Received: 17 March 2017 | Revised: 18 July 2017 | Accepted: 17 September 2017

DOI: 10.1002/aqc.2851

WILEY

RESEARCH ARTICLE

# Conservation genetics of the Mary River turtle (*Elusor macrurus*) in natural and captive populations

**Daniel J. Schmidt**[1] | **Thomas Espinoza**[2] | **Marilyn Connell**[3] | **Jane M. Hughes**[1]

[1] Australian Rivers Institute, Griffith University, Nathan, QLD, Australia

[2] Queensland Department of Natural Resources and Mines, Bundaberg, QLD, Australia

[3] Tiaro & District Landcare Group, Tiaro, Qld, Australia

**Correspondence**
Daniel J Schmidt, Australian Rivers Institute, Griffith University, Nathan, QLD, 4111, Australia
Email: d.schmidt@griffith.edu.au

**Funding information**
Everyone's Environment grants, Grant/Award Number: EER140016

## Abstract

1. Many thousands of Mary River turtle eggs were harvested for the pet trade in the 1960s and 1970s before it was recognized as a new species in a unique genus. Pet turtles and their descendants still survive in captive collections. *Elusor macrurus* is now an endangered species after suffering dramatic population declines along the single Australian river that constitutes its entire range.
2. A conservation genetic assessment was conducted to evaluate population subdivision within the remaining wild population of the Mary River turtle; to compare diversity of the wild population with a captive sample derived from the pet trade; and to establish a baseline estimate of effective population size ($N_e$) to assist with future monitoring and recovery.
3. Microsatellite analysis indicated panmixia throughout most of the Mary River catchment with the exception of one downstream tributary –Tinana Creek (pop. Specific $F_{ST}$ = 0.154). Subdivision between Tinana Creek and Mary River is a feature common to multiple co-distributed freshwater taxa including the threatened Australian lungfish and Mary River cod. Microsatellite diversity of the wild adult population was low (average $H_S$ = 0.554) and not significantly different from that of a sample of captive turtles from the pet trade – indicating genetic diversity may be well represented in captive stocks. Mitochondrial DNA diversity was extremely limited, with only two haplotypes found in the wild and a single shared haplotype in captive turtles.
4. Estimates of $N_e$ applicable to the entire species in the wild were ~136 and ~158 using two independent methods. A reasonable management objective should be retention of $N_e$ levels >100 during recovery of the species. Additional recommendations include that Mary River turtles be listed as Critically Endangered, and that a recovery plan be developed that considers 'headstarting' – using captive bred stocks to supplement the wild population.

**KEYWORDS**

endangered species, genetics, impoundment, reptiles, river, salinity

## 1 | INTRODUCTION

Assessment of genetic diversity and genetic structuring of threatened species provides fundamental information for effective conservation management (Palsboll, Berube, & Allendorf, 2007). Nearly half of all freshwater turtle species worldwide are threatened, and many exhibit low levels of genetic variability, so identification of genetic subdivision within natural populations is important for conservation strategies that aim to preserve diversity (Alacs, Janzen, & Scribner, 2007; Ihlow et al., 2014). The Mary River turtle (MRT), *Elusor macrurus*, represents a monotypic genus of ancient short-necked turtles restricted to a single coastal drainage of eastern Australia (Georges & Thomson, 2006). The narrow distribution of *E. macrurus* is limited to the Mary River in south-eastern Queensland (SEQ). This species is a river specialist, capable of bimodal respiration via cloacal ventilation, and depends upon flowing streams and pool habitats available along the Mary River (Clark, Gordos, & Franklin, 2009). The Mary River turtle experienced significant population declines resulting from a history of habitat alteration, exploitation for the pet trade and recruitment failure due to nest predation. Consequently, the MRT is one of the most threatened species of freshwater turtle in Queensland (Limpus, 2012; Micheli-Campbell, Campbell, Connell, Dwyer, & Franklin, 2013). MRT is listed as

  

'Endangered' at state level under the Nature Conservation Act 1992 and at national level under the EPBC Act 1999, and recognized as endangered globally in the IUCN Red List of Threatened Species 2015–4. From 1962 to 1974 as many as 12 000 MRT eggs were harvested annually from nesting banks in the mid to lower reaches of the Mary River for supply to the pet trade as 'penny turtles' (Flakus, 2002). The practice was made illegal, but it was not until the 1990s that the species was formally described and its geographic origin discovered (Cann & Legler, 1994). Egg harvesting for the pet trade appears no longer a major threat (Flakus, 2002), although egg predation by introduced foxes and native goannas is now a serious threat to recruitment of MRTs and other Australian freshwater turtles (M. Connell pers. obs.; Spencer, Van Dyke, & Thompson, 2017). *Elusor* has a long life-span, taking 20–30 years to reach maturity (Limpus, 2012), so turtles derived from the pet trade (and their descendants) still exist in captivity at zoos and private collections.

Molecular markers suitable for conservation genetic assessment of MRT were lacking before this project. The MRT mitochondrial genome exhibits very low variability, with only two haplotypes detected in wild populations (Schmidt, Brockett, Espinoza, Connell, & Hughes, 2016). Twenty-four microsatellite loci developed for co-distributed species *Elseya albagula* and *Emydura macquarii krefftii* and reported to amplify in *E. macrurus* were tested (Todd, Blair, Hamann, & Jerry, 2011). Of these only one locus (*Ekref18*) exhibited polymorphism in a sample of eight *E. macrurus* individuals from Mary River and Tinana Creek (Schmidt unpub. Data). A species-specific set of microsatellites for *E. macrurus* was therefore needed for current and future assessment of genetic diversity.

Previous work on spatial genetic structure of Mary River cod and Australian lungfish showed significant genetic subdivision within the Mary River catchment (Bishop, Hughes, & Schmidt, in press; Huey, Espinoza, & Hughes, 2013; Hughes et al., 2015). These studies revealed boundaries between semi-isolated populations in Tinana Creek and the remainder of the Mary River. Tinana Creek flows into the Mary River not far from the mouth (Figure 1), with both localities sharing a tidal estuarine reach in the lower catchment. One potential explanation for genetic subdivision between Tinana and Mary populations of freshwater taxa is restricted migration between the two systems resulting from elevated salinities (Hughes et al., 2015).

Effective population size ($N_e$) is a key parameter for monitoring the genetic health of wildlife populations because it reflects a population's recent demographic history and future evolutionary potential (Frankham, Bradshaw, & Brook, 2014). Traditional measures of population abundance such as census counts may not represent the underlying effective size owing to historical population fluctuations (e.g. bottlenecks) and reproductive variance which can reduce $N_e$ to a small fraction of census population size (Charlesworth, 2009). Information on $N_e$ is often incorporated into conservation management programmes for endangered species. For example, the well-known '50/500 rule' adopted by the IUCN Red List is based on the idea that threshold population sizes required to prevent inbreeding depression and retain evolutionary potential are 50 and 500 respectively (Jamieson & Allendorf, 2012). Recent recommendations suggest that these values should be doubled to '100/1000' for short-term management to avoid immediate extinction and long-term retention of evolutionary potential (Frankham et al., 2014). These target values highlight the increased extinction risk associated with small effective population size. Maintaining genetic variation with these values in mind is important, but genetic considerations are just one of several factors that should influence conservation strategies and policies (Jamieson & Allendorf, 2012).

This study is the first conservation genetic assessment of the Mary River turtle and establishes baseline information and new molecular resources for future monitoring of the species. Project objectives were to: (1) develop new *Elusor*-specific microsatellite loci; (2) identify the existence of any sub-population boundaries within the Mary River catchment; (3) compare genetic diversity between the extant wild population and a sample of the captive individuals derived from the pet trade in the 1960s and 1970s; (4) estimate effective population size of the existing adult wild population as a baseline for future monitoring of the species.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area

The Mary River, in south-east Queensland, Australia, flows north for 300 km from the Conondale Ranges to its mouth at River Heads west of Fraser Island (Figure 1(a)). Multiple tributaries along its main trunk contribute an additional 2700 km of stream length. It is a relatively unregulated watercourse, providing habitat for Mary River turtles and other threatened aquatic species within a catchment area of 9595 $km^2$ (Figure 1(b)(c)). Water storage infrastructure exists on several of its tributaries although only a saltwater barrage and small weir impedes the main trunk of the Mary River. The river is considered highly significant habitat from a biodiversity and conservation perspective (Arthington, 2009). In addition to MRTs, habitats along the Mary River are critical for conservation of vulnerable Australian lungfish and endangered Mary River cod (Arthington, 2009; Huey et al., 2013; Hughes et al., 2015). Freshwater flows from the Mary influence ecological processes and ecosystems across Hervey Bay and the southern Great Barrier reef lagoon, including the internationally significant Ramsar-listed Great Sandy Strait wetlands.

### 2.2 | Field methods

In total, 150 *Elusor macrurus* individuals were sampled, including 123 wild adults from 34 sites collapsed into six geographic groups, and 27 captive individuals (adults and immatures) sourced from zoos and private collectors (Figure 1 and Supplementary File S1). Wild samples were collected using double-winged fyke nets set overnight in riffles facing both upstream and downstream (Figure 1(d)). A small section of skin (5 $mm^2$) was taken from along the webbing of the hind foot, and preserved immediately in 100% ethanol. All procedures complied with Australian animal ethics permit number ENV/08/15/AEC and DAFF Animal Experimentation Ethics Committee permit number SA 2012/11/394, 395, 398, 399, 400 and General Fisheries Permit 139122.

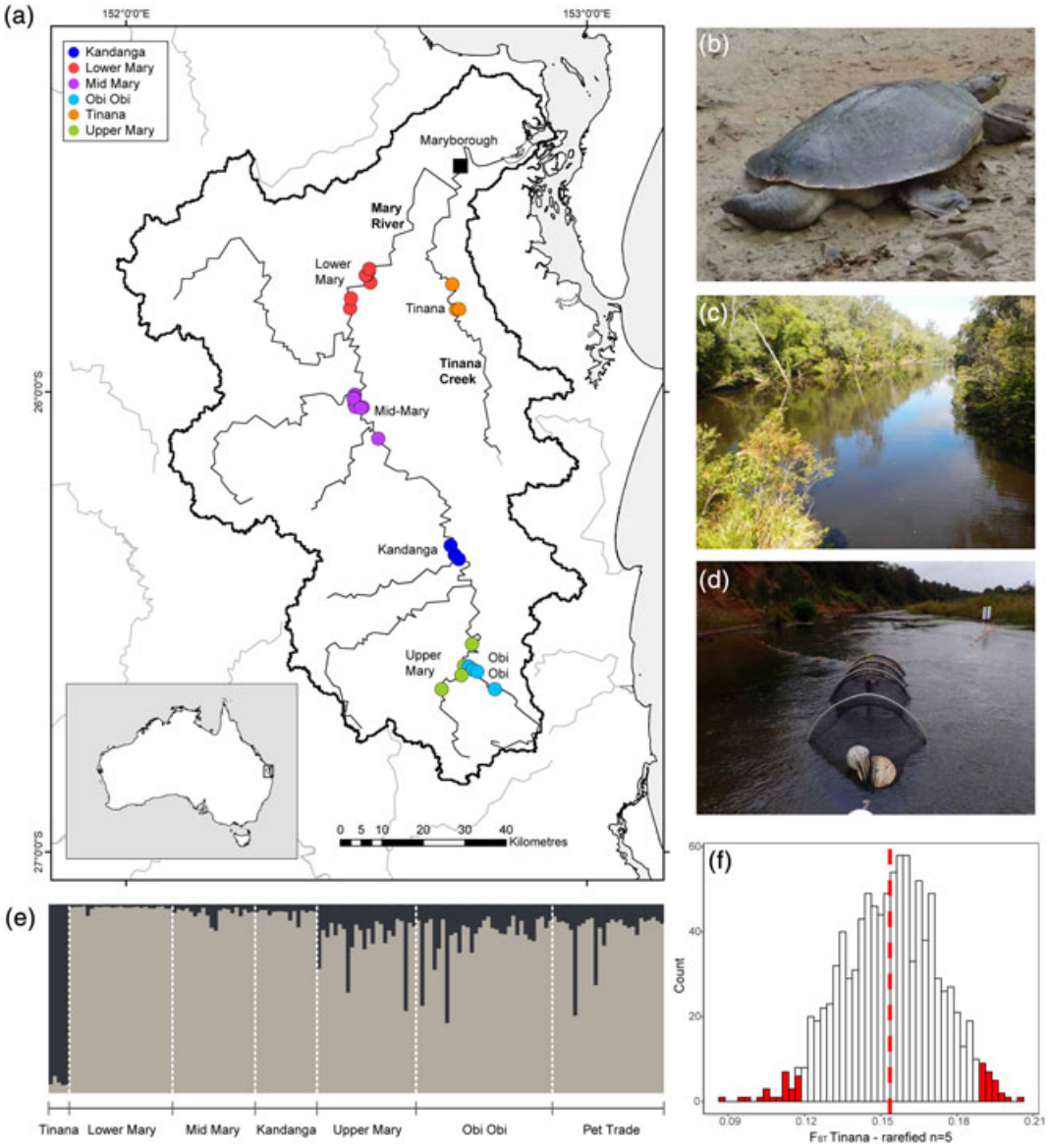


**FIGURE 1** **(a)** Map of study area, sample localities denoted by circles, colour-coded by population groupings used in the analysis. Coordinates provided in Supplementary File S1. Catchment boundary in bold. **(b)** *Elusor macrurus* adult male, highlighting large muscular tail characteristic of males. **(c)** Mary River turtle habitat, Tinana Creek. **(d)** Fyke net in position, Mary River. **(e)** STRUCTURE barplot showing outcome of admixture clustering for K = 2 groups. **(f)** Histogram of population-specific $F_{ST}$ for the Tinana Creek sample, derived from 1000 replicates with population sample size rarefied to $n$ = 5 and 95% quantiles highlighted in red. Position of observed $F_{ST}$ derived from full dataset without rarefaction marked by dashed red line

## 2.3 | Molecular methods

New microsatellites were developed by sequencing randomly sheared DNA from wild-caught voucher specimen MRT04 (Supplementary Table S1), using the iTru library protocol and a 600 cycle MiSeq v3 sequencing kit (Glenn et al., 2016; Schmidt et al., 2016). Sequences containing microsatellite motifs with uninterrupted length of > = 15 were extracted from merged paired-end reads using the QDD pipeline version 3.1 (Meglécz et al., 2014). Microsatellite genotyping and PCR protocols followed Real, Schmidt, and Hughes (2009), except that an annealing temperature of 55°C was used for all loci. Characteristics of the new loci and their primer sequences are provided in Table 1. Mitochondrial DNA (mtDNA) variation was assessed by sequencing an 800 bp fragment of the control region using primers MRT15599F and MRT16379R (Schmidt et al., 2016). Thirty-six new individuals were sequenced and combined with 22 previously sequenced by Schmidt et al. (2016) to increase geographic coverage and include a sample representing the captive population.

## 2.4 | Data analysis

Editing and alignment of mtDNA sequences was performed using Geneious v9.1.5 (Kearse et al., 2012). Microsatellite alleles were scored with GeneMapper v3.1 (Applied Biosystems). Analysis of diversity and differentiation statistics were performed using R packages: adegenet v2.0.1 (Jombart, 2008); pegas v0.9 (Paradis, 2010); hierfstat v0.04 (Goudet, 2005). A sample rarefaction procedure was developed to

**TABLE 1** Characterization of nine microsatellite loci developed for *Elusor macrurus*. Summary statistics based on genotyping 123 wild-caught individuals. Summary statistics from function basic.Stats; *hierfstat* R package. HWE test from function hw.Test; *pegas* R package

| Locus name | Genbank accession | Primer sequence (5′-3′) | Repeat motif[1] | Fwd 5′ Tail[2] | Size range[3] (bp) | $N_A$ | $H_O$ | $H_S$ | HWE *P*-value | $F_{IS}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *emac_01* | KY284058 | F: GATCAGGGAAATACTACCAAGGC<br>R: ACCATAGCCGTATGACCACA | $Ac_{(19)}$ | 1 | 124–142 | 3 | 0.372 | 0.463 | 0.228 | 0.196 |
| *emac_02* | KY284059 | F: ACATGCATCTTGGACTGCCT<br>R: GCCTCAGGTGGGTGTAAATG | $Ac_{(17)}$ | 2 | 190–210 | 8 | 0.525 | 0.542 | 0.096 | 0.031 |
| *emac_05* | KY284060 | F: CATCTCCCTGCCCTTGATT<br>R: AGGCACTGAGAACGTGGAAT | $Ag_{(20)}$ | 1 | 240–254 | 4 | 0.542 | 0.585 | 0.252 | 0.073 |
| *emac_25* | KY284063 | F: TTTCTTGTGTTGACTTGCTCTTAG<br>R: GCCCTTAAGGTCCATGTAGAAA | $Ac_{(15)}$ | 1 | 172–188 | 7 | 0.445 | 0.444 | 0.591 | −0.002 |
| *emac_26* | KY284064 | F: TGCTGCATACAAACACAAAGC<br>R: GGTTGTGCTTTCTCCTGTGG | $Ac_{(16)}$ | 2 | 129–139 | 6 | 0.661 | 0.718 | 0.228 | 0.079 |
| *emac_31* | KY284066 | F: GGTTAGCCATTTAAAGAAATGCAG<br>R: GCAGTTCAGAGAACACAAACACA | $Ac_{(16)}$ | 3 | 177–193 | 5 | 0.383 | 0.401 | 0.591 | 0.044 |
| *emac_35* | KY284067 | F: GGACGAACACTCAGTTTGTTG<br>R: GTCGTCTAGGACCATTTGGC | $Ac_{(15)}$ | 3 | 147–155 | 4 | 0.5 | 0.477 | 0.706 | −0.047 |
| *emac_39* | KY284068 | F: GCAGTGGGCAGAGACAAGTT<br>R: CGATGACTGACACAGAAGACAC | $Ac_{(17)}$ | 3 | 174–192 | 5 | 0.607 | 0.644 | 0.228 | 0.057 |
| *emac_48* | KY284069 | F: TGAATTGGATGATACAAAGAATCC<br>R: CTGAGGCATCTACCAGGGAC | $Ac_{(19)}$ | 4 | 225–253 | 8 | 0.678 | 0.713 | 0.591 | 0.049 |

[1]Repeat motif observed in original sequence from voucher specimen MRT04.

[2]code for tail added to forward primer to facilitate fluorescent labelling of PCR product (Real et al., 2009).

[3]size range includes 20 bp tail on forward primer.

$N_A$ = number of alleles; $H_O$ = observed heterozygosity; $H_S$ = expected heterozygosity; HWE *P*-value = FDR correction of Hardy–Weinberg test *P*-value using function p.adjust ('BH' method; *stats* R package); $F_{IS}$ = inbreeding coefficient.

examine the influence of low sample size on estimation of population specific $F_{ST}$. An R script and explanation of this analysis is provided in Supplementary File S3. The existence of distinct genetic groups within the microsatellite dataset was tested using model-based Bayesian clustering implemented in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The probability of an admixture model was tested for up to eight clusters (K) and sample groups were incorporated as a prior in the analysis (LOCPRIOR model). Models were tested using 20 independent MCMC simulations, each consisting of $2 \times 10^6$ iterations after a burn-in of $2 \times 10^5$ iterations. STRUCTURE output was processed with POPHELPER v1.0.10 (Francis, 2017). Estimates of effective population size ($N_e$) based on patterns of individual relatedness and linkage disequilibrium were obtained for wild-caught samples using the sibship frequency (SF) method in COLONY v2.0.6.2 (Wang, 2009) and the linkage disequilibrium method (LDNe) implemented in NeEstimator v2 (Do et al., 2014). The SF method estimates $N_e$ of the sample as a function of the frequencies of half and full siblings identified from multi-locus genotype data (Wang, 2009). The LDNe method estimates $N_e$ of the sample as a function of associations between alleles from independent loci (Waples & Do, 2008). On the assumption that the sample of wild turtles is representative of most cohorts resulting from multiple reproductive cycles across a generation, these $N_e$ estimates may be biased downwards relative to true $N_e$ (Waples & Antao, 2014); however, they serve as a useful baseline for future monitoring of the species.

## 3 | RESULTS

### 3.1 | Microsatellite DNA

Nine dimeric microsatellite loci were polymorphic with 3–8 alleles per locus and satisfied Hardy–Weinberg proportions in the sample of 123 wild-caught individuals (Table 1). Tests for linkage disequilibrium indicated that loci are independent (standardized index of association = 0.029; *P*-value = 0.08; 1000 permutations; ia function in poppr R package). The level of missing data across the full dataset of 150 individuals genotyped at nine loci was 5.9%. The full dataset is available in Supplementary material as an R S4 class 'genind' object (adegenet R package).

Genetic diversity was similar across the six geographic samples and the pet trade sample (Table 2). Expected heterozygosity ($H_S$) and allelic richness (AR) were both lowest in Tinana Creek ($H_S$ = 0.49, AR = 2.3; Table 2) and highest in the upper Mary ($H_S$ = 0.58, AR = 2.8; Table 2). Despite substantial investment in sampling effort, the Tinana sample size was low (*n* = 5) so estimates of relatively low diversity and relatively high inbreeding coefficient ($F_{IS}$) for this population may be affected by sampling error (Table 1). However, Tinana Creek had the highest number of private alleles of any sample, indicating that this tributary harbours a disproportionate amount of genetic diversity across the geographic range of MRTs (Table 1). Note that the five Tinana individuals were the product of three field trips and came from three different sites within Tinana Creek. Average expected heterozygosity (± SE) of the pooled wild sample ($H_S$ = 0.55 ± 0.04; *n* = 123) was similar to the captive sample ($H_S$ = 0.54 ± 0.04; *n* = 27). Comparison of

**TABLE 2** Genetic diversity statistics per population group for the Mary River turtle. Summary statistics from *hierfstat* R package

| Population group | | Microsatellites - nine loci | | | | | | | | mtDNA | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_{Total}$ | $n_{avg.\ Per\ locus}$ | $H_O$ | $H_S$ | AR | Priv. | $F_{IS}$ | $F_{ST}$ | $F_{ST\ rar.\ n\ =\ 5}$ | n | $H_D$ |
| Tinana | 5 | 5.0 | 0.378 | 0.489 | 2.333 | 3 | 0.222 (−0.049, 0.572) | 0.154 | 0.154 (0.117, 0.186) | 4 | 0.000 |
| Lower Mary | 25 | 24.0 | 0.542 | 0.565 | 2.695 | 0 | 0.041 (−0.055, 0.130) | −0.002 | −0.002 (−0.110, 0.119) | 9 | 0.389 |
| Mid Mary | 20 | 18.8 | 0.513 | 0.529 | 2.693 | 0 | 0.017 (−0.070, 0.148) | 0.061 | 0.063 (−0.039, 0.188) | 7 | 0.571 |
| Kandanga | 16 | 15.1 | 0.448 | 0.534 | 2.708 | 0 | 0.153 (0.081, 0.243) | 0.057 | 0.065 (−0.070, 0.235) | 8 | 0.000 |
| Upper Mary | 24 | 23.8 | 0.603 | 0.575 | 2.849 | 2 | −0.054 (−0.089, −0.005) | −0.023 | −0.028 (−0.133, 0.091) | 14 | 0.000 |
| Obi Obi | 33 | 31.8 | 0.516 | 0.529 | 2.684 | 0 | 0.022 (−0.055, 0.087) | 0.062 | 0.066 (−0.088, 0.214) | 7 | 0.000 |
| Pet trade | 27 | 22.7 | 0.532 | 0.542 | 2.772 | 0 | 0.027 (−0.104, 0.149) | 0.038 | 0.040 (−0.144, 0.211) | 9 | 0.000 |

N = total number of individuals genotyped; n = number of individuals successfully genotyped averaged over loci; $H_O$ = average observed heterozygosity; $H_S$ = average expected heterozygosity; AR = average allelic richness rarified to sample size of 10 alleles; Priv = number of unique alleles; $F_{IS}$ = average inbreeding coefficient (95% CI); $F_{ST}$ = population specific $F_{ST}$; $F_{ST\ rar}$ = median population specific $F_{ST}$ based on sample rarefaction (95% CI); $H_D$ = haplotype diversity.

diversity between the pet trade sample and the pooled wild sample found no significant difference based on a permutation test of expected heterozygosity (Hs diff. = 0.016; *P*-value = 0.83; 1000 Monte-Carlo simulations; Hs.test function in adegenet R package).

Global $F_{ST}$ among the six geographic groups sampled from the wild was 0.016 (95% CI = 0.006, 0.024; varcomp.glob function, adegenet R package). Pairwise $F_{ST}$ values indicated little or no genetic subdivision among five geographic groups sampled within the main stem of the Mary River as most pairwise estimates of $F_{ST}$ were close to zero, with 95% confidence intervals overlapping zero (Table 3). This result indicates that no genetic subdivision exists along the main stem of the Mary River. Samples derived from the pet trade were also indistinguishable from populations on the main stem of the Mary, consistent with harvesting of eggs during the 1960s and 1970s from nesting banks in this area. Significant genetic differentiation was observed between the Tinana Creek sample and all other samples (pop. Specific $F_{ST}$ = 0.154; Table 2). Pairwise $F_{ST}$ estimates were ~10-fold higher in all comparisons involving Tinana Creek, and all of these comparisons had non-zero lower 95% confidence intervals (Table 3). The low number of Tinana samples (*n* = 5) may not represent the full extent of genetic diversity within this population, but small sample size is unlikely to be wholly responsible for the observed high $F_{ST}$ values because the $F_{ST}$ calculation used corrects for uneven sample size (Weir & Cockerham, 1984). In addition, a sample rarefaction procedure was applied, showing that reduction of all populations to n = 5 gave median $F_{ST}$ values very close to values obtained with the full dataset (Table 1; Supplementary File S3). In particular, the population-specific $F_{ST}$ value for Tinana obtained via this rarefaction analysis was 0.154 with lower and upper 95% quantiles of 0.117, 0.186 (Table 1; Supplementary File S3).

Admixture-based clustering using STRUCTURE showed the best clustering solution involved dividing samples into two groups (Figure 1(e)). This inference was supported by K = 2 receiving both the highest mean likelihood score and highest ΔK. Individuals from Tinana Creek belong in one group, and samples from mid to lower Mary constitute the other (Figure 1(e)). Upstream samples and the pet trade samples exhibited varying degrees of apparent admixture with the Tinana group, but were most similar to the lower Mary (Figure 1(e)). Potential relationships between Tinana and upstream Mary samples were further assessed using discriminant analysis of principal components (DAPC; Jombart, Devillard, & Balloux, 2010). DAPC supported distinct subdivision between Tinana and all Mary samples, with no evidence for closer affinity between Tinana and upper Mary samples as suggested by the pattern of admixture in the STRUCTURE plot (Supplementary file S2).

Results of population structure analysis suggest that MRTs are a single genetically homogeneous population over most of their range, with the exception of Tinana Creek. Therefore, a single estimate of effective population size ($N_e$) was made for the species as a whole based on 123 wild-caught adults. Maximum likelihood estimation of sibship dyads among 7503 individual pairwise comparisons found 95 potential cases of full-sibs. For the SF method, this rate of population-wide sibship translates to an estimated $N_e$ of 158, with a 95% confidence interval of 120 to 210. The LDNe method gave a similar $N_e$ estimate of 136, with a 95% confidence interval of 72 to 416. Repeating $N_e$ calculations with five Tinana individuals excluded had little influence on the SF-based estimate ($N_e$ = 157; 95% CI = 120–211), and a slightly higher estimate for the LDNe method ($N_e$ = 155; 95% CI = 78–750).

### 3.2 | Mitochondrial DNA

Only two unique haplotypes were found among the 58 mtDNA sequences, with an overall haplotype diversity of 0.16 (Table 1). Haplotype A (GenBank: KX369542) was found in 53 individuals and was common in all the six geographic locations as well as the pet trade sample (Table 2). Haplotype B (GenBank: KX369543) differs from haplotype A by a single base substitution and by contraction of an $(AT)_5$ tandem repeat unit to $(AT)_4$. Haplotype B was found in five individuals from the lower and mid-Mary (Table 2). Overall estimates of $\Phi_{ST}$ (0.226; *P* = 0.001) and $F_{ST}$ (0.226; *P* = 0.013) were significant owing to the frequency of haplotype B in the lower and mid-Mary samples (Table 2); however, limited polymorphism resulted in only one significant pairwise comparison between mid-Mary and upper-Mary.

## 4 | DISCUSSION

Within-catchment genetic subdivision in the Mary River turtle might be unexpected given a general lack of genetic structure found in other Australian freshwater turtles within catchments (Hodges, Donnellan, & Georges, 2014; Todd et al., 2013; Todd, Blair, Georges, Lukoschek, & Jerry, 2014). However, genetic subdivision in MRTs is spatially identical to the pattern seen in co-distributed freshwater taxa. The tidal

**TABLE 3** Pairwise $F_{ST}$ among seven population groupings of *Elusor macrurus* based on nine microsatellite loci. $F_{ST}$ above diagonal, 95% confidence interval below diagonal, based on 1000 bootstrap replicates. Methods = function pairwise.WCfst; function boot.Ppfst; *hierfstat* R package. $F_{ST}$ estimates with lower 95% CI above zero highlighted in bold font

| Group | Tinana | Lower Mary | Mid Mary | Kandanga | Upper Mary | Obi Obi | Pet trade |
|---|---|---|---|---|---|---|---|
| Tinana | - | **0.155** | **0.164** | **0.128** | **0.117** | **0.113** | **0.118** |
| Lower Mary | 0.087, 0.209 | - | 0.014 | 0.010 | 0.014 | **0.020** | **0.019** |
| Mid Mary | 0.084, 0.244 | −0.003, 0.033 | - | −0.005 | 0.010 | 0.001 | 0.006 |
| Kandanga | 0.053, 0.203 | −0.009, 0.031 | −0.021, 0.011 | - | 0.003 | −0.003 | 0.008 |
| Upper Mary | 0.037, 0.201 | −0.001, 0.026 | −0.01, 0.038 | −0.007, 0.016 | - | −0.003 | −0.003 |
| Obi Obi | 0.033, 0.194 | 0.004, 0.036 | −0.01, 0.042 | −0.016, 0.016 | −0.01, 0.006 | - | −0.004 |
| Pet trade | 0.053, 0.176 | 0.003, 0.037 | −0.013, 0.032 | −0.005, 0.024 | −0.013, 0.005 | −0.009, 0 | - |

estuarine reach shared between Tinana Creek and Mary River divides genetically distinct populations of the endangered Mary River cod, *Maccullochella mariensis* (Huey et al., 2013), the threatened Australian lungfish, *Neoceratodus forsteri* (Bishop et al., in press; Hughes et al., 2015) and a freshwater crayfish, *Cherax dispar* (Bentley, Schmidt, & Hughes, 2010). Salinity levels above the confluence of these streams may be a significant barrier that impedes gene flow between Tinana and Mary populations of freshwater taxa. Freshwater populations inhabiting these systems are separated at present by two tidal barrages and ~20 km of salt water. A turtle that engages in overland dispersal should be less susceptible to a barrier of this nature (Hughes, Huey, & Schmidt, 2013), but overland dispersal is unlikely in MRTs, which are a strongly stream-dependent species, not recorded from peripheral water bodies (Limpus, 2012). Radio-tracked females are only known to leave the water for nesting or basking (Flakus, 2002). The sample size from Tinana Creek was low ($n$ = 5), despite multiple sampling trips targeting this area. Distribution and abundance of MRTs in Tinana Creek is poorly documented (Limpus, 2012) but deserving of immediate attention given the genetic evidence presented here.

Genetic divergence between Tinana and Mary subpopulations was restricted to the microsatellite data, with mtDNA resolution limited by an overall lack of diversity (Schmidt et al., 2016). Deep phylogeographic 'breaks' in mtDNA are often observed between catchments in freshwater turtles, indicating long-term subdivision (Hodges et al., 2014; Todd et al., 2014; Walker & Avise, 1998). Lack of phylogeographic mtDNA divergence in MRTs indicates that the Tinana–Mary barrier is not an old one. Likewise, the timescale of divergence between Tinana and Mary populations of Australian lungfish is relatively recent (less than 10,000 years) and linked to periods of lowered sea level in the late Pleistocene (Bishop et al., in press).

Overall estimates of genetic diversity based on microsatellite data (average Hs = 0.55) were similar to values estimated for *Elseya albagula*, an endangered freshwater turtle co-distributed with MRT (average Hs = 0.57; Todd et al., 2013), but lower than values observed in a range of other threatened turtle species (Davy, Bernardo, & Murphy, 2014; Escalona et al., 2009; Petre, Selman, Kreiser, Pearson, & Wiebe, 2015). The low microsatellite-based estimate of heterozygosity (Hs = 0.55) found in MRTs is close to the threshold of 0.54 identified by Willoughby et al. (2015) as symptomatic of a species that merits conservation concern as Critically Endangered.

The estimate of $N_e$ obtained for the extant wild population of MRTs (~150) is relatively low for a turtle species. A comparable study of a threatened species restricted to a single drainage in the USA (yellow-blotched sawback, *Graptemys flavimaculata*) found minimum $N_e$ estimates >800 (Selman, Kreiser, & Qualls, 2013). Effective population size estimates derived from microsatellite data in turtle species distributed across multiple drainages are also higher than those obtained for MRTs (Petre et al., 2015; Pittman, King, Faurby, & Dorcas, 2011; Spradling, Tamplin, Dow, & Meyer, 2010). The relatively low $N_e$ estimate for MRTs may be attributed to an estimated decline in adult population size of 95% over the last 30–40 years (Flakus, 2002; Kuchling, 2008). This dramatic decline is probably related to habitat alteration following installation of impoundments, egg harvesting for the pet trade and nest predation (Kuchling, 2008). Age structure of the population is now skewed towards ageing adults (Limpus, 2012). In light of this, a reasonable target for conservation management of MRTs is to maintain $N_e \geq 100$ while the census size of the population recovers. Effective population size ≥100 is recommended to prevent inbreeding depression in the short term and limit loss of fitness to ≤10% over five generations (Frankham et al., 2014). Since MRTs are long-lived and delay sexual maturity to 25–30 years, it may take a long time for the population to recover to a point where $N_e$ of the adult population might actually increase. An available option for consideration is to supplement the wild population with individuals derived from captivity. The small sample of captive MRT examined here appears genetically compatible with the wild population, at least with the main stem of the Mary. This is not surprising; the vast numbers of eggs collected over many years appears to have captured genetic diversity of the wild population reasonably well. Captive rearing of MRTs derived from the pet trade could be considered as part of an *ex situ* conservation strategy to enhance the effective population size of the Mary river population. Modelling studies have recently recommended this 'headstarting' approach as a viable management tool for stopping the declines of freshwater turtles (Spencer et al., 2017). Reintroduction of captive-bred turtles into the Tinana Creek population should be avoided as this appears to be an isolated and genetically distinct subpopulation. Of course other factors beyond genetics would need to be evaluated if reintroduction was considered, including survival prospects of captive releases (Araki, Cooper, & Blouin, 2007; Heppell, Crowder, & Crouse, 1996; Micheli-Campbell et al., 2013).

### 4.1 | Recommendation

We propose that the Mary River turtle (*Elusor macrurus*) should be listed as Critically Endangered under the Environment Protection Biodiversity Conservation Act 1999 with a suitable recovery plan developed. Based on low levels of microsatellite diversity observed in the wild population, Mary River turtles meet criteria for Critically Endangered proposed by Willoughby et al. (2015). Populations in Tinana Creek should be considered a separate management unit distinct from the main Mary population, translating into suitable prioritization under natural resource management legislation in this area.

#### ACKNOWLEDGEMENTS

We thank Tiaro and District Landcare group members for support. We thank Lone Pine Koala Sanctuary (Rory Keenan), David Fleays Wildlife Park (Jacqui Seal), Geckos Wildlife (Martin Fingland), Gavin Brown and Marc Dorse for kindly allowing access to captive turtles. The assistance of Kathryn Real (GU), Jemma Somerville (GU), Sharon Marshall (Qld. Dept. Natural Resources and Mines), Kate Hodges (Department of Science, Innovation, Technology), Brittany Brockett (GU), Nicole Hogg and Fraxa Caraiani (Griffith University DNA sequencing facility) is gratefully acknowledged. This project was funded by the Queensland Government 'Everyone's Environment grants' scheme, EER140016 and Tiaro and District Landcare group. Authors declare no conflict of interest.

#### ORCID

*Daniel J. Schmidt* http://orcid.org/0000-0001-5638-497X

## REFERENCES


Alacs, E. A., Janzen, F. J., & Scribner, K. T. (2007). Genetic issues in freshwater turtle and tortoise conservation. *Chelonian Research Monographs*, *4*, 107–123.

Araki, H., Cooper, B., & Blouin, M. S. (2007). Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, *318*, 100–103.

Arthington, A. H. (2009). Australian lungfish, *Neoceratodus forsteri*, threatened by a new dam. *Environmental Biology of Fishes*, *84*, 211–221.

Bentley, A. I., Schmidt, D. J., & Hughes, J. M. (2010). Extensive intraspecific genetic diversity of a freshwater crayfish in a biodiversity hotspot. *Freshwater Biology*, *55*, 1861–1873.

Bishop, C. R., Hughes, J. M., & Schmidt, D. J. (in press). Mitogenomic analysis of the Australian lungfish (*Neoceratodus forsteri*) reveals structuring of indigenous riverine populations and late Pleistocene movement between drainage basins. *Conservation Genetics*.

Cann, J., & Legler, J. (1994). The Mary River tortoise: A new genus and species of short-necked chelid from Queensland, Australia. *Chelonian Conservation and Biology*, *1*, 81–96.

Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, *10*, 195–205.

Clark, N. J., Gordos, M. A., & Franklin, C. E. (2009). Implications of river damming: the influence of aquatic hypoxia on the diving physiology and behaviour of the endangered Mary River turtle. *Animal Conservation*, *12*, 147–154.

Davy, C. M., Bernardo, P. H., & Murphy, R. W. (2014). A Bayesian approach to conservation genetics of Blanding's turtle (*Emys blandingii*) in Ontario, Canada. *Conservation Genetics*, *15*, 319–330.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J., & Ovenden, J. R. (2014). NEESTIMATOR v2: Re-implementation of software for the estimation of contemporary effective population size (N-e) from genetic data. *Molecular Ecology Resources*, *14*, 209–214.

Escalona, T., Engstrom, T. N., Hernandez, O. E., Bock, B. C., Vogt, R. C., & Valenzuela, N. (2009). Population genetics of the endangered South American freshwater turtle, *Podocnemis unifilis*, inferred from microsatellite DNA data. *Conservation Genetics*, *10*, 1683–1696.

Flakus, S. (2002). *The ecology of the Mary River turtle, Elusor macrurus*. Master of Sciences thesis, University of Queensland, Brisbane.

Francis, R. M. (2017). POPHELPER: An R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, *17*, 27–32.

Frankham, R., Bradshaw, C. J. A., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, *170*, 56–63.

Georges, A., & Thomson, S. (2006). Evolution and zoogeography of Australian freshwater turtles. In J. R. Merrick, M. Archer, G. M. Hickey, & M. S. Y. Lee (Eds.), *Evolution and zoogeography of Australasian vertebrates* (pp. 291–308). Sydney: Australia: Auscipub Pty Ltd.

Glenn, T. C., Nilsen, R., Kieran, T. J., Finger, J. W., Pierson, T. W., Bentley, K. E., … Faircloth, B. C. (2016). Adapterama I: Universal stubs and primers for thousands of dual-indexed Illumina libraries (iTru & iNext). *bioRxiv*.. https://doi.org/10.1101/049114

Goudet, J. (2005). HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, *5*, 184–186.

Heppell, S. S., Crowder, L. B., & Crouse, D. T. (1996). Models to evaluate headstarting as a management tool for long-lived turtles. *Ecological Applications*, *6*, 556–565.

Hodges, K., Donnellan, S., & Georges, A. (2014). Phylogeography of the Australian freshwater turtle *Chelodina expansa* reveals complex relationships among inland and coastal bioregions. *Biological Journal of the Linnean Society*, *111*, 789–805.

Huey, J. A., Espinoza, T., & Hughes, J. M. (2013). Natural and anthropogenic drivers of genetic structure and low genetic variation in the endangered freshwater cod, *Maccullochella mariensis*. *Conservation Genetics*, *14*, 997–1008.

Hughes, J. M., Huey, J. A., & Schmidt, D. J. (2013). Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? *Freshwater Biology*, *58*, 951–966.

Hughes, J. M., Schmidt, D. J., Huey, J. A., Real, K. M., Espinoza, T., McDougall, A., … Roberts, D. T. (2015). Extremely low microsatellite diversity but distinct population structure in a long-lived threatened species, the Australian lungfish *Neoceratodus forsteri* (Dipnoi). *Plos One*, *10*(4). https://doi.org/10.1371/journal.pone.0121858

Ihlow, F., Ahmadzadeh, F., Ghaffari, H., Taskavak, E., Hartmann, T., Etzbauer, C., & Rodder, D. (2014). Assessment of genetic structure, habitat suitability and effectiveness of reserves for future conservation planning of the Euphrates soft-shelled turtle *Rafetus euphraticus* (Daudin, 1802). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*, 831–840.

Jamieson, I. G., & Allendorf, F. W. (2012). How does the 50/500 rule apply to MVPs? *Trends in Ecology & Evolution*, *27*, 578–584.

Jombart, T. (2008). adegenet: An R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*. https://doi.org/10.1186/1471-2156-11-94

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*, 1647–1649.

Kuchling, G. (2008). *Independent expert review of the information provided in the Traveston Crossing Dam EIS that relates to the Mary River turtle (EPBC referral 2006/3150)*. Canberra: Australian Government Retrieved from http://www.environment.gov.au/epbc/notices/assessments/2006/3150/pubs/independent-expert-report-on-the-mary-river-turtle-kuchling.pdf.

Limpus, C. (2012). *Freshwater Turtles in the Mary River: Review of biological data for turtles in the Mary River, with emphasis on* Elusor macrurus *and* Elseya albagula. Brisbane: Queensland Government Retrieved from https://www.ehp.qld.gov.au/wildlife/pdf/mary-river-turtles.pdf.

Meglécz, E., Pech, N., Gilles, A., Dubut, V., Hingamp, P., Trilles, A., … Martin, J.-F. (2014). QDD version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: experimental validation of variables determining genotyping success rate. *Molecular Ecology Resources*, *14*, 1302–1313.

Micheli-Campbell, M. A., Campbell, H. A., Connell, M., Dwyer, R. G., & Franklin, C. E. (2013). Integrating telemetry with a predictive model to assess habitat preferences and juvenile survival in an endangered freshwater turtle. *Freshwater Biology*, *58*, 2253–2263.

Palsboll, P. J., Berube, M., & Allendorf, F. W. (2007). Identification of management units using population genetic data. *Trends in Ecology & Evolution*, *22*, 11–16.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*, 419–420.

Petre, C., Selman, W., Kreiser, B., Pearson, S. H., & Wiebe, J. J. (2015). Population genetics of the diamondback terrapin, *Malaclemys terrapin*, in Louisiana. *Conservation Genetics*, *16*, 1243–1252.

Pittman, S. E., King, T. L., Faurby, S., & Dorcas, M. E. (2011). Demographic and genetic status of an isolated population of bog turtles (*Glyptemys muhlenbergii*): Implications for managing small populations of long-lived animals. *Conservation Genetics*, *12*, 1589–1601.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959. Retrieved from <Go to ISI>://WOS:000087475100039

Real, K. M., Schmidt, D. J., & Hughes, J. M. (2009). *Mogurnda adspersa* microsatellite markers: Multiplexing and multi-tailed primer tagging. *Conservation Genetics Resources*, *1*, 411–414.

Schmidt, D. J., Brockett, B., Espinoza, T., Connell, M., & Hughes, J. M. (2016). Complete mitochondrial genome of the endangered Mary River turtle (*Elusor macrurus*) and low mtDNA variation across the species' range. *Australian Journal of Zoology*, *64*, 117–121.

Selman, W., Kreiser, B., & Qualls, C. (2013). Conservation genetics of the yellow-blotched sawback *Graptemys flavimaculata* (Testudines: Emydidae). *Conservation Genetics*, *14*, 1193–1203.

Spencer, R. J., Van Dyke, J. U., & Thompson, M. B. (2017). Critically evaluating best management practices for preventing freshwater turtle extinctions. *Conservation Biology*. doi:https://doi.org/10.1111/cobi.12930

Spradling, T. A., Tamplin, J. W., Dow, S. S., & Meyer, K. J. (2010). Conservation genetics of a peripherally isolated population of the wood turtle (*Glyptemys insculpta*) in Iowa. *Conservation Genetics*, *11*, 1667–1677.

Todd, E., Blair, D., Farley, S., Farrington, L., Fitzsimmons, N. N., Georges, A., … Jerry, D. R. (2013). Contemporary genetic structure reflects historical drainage isolation in an Australian snapping turtle, *Elseya albagula*. *Zoological Journal of the Linnean Society*, *169*, 200–214.

Todd, E., Blair, D., Georges, A., Lukoschek, V., & Jerry, D. R. (2014). A biogeographical history and timeline for the evolution of Australian snapping turtles (Elseya: Chelidae) in Australia and New Guinea. *Journal of Biogeography*, *41*, 905–918.

Todd, E., Blair, D., Hamann, M., & Jerry, D. (2011). Twenty-nine microsatellite markers for two Australian freshwater turtles, *Elseya albagula* and *Emydura macquarii krefftii*: development from 454-sequence data and utility in related taxa. *Conservation Genetics Resources*, *3*, 449–456.

Walker, D., & Avise, J. C. (1998). Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annual Review of Ecology and Systematics*, *29*, 23–58.

Wang, J. L. (2009). A new method for estimating effective population sizes from a single sample of multilocus genotypes. *Molecular Ecology*, *18*, 2148–2164.

Waples, R. S., & Antao, T. (2014). Intermittent breeding and constraints on litter size: Consequences for effective population size per generation (Ne) and per reproductive cycle (Nb). *Evolution*, *68*, 1722–1734.

Waples, R. S., & Do, C. (2008). LDNE: A program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, *8*, 753–756.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population-structure. *Evolution*, *38*, 1358–1370.

Willoughby, J. R., Sundaram, M., Wijayawardena, B. K., Kimble, S. J. A., Ji, Y. Z., Fernandez, N. B., … DeWoody, J. A. (2015). The reduction of genetic diversity in threatened vertebrates and new recommendations regarding IUCN conservation rankings. *Biological Conservation*, *191*, 495–503.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Schmidt DJ, Espinoza T, Connell M, Hughes JM. Conservation genetics of the Mary River turtle (*Elusor macrurus*) in natural and captive populations. *Aquatic Conserv: Mar Freshw Ecosyst*. 2017;1–9. https://doi.org/10.1002/aqc.2851