punctularia × *Rhinoclemmys melanosterna** (FRITZ 1995). Origin: captivity.

Discussion

The compiled list is incomplete because most cases of batagurid hybrids are not reported in the literature. We predict that additional cases and parental combinations will be discovered. Our list, incomplete as it is, still allows us to see some patterns. For example, *Mauremys reevesii* is involved in nearly a third (n=6) of the 19 hybridisations. We do not suggest there is something special about *M. reevesii* among batagurids. Instead, we hypothesise that *M. reevesii* hybrids are most common because *M. reevesii* is one of the most widely reared and bred batagurid turtles. In Japan and China it has been kept along with a diversity of other local batagurids (OTANI 1995a, SHI & PARHAM 2001). Consequently it has had the chance to hybridise with more batagurids than other turtles. *Sacalia* species are not as common in captivity and so captive-produced hybrids are correspondingly rare.

Fourteen of the 19 known batagurid hybrids are restricted to the *Mauremys* + *Cuora* clade. The hybridisation of *Sacalia* and *Mauremys* is important because *Sacalia* is far removed from the *Cuora* + *Mauremys* clade (BARTH et al. 2004, SPINKS et al. 2004). This is the first documentation of a hybridisation involving *Sacalia*. Some authors (SHI & PARHAM 2001, PARHAM & SHI 2001, PARHAM et al. 2001) have suggested that *Sacalia pseudocellata* may be a hybrid between *Sacalia* and *Cuora trifasciata*. Molecular data remain ambiguous on this point (SPINKS et al. 2004), but the hybrids reported here demonstrate that *Sacalia* can hybridise with a member of the *Mauremys* + *Cuora* clade.

The hybridisation reported here is significant in light of the large phylogenetic distance separating *Sacalia* and *Mauremys* (BARTH et al. 2004, SPINKS et al. 2004). In this respect, the *Sacalia* × *Mauremys* hybrid is as significant as the *Mauremys* × *Cyclemys* hybrid reported by SCHILDE et al. (2004). The most distant batagurid union occurred between a male *Cuora flavomarginata* and a female *Geoemyda japonica* (OTANI 1995a, b). According to BARTH et al. (2004) and SPINKS et al. (2004), *Geoemyda* is more distantly related to the *Mauremys* + *Cuora* clade than is either *Sacalia* or *Cyclemys*.

Acknowledgements

JRB would like to thank KINJI HAYASHI, MARK BARDOEL, and JODY KARLIN. This work was funded by an ANNIE ALEXANDER fellowship to JFP. MIKE PFRENDER and PAUL WOLF (Utah State University) provided lab space for CRF. This is UCMP contribution 1846 and LBNL-54656 and was performed under the auspices of the U.S. Department of Energy, Office of Biological and Environmental Research.

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Manuscript received: 24 April 2004

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