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REPORT

Bellinger River Turtles: Assessment of genetic diversity and hybridization in a species under threat

Report prepared for the Ecosystems and Threatened Species Unit, Office of Environment and Heritage, PO Box A290, Sydney South, NSW 1232

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Bellinger River Turtles: Assessment of genetic diversity and hybridization in a species under threat

Agency: NSW Ecosystems and Threatened Species Unit

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Brief

The aims of this project were to undertake genetic analysis of captive specimens of *Myuchelys georgesii* to determine if

- (a) the genetic variability among captive individuals is representative of the variability of the population as a whole;
- (b) there are any hybrids between *Myuchelys georgesii* and the introduced *Emydura macquarii*; and
- (c) the Kalang population is a suitable insurance colony should the Bellinger population of *Myuchelys georgesii* become threatened with extinction.

Recommendations

1. Strong evidence of hybridization between the endemic *Myuchelys georgesii* and the introduced *Emydura macquarii* requires reconsideration of the management of the turtle populations in the Bellinger and Kalang Rivers, with particular consideration given to options for managing the impact of hybridization and introgression on the integrity of *Myuchelys georgesii* as a species.
2. The captive individuals show genetic variability representative of that found in the native Bellinger population. Captive female individual AA70217 (Kalang) should be removed from the breeding program as strong evidence points to it being an F2 hybrid between *Myuchelys georgesii* and *Emydura macquarii*.
3. The Kalang population of *Myuchelys georgesii* is not considered suitable as an insurance colony to minimize the risk of extinction should the disease in the Bellinger River lead to local extirpation there. This is because of the high level hybridization and introgression demonstrated in individuals collected from the Kalang.
4. If funding permits, additional plates of specimens including all samples of both species from the Kalang River, additional samples of both species from the Bellinger, and samples

from the suspected source populations of *Emydura macquarii* introductions, should be processed to better quantify the frequency and extent of introgression.

5. Further analyses should be undertaken to bring these findings up to a standard suitable for publication in the peer reviewed literature, as an exemplary example of the consequences of human-assisted dispersal of native fauna outside their historical range.

Background to the Aims

The Bellinger River, and the associated Kalang Creek, is a small isolated drainage arising in the Dorrigo Plateau and discharging via the coast of NSW. It is home to three species of freshwater turtle – the endemic Bellinger River *Myuchelys georgesii*, the Eastern Long-necked Turtle *Chelodina longicollis* and the Southern Shortneck River Turtle *Emydura macquarii*. *Chelodina longicollis* is rarely encountered in the Bellinger catchment.

We would prefer to restrict the common name of Snapping Turtle to the species in the genus *Elseya*, and restrict the common name of SawShell Turtle to species in the genus *Myuchelys*, but to be consistent with other documents on the disease outbreak and public media releases, we refer to *Myuchelys georgesii* as the Bellinger Snapping Turtle.

The Bellinger River Snapping Turtle

Myuchelys georgesii was for many years regarded as a distinct but undescribed species restricted to the Bellinger and Manning rivers of coastal NSW (Legler, 1981). Its biology has been reviewed by Cann et al. (2015). Examination of species boundaries and phylogeny using allozyme electrophoresis demonstrated that the Bellinger and Manning populations were very distinctive despite their cryptic morphological distinction (Georges & Adams, 1996), and that they were not sibling species (Georges & Adams, 1992). *Myuchelys latisternum* and *M. belli* were among the descendent species of the most recent common ancestor of what is now known as *M. georgesii* (from the Bellinger, Cann, 1997) and *M. purvisi* (from the Manning River, named by Wells & Wellington, 1985, but as yet lacking an adequate description).

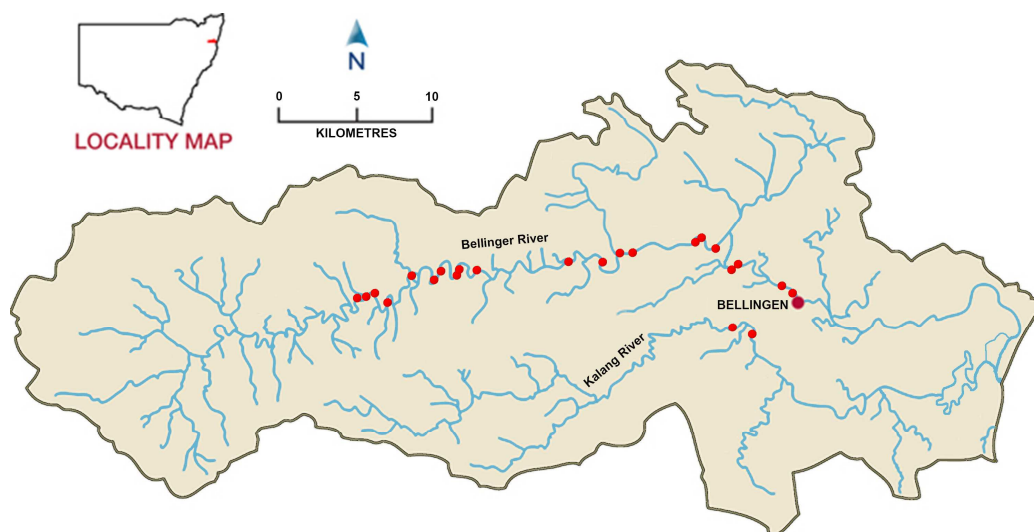


Figure 4. Distribution of *Myuchelys georgesii*.

The genus *Myuchelys* was erected to reflect the definitive results of the allozyme studies to include the saw-shelled turtles – the Eastern SawShell Turtle *Myuchelys latisternum*, the Western SawShell Turtle *Myuchelys bellii*, the Manning River *Myuchelys purvisi* (but see Le et al., 2013; Spinks, Georges & Shaffer, 2015), and the Bellinger Snapping Turtle *Myuchelys georgesi*. *Myuchelys georgesi* is vulnerable by virtue of its extremely restricted distribution (Georges, 1994, Figure 1), though this has not been reflected in formal lists of threatened species at state or federal level.

A catastrophic recent die-off of *M. georgesi* in the Bellinger River has been reported, causes as yet unknown. On February 18th 2015, distressed and dead turtles were found by canoeists in the Bellinger River. Mortality rate of infected animals is 100%. The spread of the disease (both up and downstream) has been rapid and at least 95% of the species range is now affected. Currently, the cause of the disease is unknown despite extensive research, although affected turtles present with acute, sudden and inflammatory lesions, which is most consistent with the presence of an infectious or parasitic agent (NSW_DPI, 2015).

An insurance colony of individuals collected from an as yet unaffected area has been established at the University of Western Sydney (18 animals) working with limited information available on the captive husbandry of the species. ***It is important to know that the captive individuals are representative of the genetic diversity of the natural population.*** The insurance plan is to breed from these individuals and head-start the hatchlings for release.

This incident has brought focus and urgency to the plight of the endemic Bellinger Snapper.

The Bellinger River *Emydura*

Emydura macquarii from the Bellinger was listed as vulnerable in the Reptile Action Plan (Cogger et al., 1993) as *Emydura signata*, vulnerable in the Schedules of the Threatened Species Act, New South Wales (NSW_NPWS, 2001) as *E. macquarii*, Bellinger River Form, and vulnerable in the national EPBC Act (Commonwealth of Australia 1999) as *E. macquarii signata* (Bellinger River, NSW). This 'species' of turtle was used as a flagship to marshal community support for a range of initiatives in riparian and riverine restoration and invasive fox control (NSW_NPWS, 2001).

For a time, the question of whether the Bellinger River *Emydura* is a distinctive taxon or an unremarkable representative of the more widespread *E. macquarii macquarii* (sensu Georges & Thomson, 2010) remained unanswered. Discovered in 1990 in a single waterhole in the middle reaches of the Bellinger River by Peter King as part of broader studies (King & Heatwole, 1994b; King & Heatwole, 1994a) and again at the same location in 1992 by Cann (1998), captures of the Bellinger *Emydura* were initially extremely rare. Recent surveys funded by the NSW Government have shown progressive increases in abundance and distribution in the Bellinger River, to the point where the turtles are now widespread and common in the drainage. Examination of mitochondrial DNA markers revealed haplotypes in common with those of the Clarence and MacLeay rivers, and from the vicinity of the township of Coffs Harbour (Georges et al., 2011). The distribution of some of these haplotypes was patchy and coincided with regions of known accidental releases of *Emydura* to the river. The *Emydura* has been introduced to the Bellinger

catchment, and indeed, is an unremarkable population of a common and widespread species, *Emydura macquarii* (Georges et al., 2011).

Risks Posed by the Introduced *Emydura*

The introduction of *Emydura macquarii* to the Bellinger River can have several consequences for the endemic *Myuchelys georgesii*. First, their diets overlap, *Emydura macquarii* being more catholic in its diet than *Myuchelys georgesii* (Allanson & Georges, 1999; Spencer et al., 2014), which opens the possibility that they will compete for scarce resources in this oligotrophic river. Second, hybridization between chelid turtle species is widespread in the wild (Georges & Adams, 1996; Georges, Adams & McCord, 2002) even between species in different genera. ***The possibility that Emydura macquarii and Myuchelys georgesii could interbreed, compromising the integrity of the endemic M. georgesii is of considerable concern***, but could not be tested with mitochondrial data alone. Multiple independent nuclear DNA markers are required to address this issue. ***It is important to ensure that no hybrids between M. georgesii and the introduced Emydura macquarii are among the captives in the insurance colony.***

Material Examined

A total of 94 individuals were available for examination. Thirteen *Emydura macquarii* and 59 *Myuchelys georgesii* were collected from the Bellinger and Kalang Rivers as part of a survey published elsewhere (Georges et al., 2011). Tissue samples were collected from these individuals by removing a portion of the trailing webbing of the clawless toe on the rear foot. The samples were preserved and stored in 75% ethanol, and held at the University of Canberra in the UC Wildlife Tissue Collection (UC <Aus>, <http://iae.canberra.edu.au/cgi-bin/locations.cgi>) before being subsampled for this study.

An additional 18 heparinized blood samples were taken from *Myuchelys georgesii* being held as an insurance colony at the University of Western Sydney (TZ01 to TZ18 = AA70200 to AA70217). They were shipped to Canberra by courier, and immediately subsampled for analysis.

Methods

A total of 94 individuals (one plate) were screened at low coverage for polymorphic single nucleotide polymorphisms using DArT Seq (double-digest restriction fragment sequencing). DNA samples were extracted and processed in digestion/ligation reactions as described by Kilian et al. (2012). The resultant fastQ formatted sequences were processed using proprietary DArT analytical pipelines. In summary, the primary pipeline filters poor quality sequences while simultaneously applying more stringent selection criteria to the barcode region, ensuring the reliable assignment of sequences to specific samples, and then collapses identical sequences into “fastqcall” files. These are used in the secondary pipeline for DArT proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14).

Analyses

The data were converted to a matrix of SNP loci by individuals, with the contents stored as integers 0 = homozygote, reference state; 1 = heterozygote; and 2 = homozygote for the SNP state. The most common SNP state was taken as reference. The resultant matrix, suitably coloured, could be examined visually.

As a summary of genetic distances between individuals, a Principal Coordinates Analysis (PCoA) (Gower, 1966) was applied to the individuals (entities) by loci (attributes) matrix to yield a 2 dimensional ordinated space in which the individuals were plotted. The PCoA was implemented from R package [ape] using a Gower distance matrix across populations calculated using R package [vegan].

Finally, the data were analysed with software package NewHybrids (Anderson & Thompson, 2002) to identify individuals representing F1 and F2 hybrids and backcrosses with one or the other parental species. Strictly, this software identifies F1 hybrids and groups gene frequencies into classes representing different levels of hybridization (F2) and backcrosses, some of which can overlap. In the case of the Bellinger turtles, where the introduction of the *Emydura* is relatively recent, the interpretation of the F1, F2 and associated backcrosses is likely to be unambiguous.

Results

A total of 32,569 polymorphic SNP loci identified for 93 individuals, comprising 59 wild caught Bellinger *Myuchelys georgesii*, 13 Bellinger *Emydura macquarii*, 4 wild caught Kalang *M. georgesii* and 17 captive *M. georgesii*. DNA from one captive individual (TZ_14 = AA70213) failed to yield useable data. Additional blood specimens need to be obtained from this sample.

Software package NewHybrids assigned most individuals to one or the other parental populations when searching for F1 and F2 hybrids and associated backcrosses (Attachment A). In addition, three of the individuals from the Kalang River suspected on a priori superficial morphological examination to be hybrids, were identified as F2 hybrids (Table 1).

Table 1. Hybrid individuals identified among the samples tested using software package NewHybrids (Anderson & Thompson, 2002).

	Parental Mygeo	Parental Emmac	F1 hybrid	F2 hybrid	Back cross to Mygeo	Back cross to Emmac
AA36212	0.0005	0.0000	0.0000	0.0002	0.0000	0.9993
AA48061	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
AA48159	0.0001	0.0000	0.0000	0.9998	0.0000	0.0001
AA48175	0.0001	0.0000	0.0000	0.9999	0.0000	0.0000
AA70217	0.0001	0.0000	0.0000	0.9999	0.0000	0.0000

In addition, one of the animals retained in the insurance colony (TZ_18 = AA70217, Kalang River, Table 1) is an F2 hybrid, and needs to be eliminated from the breeding program. A specimen was also identified as a backcross of an F1 hybrid to the parental species *Emydura macquarii* (Table 1).

The NewHybrids program was unable to utilize the 32,569 specimens screened, in that software capability lags behind our ability to generate data. Other evidence of introgression was obtained on visual examination of the SNP dataset. The first 100 loci are presented in Attachment 2 for illustration. SNP state 0 (homozygous for reference state) is shown in black, SNP state 2 (homozygote for SNP state) in white, missing values in blue, and heterozygotes in pink. Missing values, arising by chance because of the low coverage or because of mutations at restriction sites (restriction unsuccessful in some individuals), have been filled by consensus, where the consensus was unambiguous, in this visual assessment, but not in subsequent analyses using NewHybrids or PCoA. Here in Attachment 2, you can see clearly the status of the F2 hybrids identified by NewHybrids in Table 1, as distinct from both parental taxa, with an excess of heterozygosity (see the graph at the bottom of the table). The captive F2 also stands out clearly.



Figure 2. Locations of *Myuchelys* individuals thought to have been subject to introgression of *Emydura* genes. They are found in a 10 km stretch of river between Thora and Gordonville, and approximately 10 km from the site of first capture of *Emydura* in the Bellinger River (Cann, 1998).

Similarly, the status of the backcross (AA36212) is clearly evident in the table, but there are also a number of other *Myochelys georgesi* with strong indications of introgression, presumably in the third generation not tested by NewHybrids. All of these individuals were collected in a restricted region of the Bellinger River, between Thora and Gordonville Cutting (Figure 2), approximately 10 km downstream from the first sighting of *Emydura* in the Bellinger catchment (Cann, 1998). This, and the lack of F1 individuals in our sample, suggests that breeding between *Myuchelys georgesi* and *Emydura macquarii* is a rare event, or rarely leads to production of viable young, a proposition that could be tested by looking at relatedness among these individuals.

Finally, one specimen from the Kalang River (AA36801) identified as a prospective hybrid by Darren Fielder had a SNP profile consistent with *Emydura macquarii*, to which it was assigned with 0.9995 probability by NewHybrid (Attachment A).

A summary of the genetic similarity among the 93 individuals is shown in Figure 3. This plot represents the genotypes in a “locus space” reduced in dimensionality by ordination using Principal Coordinates analysis (PCoA). The first principal coordinates axis was aligned in the direction of 98.9% of variation among individuals, and so is the axis that should be the focus of attention. The second axis in the ordination explained negligible variation (0.16%) and is included only to draw the points out visually. No significance should be placed on displacement in the direction of the second PCoA axis.

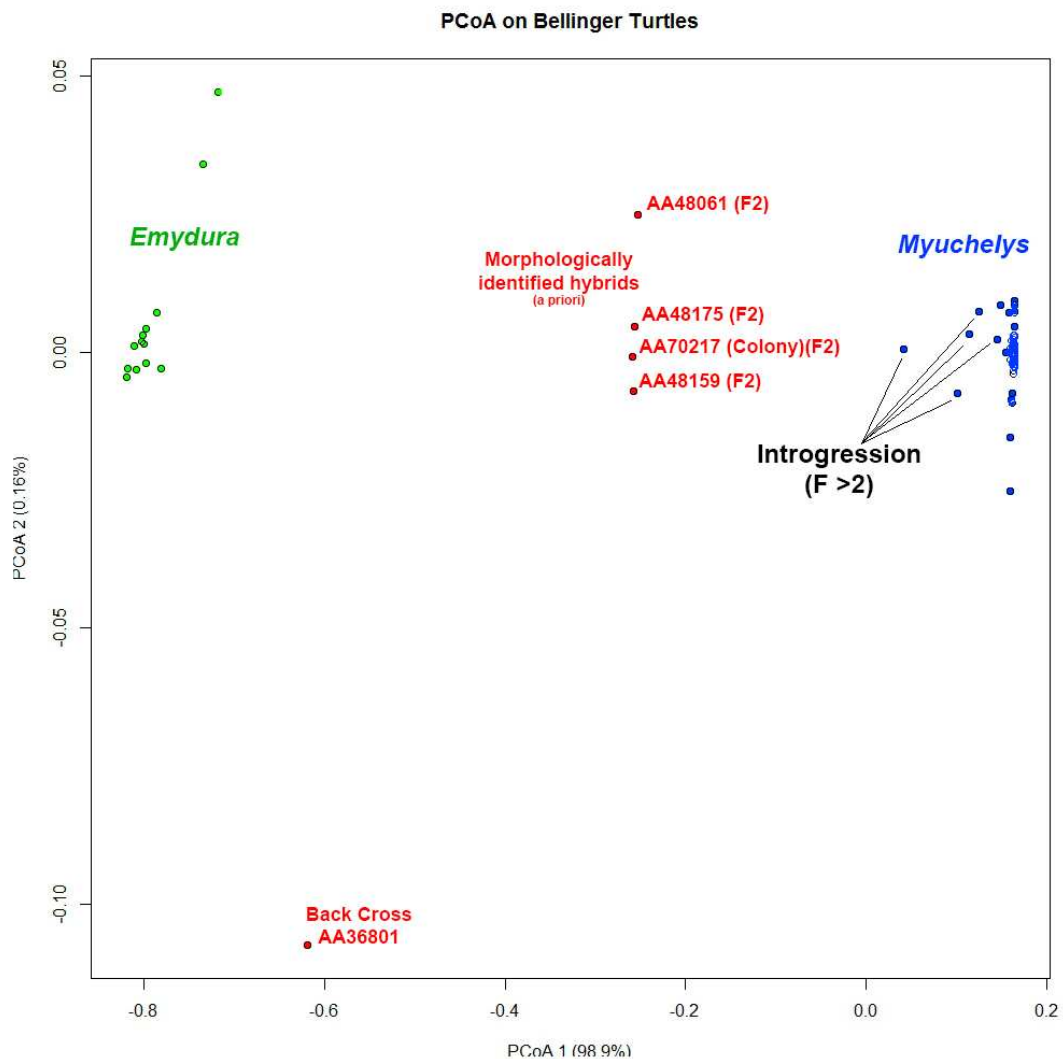


Figure 3. A plot of individual turtles in the first two dimensions of a reduced ordinated space of SNP loci. The second axis contains negligible information (0.16%) and is included only to draw out the points representing individual turtles. Consider position along the X-axis only.

This analysis qualitatively supports that of NewHybrids. Specimens allocated to the parental species *Myuchelys georgesii* and *Emydura macquarii* are displaced maximally on axis 1 representing the strong divergence between the two that is evident by the almost universally

reciprocal SNP states (reference verses alternate) in *Emydura macquarii* versus *Myuchelys georgesii* (Attachment 2). The F2 hybrids all align almost concurrently on axis 1 and intermediate between the two parental species. The backcrossed individual also appears intermediate to the parental species on axis 1, but closer to *Emydura macquarii* as expected. The specimens of *Myuchelys georgesii* suspected of arising from introgression in the third or later generations, appear in the PCoA plot as displaced from the tight cluster of *Myuchelys georgesii* in the direction of *Emydura macquarii*. Thus, the PCoA, using all the data, supports the conclusions of the visual examination of the first 100 loci (Attachment A) and the statistical analysis of the first 200 loci using NewHybrids.

Variability among specimens of *Myuchelys georgesii* is exceptionally low, and order of magnitude lower than in *Emydura macquarii*. This result is consistent with the mitochondrial data which identified 7 mt haplotypes in *Emydura macquarii* from the Bellinger River, but only one for *Myuchelys georgesii* (Georges et al., 2011). The 16 captive *Myuchelys georgesii* (that is, the captive specimens minus the F2 hybrid and the individual that generated no data) are representative of variation in the wild population of the Bellinger River.

Conclusions

The captive colony appears to capture what is exceptionally low genetic variability in *Myuchelys georgesii*.

Myuchelys georgesii is hybridising with the introduced *Emydura macquarii*, which presents a major challenge for management. Hybrids with roughly equal representation of the two species can be identified morphologically, but those with greater degrees of unbalanced introgression are morphologically cryptic.

The hybridization and introgression we detected in the Bellinger River appears curiously restricted geographically and, coupled with the failure to identify F1 individuals, may suggest that hybridization events between the two species occurs only rarely, or with low survivorship of the F1 hybrids. When fertile F1 hybrids do arise, they clearly breed with each other and back to at least one of the parental species.

The frequency of hybridization and introgression in the Kalang River precludes this population from being used as a natural insurance population for the Bellinger.

The captive colony contains a hybrid individual from the Kalang River (specimen AA70217, female), which must be excluded from any breeding program.

Now that we have established that *Myuchelys georgesii* and *Emydura macquarii* are hybridizing and that this is leading to detectable introgression, a sensible next step would be to include specimens of *Emydura macquarii* from the suspected source populations. This would increase the power of analyses to detect introgression because currently, our “parental” populations each may contain individuals already subject to some introgression.

It would be wise to screen more individuals from the Kalang and Bellinger Rivers and the suspected source populations for the *Emydura*, to ascertain the frequency of hybridization and rate of introgression, and whether or not the introgression is bidirectional.

A decision needs to be made as to whether specimens *Emydura macquarii* should be actively removed from the Bellinger and Kalang Rivers, whether this is indeed feasible, and whether such action would lead to adequate management of the introgression between the introduced *Emydura macquarii* and the distinctive endemic *Myuchelys georgesi*.

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	Parental Mygeo	Parental Emmac	F1 hybrid	F2 hybrid	Back cross to Mygeo	Back cross to Emmac	Status
AA45458	0.00011	0.99956	0	0.00033	0	0	Emydura macquarii
AA36018	0.0001	0.99957	0	0.00033	0	0	Emydura macquarii
AA36282	0.0001	0.99947	0	0.00043	0	0	Emydura macquarii
AA36009	0.00002	0.99957	0	0.00041	0	0	Emydura macquarii
AA36260	0.00011	0.99957	0	0.00032	0	0	Emydura macquarii
AA48054	0.00011	0.99952	0	0.00038	0	0	Emydura macquarii
AA48171	0.00007	0.99957	0	0.00036	0	0	Emydura macquarii
AA48194	0.00011	0.99946	0	0.00043	0	0	Emydura macquarii
AA36241	0.00009	0.99951	0	0.0004	0	0	Emydura macquarii
AA36242	0.00011	0.99957	0	0.00032	0	0	Emydura macquarii
AA48182	0.00011	0.99957	0	0.00032	0	0	Emydura macquarii
AA36882	0.00011	0.99957	0	0.00033	0	0	Emydura macquarii
AA36888	0.00005	0.99957	0	0.00038	0	0	Emydura macquarii
AA36068	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36128	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36154	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36058	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36212	0.00054	0	0	0.00021	0	0.99925	Backcross of F1 hybrid to E. macquarii
AA36213	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36008	0.99975	0	0	0.00025	0	0	Myuchelys georgesi
AA36029	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36069	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36059	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36078	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36079	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36019	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA36028	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36038	0.99981	0	0	0.00019	0	0	Myuchelys georgesi
AA36098	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36893	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA36894	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36245	0.99977	0	0	0.00023	0	0	Myuchelys georgesi
AA36246	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36249	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36281	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36297	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36298	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36858	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36859	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36860	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48197	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36862	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36863	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA48083	0.99984	0	0	0.00016	0	0	Myuchelys georgesi
AA48085	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48093	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36872	0.99983	0	0	0.00017	0	0	Myuchelys georgesi
AA36873	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36880	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48153	0.99981	0	0	0.00019	0	0	Myuchelys georgesi
AA48161	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
UC_0131	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36181	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36183	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36802	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36803	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36804	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36247	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA36248	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36257	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36881	0.99977	0	0	0.00023	0	0	Myuchelys georgesi
AA36883	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36884	0.99988	0	0	0.00012	0	0	Myuchelys georgesi
AA48102	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48103	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48105	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA36876	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA36877	0.99981	0	0	0.00019	0	0	Myuchelys georgesi
AA36878	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA48041	0.99976	0	0	0.00024	0	0	Myuchelys georgesi
AA48042	0.99977	0	0	0.00023	0	0	Myuchelys georgesi
AA48059	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA36801	0.00011	0.99946	0	0.00043	0	0	Emydura macquarii
AA48061	0.00004	0	0	0.99996	0	0	F2 generation hybrid
AA48159	0.00011	0	0	0.99979	0	0.00011	F2 generation hybrid
AA48175	0.0001	0	0	0.9999	0	0	F2 generation hybrid
AA70200	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA70201	0.99986	0	0	0.00014	0	0	Myuchelys georgesi
AA70202	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA70203	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA70204	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA70205	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA70206	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA70207	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA70208	0.9997	0	0	0.0003	0	0	Myuchelys georgesi
AA70209	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA70210	0.99979	0	0	0.00021	0	0	Myuchelys georgesi
AA70211	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA70212	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA70214	0.99978	0	0	0.00022	0	0	Myuchelys georgesi
AA70215	0.9998	0	0	0.0002	0	0	Myuchelys georgesi
AA70216	0.99989	0	0	0.00011	0	0	Myuchelys georgesi
AA70217	0.00011	0	0	0.99986	0	0.00003	F2 generation hybrid

ATTACHMENT 1.

Results of an assignment of individuals to two generation hybrid classes using software package NewHybrids (Anderson & Thompson, 2002).

ATTACHMENT 2

A spreadsheet showing the first 100 of 32,569 SNP loci screened for 13 *Emydura macquarii*, 59 *Myuchelys georgesi*, 4 suspected hybrids, and 17 specimens held in an insurance colony at the University of Western Sydney. Loci homozygous for the reference SNP (0, typical of *Myuchelys*) are shaded in black, loci homozygous for the alternate SNP (2, typical of *Emydura*) are shaded in white, heterozygous loci (1) are shaded in pink, and missing data are shaded in blue. The graph at the bottom of the table shows a count of the number of heterozygotes. The hybrids identified a priori (wild and captive) show a very distinctive pattern as F2 hybrids. There is evidence of introgression in generation F2 (one individual AA36212) and F3 or above in both *Emydura* and *Myuchelys*. These patterns, evident in the first 100 loci are representative of the patterns across all loci.

