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The status of the critically endangered freshwater crayfish *Engaewa pseudoreducta* (Crustacea: Parastacidae) in south-western Australia

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ABSTRACT – An update of taxonomic, distribution and conservation information for the freshwater crayfish *Engaewa pseudoreducta* Horwitz and Adams is presented. *Engaewa pseudoreducta* is formally listed as Critically Endangered by the state of Western Australia and under Australian national legislature, and informally in a recently completed international (IUCN Red List) global assessment of freshwater crayfish. *Engaewa pseudoreducta* was first described on the basis of morphological characters of a small number of individuals from a single site, however the type locality was known to no longer support a population at the time of description and only one other nearby population was known prior to this study. This paper records two further populations and presents the first genetic analysis of the species. DNA sequence data support the recognition of *E. pseudoreducta* as a species and despite the discovery of additional populations there appears no reason to lessen the conservation concern surrounding the species, particularly in light of continuing development in the area.

KEYWORDS: *Engaewa*, conservation, molecular systematics, freshwater crayfish, 16S, south-western Australia

INTRODUCTION

The first scientific collections of obligate burrowing freshwater crayfish in south-western Australia were made in the late 1950s and, following subsequent collections in the early part of the next decade, Riek (1967) described three species, each from a single locality, and placed them within a new genus *Engaewa* Riek, 1967. As part of wide scale revisions of species in the freshwater crayfish family Parastacidae in Australia throughout the 1980s and 1990s (e.g. Austin 1986, 1996; Morgan 1986, 1988, 1997; Horwitz 1990; Horwitz, Adams and Baverstock 1990; Zeidler and Adams 1990; Austin and Knott 1996), Horwitz and Adams (2000) reviewed the status of the genus *Engaewa*. In doing so they described an additional two species (*E. pseudoreducta* Horwitz and Adams, 2000 and *E. walpolea* Horwitz and Adams, 2000) and proposed the first realistic species boundaries, after Riek (1967) initially suggested species boundaries of the three original species (*E. reducta* Riek, 1967, *E. similis* Riek, 1967 and *E. subcoerulea* Riek, 1967) based on collections from only four localities.

Horwitz and Adams (2000) recorded the occurrence of 41 populations across all five species (*E. pseudoreducta* – 1; *E. reducta* – 5; *E. similis* – 19; *E. subcoerulea* – 12; *E. walpolea* – 4) (based on their distribution map). All species in the genus show narrow geographical distributions with *E. pseudoreducta* being the quintessential example – its entire known range prior to this study constituted significantly less than 3 km^2. The species was originally described from a collection made in 1985 in a clay-based wetland at the headwaters of a stream, some 15 km ENE of Margaret River. This site became the type locality for the species, but has subsequently been converted to a farm dam. The local catchment was converted to a blue gum (*Eucalyptus globulus*) plantation. Concern for *Engaewa pseudoreducta* was raised by Horwitz and Adams (2000) since it could no longer be found at the type locality following the modification of habitat and had only been found at one other site in the next drainage line to the west. *Engaewa pseudoreducta* was subsequently gazetted on Schedule 1 (Fauna that is rare or is likely to become extinct) (Wildlife Conservation (Specially Protected Fauna) Notice 2006), under the Western Australian Wildlife Conservation Act 1950, on the criteria that it had very restricted areas of occurrence and occupancy, with extreme fluctuations in area, extent and/or quality of habitat, and number of locations or subpopulations.

Recovery planning by the State's Department of

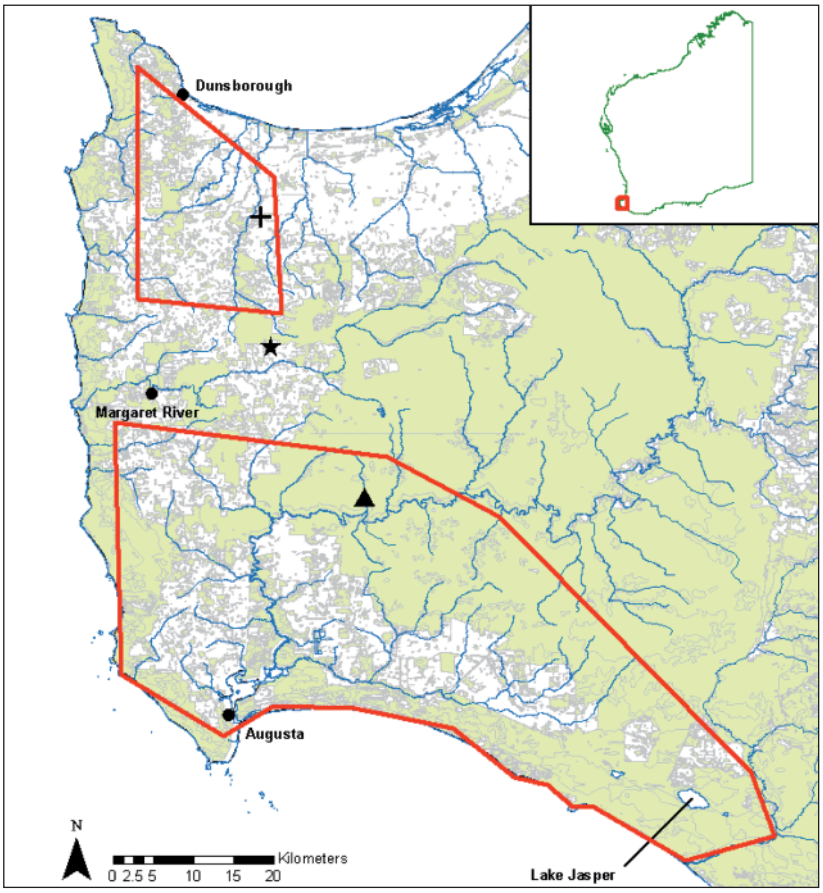

FIGURE 1 Location of important populations for this study (showing known *E. pseudoreducta* population (star), a new population at Payne Rd (cross), and *Clade A* population (triangle). Also shown are the approximated distribution polygons for *E. reducta* (northern) and *E. similis* (southern) as considered by Riek (1967) and Horwitz and Adams (2000). The search area for this study encompassed suitable habitat between Dunsborough and Lake Jasper.

Environment and Conservation commenced in 2007 for this species (and two others in the genus listed as threatened), during which time nominations were prepared for federal recognition and in 2009 the species was gazetted as Critically Endangered under the Commonwealth of Australia's EPBC Act (1999). Recovery operations also included an extensive survey of the geographical range of the genus by the lead author, in the hope that additional populations of *E. pseudoreducta* could be found, and to determine the genetic variability between this species and others in the genus, for any populations found. This paper presents the findings of these investigations and discusses their significance in view of the conservation status of the species.

MATERIALS AND METHODS

As *Engaewa* spend virtually their entire life below ground, one immediate challenge is to confirm their presence at a site. In order to direct collection efforts, in the first instance potential habitat was identified using a combination of maps and satellite imagery, looking for small creeks or potentially larger swamp systems that possessed a significant canopy of native vegetation. This approach created a list of potential habitat sites that required ground-truthing. Areas that appeared to be suitable from maps and imagery were often not so once visited and vice versa, hence the only reliable way to assess an area was to visit it. As such, virtually every accessible creek, drainage line, swamp or seepage with some degree of native vegetation remaining within the search area was examined.

An important corollary here is that non-detection of *Engaewa* at a particular site cannot be taken as definitive evidence of its absence (i.e. a false negative), which is true of all presence-absence records (MacKenzie 2005). However, significant limitations to the likelihood of detection notwithstanding intense survey effort are especially pertinent for *Engaewa*, and of great importance for a critically endangered species such as *E. pseudoreducta*. Notable impediments to detection of *Engaewa* are the species' cryptic, burrowing nature, the difficulty seeing and accessing burrows in often dense vegetation and gaining access (by road) to potential sites during the wet season when the animals are most active and the soil is suitable for digging.

For *Engaewa pseudoreducta* the search area was defined as being between Dunsborough and Lake Jasper, east of Augusta (Figure 1), a distance of approximately 150 km following the coastline and extending 40 km inland. This is far beyond its known distribution and also encompasses the ranges of two other species considered, with *E. pseudoreducta*, to represent a species-complex in the genus (Horwitz and Adams 2000). All areas of potentially suitable habitat occurring on Crown Lands were examined, as well as a number of sites located on private property throughout the assumed potential distribution of the species. On site, the presence of *Engaewa* species was indicated by the occurrence of 'chimneys' of pelleted soil at the entrance to a burrow system (Figure 2). These piles of soil are formed when material is expelled from the burrows dug by the crayfish. The individual pellets can be up to 1–2 cm in diameter, though generally are much smaller, and the chimney can range from less than half a dozen small pellets surrounding a small hole to a conical shaped chimney up to 35 cm high and formed from tens or even hundreds of individual pellets. The soil forming the chimney may be distinctly pelleted or it may appear as a simple pile of soil, due to the effect of weathering. Where obvious chimneys were lacking, closer attention was paid to any patches of different coloured soil or even simple holes in the ground that may also signal the entrance to a burrow should the chimney have weathered away entirely.

The presence of chimneys is not definitive evidence of inhabitation by *Engaewa* species because members of the freshwater crayfish genus *Cherax* also construct chimneys in particular soil types. An experienced surveyor, however, can usually recognise slight variations in the characteristics of the chimneys produced by these different crayfish. *Engaewa* chimneys are usually far more substantial and the pellets of soil much smaller. *Cherax* species typically dig short, straight tunnels and, as such, have small chimneys with much larger pellets due to their larger body size. *Cherax* chimneys also often form a miniature caldera, whereas *Engaewa* chimneys almost always appear conical. The diameter of the tunnel extending vertically from the chimney is also characteristic as *Engaewa* burrows are much smaller in diameter (approximately a 'pinky' finger in width) when compared to a *Cherax* burrow (often in the range of middle finger to thumb in width and, at times, larger). Once a potential *Engaewa* species burrow had been identified, it was excavated, and if crayfish belonging to the genus *Engaewa* were found, they were collected for morphological and molecular analyses.

FIGURE 2 Two 'chimneys', formed by spherical pellets of soil, which indicate the entrance to an *Engaewa* burrow system.

Crayfish were initially identified according to the diagnostic morphological characters described by Horwitz and Adams (2000) (for *E. pseudoreducta* the most easily recognised diagnostic characters were the presence of patches of setae on the ventral surface of the merus, ventrally and distally on the carpus, laterally adjacent to cutting edges, and occasionally on the propodal palm as well). Genetic characterization was then undertaken to confirm or refute the species descriptions of Horwitz and Adams (2000).

DNA was isolated using the DNeasy Blood and Tissue Kit (QIAGEN) from tail or gill tissue of ethanol preserved specimens. PCR was used to amplify part of the mitochondrial large rDNA (16S rDNA), using total genomic DNA as a template, and primers 1471 (5′–CCTGTTTANCAAAAACAT–3′) and 1472 (5′–AGATAGAAACCAACCTGG–3′) (Crandall, Lawler and Austin 1995; Crandall and Fitzpatrick 1996), and HotStarTaq Plus Master Mix (QIAGEN). The cycling conditions were an initial denaturing step (94°C for 5 min), 35 cycles of denaturing (94°C for 30 sec), annealing (46°C for 30 sec) and extension (72°C for 45 sec), and a final extension step (72°C for 7 min). PCR products were sent to Macrogen Inc. (Seoul, South Korea) to be purified and sequenced automatically and directly using the ABI BigDye chemistry. Chromatograms were checked manually and edited by comparing the sequence derived from forward and reverse primers in FinchTV v.1.4 (http://www.geospiza.com/Products/finchtv.shtml). The consensus sequences were then aligned using MUSCLE (Edgar 2004) as implemented in MEGA5 (Tamura *et al*. 2011).

The phylogenetic relationships between specimens were inferred using Maximum Likelihood (ML) analyses, conducted in PhyML v2.4.4 (Guindon and Gascuel 2003) based on the best substitution model selected by jModeltest v3.7 (Posada 2008) under the Akaike Information Criterion (Akaike 1974) with support for nodes assessed by non-parametric bootstrap (Felsenstein 1985) with 1000 bootstrap replicates. The non-rooted bootstrap tree was visualised with Figtree v.1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). In order to further discuss species boundaries we calculated genetic distances within and between clades identified in this study and *Engaeus* Erichson, 1846 using maximum composite likelihood in MEGA5 (Tamura *et al*. 2011). Following Schultz *et al*. (2009) the genus *Engaeus* was viewed as two distinct clades, *Engaeus sensu stricto* and *Engaeus lyelli*.

The appropriate conservation status of *Engaewa pseudoreducta* was assessed based on the current IUCN/EPBC criteria. Areas of occurrence and occupancy for *E. pseudoreducta* were calculated by drawing polygons in Google Earth (version 6.0.1.2032 beta) and then the saved KML files were entered into the University of New Hampshire Cooperative Extension Tools web-based polygon program (http://extension.unh.edu/kmlTools/index.cfm).

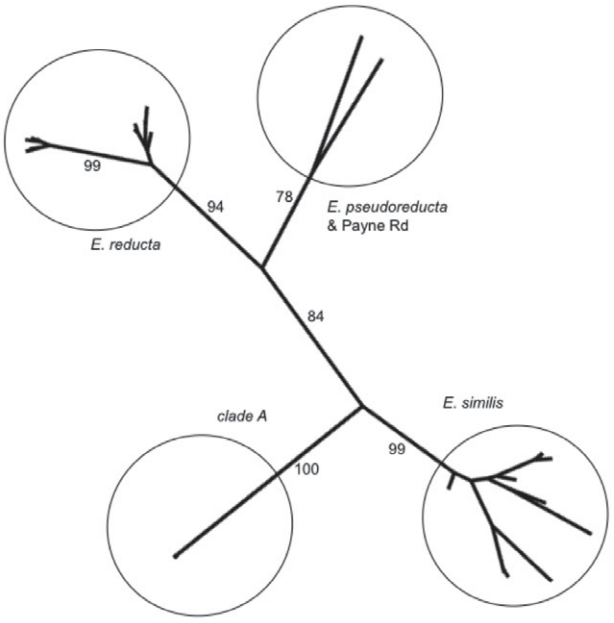

FIGURE 3 Unrooted ML tree for 47 specimens of *Engaewa* constructed from 394 basepairs of the 16S mtDNA, with boostrap support (1000 replicates) shown for the major branches in the tree. Clear molecular support for four clades identified by morphological characters (*E. pseudoreducta* (including the newly uncovered population at Payne Road), *E. reducta*, *E. similis* and *Clade A*) is evident from this tree.

RESULTS

Engaewa were collected from 26 sites throughout the study area and 47 specimens from these sites were analysed for this review. The specimens collected were deposited in the Western Australian Museum (Appendix 1). Based on the diagnostic morphological characters defined by Horwitz and Adams (2000), 22 specimens from 11 sites belong to *E. similis*, 17 specimens from 12 sites to *E. reducta*, one specimen belongs to *E. pseudoreducta* and two specimens (referred to as 'Payne Road') were morphologically consistent with the description of *E. pseudoreducta*, but occurred outside of its known distribution, while five specimens from a single locality had indeterminate character states (A full review of the genus is being prepared as part of the PhD thesis of the senior author.). The 47 new partial mitochondrial 16S rDNA nucleotide sequences were deposited on NCBI GenBank (accession numbers JQ613107 to 613153), whilst 23 *Engaeus* sequences from NCBI Genbank were included (19 *Engaeus sensu stricto* and 4 *Engaeus lyelli*) (Appendix 1).

The 16S analysis is presented as an unrooted ML tree showing that all specimens defined on the basis of their morphology also form monophyletic genetic

groups (Figure 3). Although not presented here, the same groupings are supported by other mitochondrial markers as well as nuclear markers – these data will be presented in the upcoming full review of the genus. The single specimen assumed to represent *E. pseudoreducta* and the specimens from 'Payne Road' are clearly divergent from all other species, are each others' nearest relatives and form a distinct clade. A fourth clade (referred to herein as *Clade A*) is formed solely by the representatives alluded to above as showing indeterminate character states.

With the exception of *Clade A* (representing a single population), each morphologically defined species shows at least two distinct genetic lineages within their respective clade (Figure 3). These genetic lineages correspond strongly to geographic partitioning of populations and are responsible for the relatively high genetic distances within these species (Table 1). This is particularly evident for the *reducta* and *similis* clades, which are relatively widespread (on the scale of *Engaewa* distribution), however, the significance of the relatively high genetic divergence between the two populations in the *pseudoreducta* clade (Figure 3, Table 1) is difficult to interpret without additional samples, either from the populations in question or any other populations that may be genetically similar (if any exist). Nevertheless the few specimens collected from these two sites do conform closely to the morphological description of the species *E. pseudoreducta* presented by Horwitz and Adams (2000).

Maximum composite likelihood genetic distances (Table 1) between *Engaewa* clades and *Engaeus sensu stricto* ranged from 0.294–0.324 and between *Engaewa* clades and *Engaeus lyelli* 0.369–0.396. In contrast, the distance between *Engaeus sensu stricto* and *Engaeus lyelli* was 0.279. The distances between *Engaeus sensu stricto* species ranged from 0.017–0.302 (data not shown) and those for *Engaewa* clades were in the middle of this range at 0.131–0.175. Within clade genetic distances were 0.172 for *Engaeus sensu stricto*, 0.035 for *Engaeus lyelli* and ~0.065 for *Engaewa* with the exception of *Engaewa Clade A*, representing a single population with a value of 0.003.

The *IUCN Red List Categories and Criteria: Version 3.1.* (2001) states that "A taxon is Critically Endangered when the best available evidence indicates that it … [is] considered to be facing an extremely high risk of extinction in the wild". The geographic range of this species, both in terms of the extent of occurrence and area of occupancy (criteria B1 and B2), falls well within the criteria for the category of Critically Endangered (<100 km^2 and <10 km^2 respectively). Furthermore, to fully satisfy criterion B2 and thus be validly considered as Critically Endangered, the species in question must conform to at least two of three further requirements. Whilst *E. pseudoreducta* are no longer believed to exist at only a single location there is no doubt that the distribution of populations is severely fragmented (preventing a metapopulation scenario whereby migrants can replace any localised extinctions), thus satisfying criterion B2a, and the loss of the largest known population at the type locality satisfies criterion B2b (an observed decline of extent of occurrence, area of occupancy, area, extent and/or quality of habitat, number of locations or subpopulations and number of mature individuals). The degree to which the natural environment has been altered since European colonization, and the resulting severe habitat fragmentation, can be easily inferred from the image in Figure 1. It can be reasonably argued that all of these can be 'inferred or projected' based on past and on-going anthropogenic impacts in the area, particularly when combined with suggested impacts of future climate change for the region (Horwitz *et al.* 2008).

TABLE 1 Composite maximum likelihood genetic distances (16S). Values on the diagonal (bold) represent the distances within species (standard errors < 0.02), those below the diagonal are distances between clades and those above the diagonal (italicised) are the associated standard errors.

N	Clade		[1]	[2]	[3]	[4]	[5]	[6]
3	*Engaewa pseudoreducta*	*[1]*	**0.065**	*0.024*	*0.027*	*0.026*	*0.046*	*0.067*
17	*Engaewa reducta*	*[2]*	0.175	**0.065**	*0.025*	*0.026*	*0.051*	*0.068*
22	*Engaewa similis*	*[3]*	0.153	0.161	**0.068**	*0.022*	*0.047*	*0.067*
5	*Engaewa Clade A*	*[4]*	0.156	0.164	0.131	**0.003**	*0.046*	*0.067*
19	*Engaeus sensu stricto*	*[5]*	0.312	0.324	0.304	0.294	**0.172**	*0.045*
4	*Engaeus lyelli*	*[6]*	0.386	0.396	0.387	0.369	0.279	**0.035**

TABLE 2 Locality and collection details for all known specimens of *E. pseudoreducta*.

Locality	Year collection made	Specimens collected	Site description and notes
"… near Osmington, north-east of Margaret River" [1]	*1985*	*5 adults* *5 juveniles*	"… burrows around a recently constructed farm dam, in the middle of a swamp with remnant vegetation consisting of tall ti-trees and some eucalypts; the soil in the area was a yellow–light brown silty sandy clay; in undisturbed parts of the small swamp, burrows ramified laterally just below the surface, and water was found in pools at the surface in August 1985. This site has undergone substantial change since then."[1]
Treeton Reserve Site 1	*2003*	*1 juvenile*	Burrows excavated in broad section of a densely-vegetated creek line, with high water levels, in November.
Treeton Reserve Site 2	*2007*	*1 adult*	"…found in the heavy clay soils of narrow valleys in and adjoining Treeton reserve. The burrows found during this study were identified by small piles of slightly different coloured soil. This soil is likely to have represented washed down chimneys as there had been significant rainfall and the burrows were within a small creek line. As the water table was so high at the time of collecting this species the burrow systems were not fully explored though they appeared to branch laterally at a shallow depth as well as possessing tunnels proceeding deeper."[2]
Payne Rd	*2007*	*2 adults*	Burrows indicated by partially weathered sandy chimneys. Crayfish dug out of coarse sand in a broad, flat area with sluggish and intertwined shallow draining channels with the water table just below ground level.

[1] From Horwitz and Adams (2000). [2] From Burnham et al. (2007).

DISCUSSION

Our findings suggest that *Engaewa pseudoreducta* should be recognised as a valid species on the basis of morphological and molecular (DNA sequence) data. In fact, morphological characters and DNA sequence data support the recognition of all three current species of *Engaewa* (*E. pseudoreducta, E. similis* and *E. reducta*) within the northern part of the range of the genus (roughly bounded by the region searched in this study) and suggest that an additional species (*Clade A*) should be recognised (species descriptions for this candidate species and another from the southern part of *Engaewa*'s range are currently being undertaken by the lead author). Prior to this study it had been recognised that a specific site within the study region contained "errant specimens [that] warrant closer examination" (Horwitz and Adams 2000, p. 677) and *Clade A* corresponds to the population referred to in that publication.

A comparison within and between the *Engaewa* species included in this study and a number of species from the closely related genus *Engaeus* (considered as two distinct clades *Engaeus sensu stricto* and *Engaeus lyelli* following Schultz *et al*., 2009) is made using 16S genetic distances. The pairwise distances between *Engaewa*, *Engaeus sensu stricto* and *Engaeus lyelli* are similar and support the presence of three clades, with *Engaeus sensu stricto* and *Engaeus lyelli* more similar to each other than either to *Engaewa*. The genetic distances within *Engaewa* clades had low variation (except *Clade A* which is from a single population and shows low diversity) and the distances between *Engaewa* clades are in the middle of the range for *Engaeus sensu stricto*. The genetic distances therefore support the current *Engaewa* molecular species groupings and morphological species descriptions, whilst also indicating the presence of an additional, undescribed species (*Clade A*).

Considering the conservation concern regarding *Engaewa pseudoreducta*, the dearth of specimens/populations to study and the lack of diagnostic morphological characters to distinguish between *E. pseudoreducta* specimens from the one drainage line which includes the type locality, and the specimen at Payne Road, it seems prudent to treat them as representatives of a single species until such time as the treatment of further specimens and/or other analyses can be undertaken. The genetic divergence between these

two samples hints at the possibility that interpopulation mtDNA diversity is extremely high for *E. pseudoreducta* (and the forthcoming review of the genus suggests that this is generally true for *Engaewa* as a whole); thus, making these existing populations even more significant from a conservation viewpoint. We therefore suggest that the currently acknowledged geographic range of *E. pseudoreducta* should include the drainage system from which the original description was made and be extended to include the population at Payne Road, some 16 km north (details of the sites from which *E. pseudoreducta* have been collected are presented in Table 2). We also note the presence of both *E. similis* and *E. reducta* at several sites very close to the drainage lines inhabited by *E. pseudoreducta*, though they have never been found in sympatry.

Including the population at Payne Road increases *E. pseudoreducta*'s known range twenty-fold, to a region of about 60 km^2. However, this figure somewhat misrepresents the distribution of the species; once unsuitable habitat is removed, the potential area of occupancy is probably less than 2.5 km^2 and the actual area of occupancy may well be significantly less again. Additional sampling should continue in the region in the hope of closing the geographic gap between the *E. pseudoreducta* populations. As noted above, *Engaewa* are highly cryptic due to their small size and almost exclusively subterranean existence, which makes the task of confirming their absence from potential habitat difficult. While we are confident that we have searched extensively, it is not impossible that one or more isolated, small populations remain undetected in this fragmented landscape.

The conservation status of Critically Endangered assigned to *E. pseudoreducta* under the EPBC Act can be re-examined using the data presented in this paper (and indeed this has been done for a recent review of the conservation status of all freshwater crayfish conducted for the IUCN Red List). The discovery of additional populations potentially bodes well for the survival of the species as a whole, although an increase from one to three populations is obviously not a reason to reduce concern, particularly as downstream habitat alteration has isolated all populations into small pockets of suitable habitat.

The inland aquatic biodiversity of south-western Australia, particularly in the coastal margins, is facing significant and increasing survival pressure due to large-scale human endeavours (Horwitz *et al.* 2008). Agriculture, urbanization, groundwater extraction and mining have all altered the natural character of the region. These changes increase the vulnerabilities of a species such as *E. pseudoreducta,* which appears to possess low dispersal ability, is wedded to highly restricted and disjunct habitat, and persists in only a few isolated populations. Based on our data we conclude that the conservation status of *E. pseudoreducta* (Critically Endangered) should remain unchanged.

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REFERENCES

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.

Austin, C.M. (1986). *Electrophoretic and morphological systematic studies of the genus Cherax (Decapoda: Parastacidae) in Australia*. PhD Thesis. (Department of Zoology, University of Western Australia: Perth.)

Austin, C.M. (1996). Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia: Electrophoretic and morphological variation. *Australian Journal of Zoology* **44**: 259–296.

Austin, C.M. and Knott, B. (1996). Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in south-western Australia: electrophoretic, morphological and habitat variation. *Australian Journal of Zoology* **44**: 223–258.

Burnham, Q., Koenders, A. and Horwitz, P. (2007). *Field studies into the biology and conservation requirements of Engaewa species in the South West and Warren DEC regions*. Final Report Prepared for the Department of Environment and Conservation, Perth.

Crandall, K.A., Lawler, S.H. and Austin, C.M. (1995). A preliminary examination of the molecular phylogenetic relationships of some crayfish genera from Australia (Decapoda: Parastacidae). *Freshwater Crayfish* **10**: 18–30.

Crandall, K.A. and Fitzpatrick, J.F. (1996). Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology* **45**: 1–26.

Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the Bootstrap. *Evolution* **39**: 783–791.

Guindon, S. and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Horwitz, P. (1990). A taxonomic revision of species in the freshwater crayfish genus *Engaeus* Erichson (Decapoda: Parastacidae). *Invertebrate Taxonomy* **4**: 427–614.

Horwitz, P. and Adams, M. (2000) The systematics, biogeography and conservation status of the species in the freshwater crayfish genus *Engaewa* Riek (Decapoda: Parastacidae) from south-western Australia. *Invertebrate Taxonomy* **14**: 655–680.

Horwitz, P., Adams, M. and Baverstock, P. (1990). Electrophoretic contributions to the systematics of the freshwater crayfish genus *Engaeus* Erichson (Decapoda: Parastacidae). *Invertebrate Taxonomy* **4**: 615–641.

Horwitz, P., Bradshaw, D., Hopper, S.D., Davies, P.M., Froend, R. and Bradshaw, F. (2008). Hydrological change escalates risk of ecosystem stress in Australia's threatened biodiversity hotspot. *Journal of the Royal Society of Western Australia* **91**: 1–11.

IUCN (2001). *IUCN Red List Categories and Criteria: Version 3.1.* IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

MacKenzie, D.I. (2005). What Are the Issues with Presence-Absence Data for Wildlife Managers? *The Journal of Wildlife Management* **69**: 849–860.

Morgan, G.J. (1986). Freshwater crayfish of the genus *Euastacus* Clark (Decapoda, Parastacidae) from Victoria. *Memoirs of the Museum of Victoria* **47**: 1–57.

Morgan, G.J. (1988). Freshwater crayfish of the genus *Euastacus* Clark (Decapoda, Parastacidae) from Queensland. *Memoirs of the Museum of Victoria* **49**: 1–49.

Morgan, G.J. (1997). Freshwater crayfish of the genus *Euastacus* Clark (Decapoda: Parastacidae) from New South Wales, with a key to all species of the genus. *Records of the Australian Museum, Supplement* **23**: 1–110.

Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25**: 1253–1256.

Pybus, O.G. (2006). Model selection and the molecular clock. *PloS Biology* **4**: 686–688.

Riek, E.F. (1967). The freshwater crayfish of Western Australia (Decapoda: Parastacidae). *Australian Journal of Zoology* **15**: 103–21.

Schultz, M.B., Smith, S.A., Horwitz, P., Richardson, A.M.M., Crandall, K.A. and Austin, C.M. (2009). Evolution underground: A molecular phylogenetic investigation of Australian burrowing freshwater crayfish (Decapoda: Parastacidae) with particular focus on *Engaeus* Erichson. *Molecular Phylogenetics and Evolution* **50**: 580–598.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* (submitted).

Zeidler, W. and Adams, M. (1990). Revision of the Australian crustacean genus of freshwater crayfish *Gramastacus* Riek (Decapoda : Parastacidae). *Invertebrate Taxonomy* **3**: 913–924.

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APPENDIX 1 Specimens used in this study and their GenBank accession numbers. All *Engaewa* sequences generated by the authors, whilst all *Engaeus* sequences were obtained from NCBI Genbank.

Taxon	Sample Identification	GenBank Accession No.
Engaewa pseudoreducta	WAM C49511	JQ613110
Engaewa pseudoreducta	WAM C49512	JQ613111
Engaewa pseudoreducta	WAM C49513	JQ613118
Engaewa reducta	WAM C49514	JQ613152
Engaewa reducta	WAM C49516	JQ613153
Engaewa reducta	WAM C49517	JQ613117
Engaewa reducta	WAM C49520	JQ613148
Engaewa reducta	WAM C49521	JQ613149
Engaewa reducta	WAM C49524	JQ613150
Engaewa reducta	WAM C49525	JQ613128
Engaewa reducta	WAM C49535	JQ613130
Engaewa reducta	WAM C49537	JQ613131
Engaewa reducta	WAM C49542	JQ613125
Engaewa reducta	WAM C49543	JQ613145
Engaewa reducta	WAM C49545	JQ613127
Engaewa reducta	WAM C49547	JQ613129
Engaewa reducta	WAM C49548	JQ613132
Engaewa reducta	WAM C49549	JQ613136
Engaewa reducta	WAM C49555	JQ613109
Engaewa reducta	WAM C49558	JQ613123
Engaewa similis	WAM C49560	JQ613146
Engaewa similis	WAM C49561	JQ613147
Engaewa similis	WAM C49562	JQ613112
Engaewa similis	WAM C49565	JQ613119
Engaewa similis	WAM C49566	JQ613120
Engaewa similis	WAM C49567	JQ613121
Engaewa similis	WAM C49568	JQ613133
Engaewa similis	WAM C49570	JQ613134
Engaewa similis	WAM C49571	JQ613108
Engaewa similis	WAM C49573	JQ613126
Engaewa similis	WAM C49575	JQ613135
Engaewa similis	WAM C49577	JQ613137
Engaewa similis	WAM C49578	JQ613138
Engaewa similis	WAM C49579	JQ613139
Engaewa similis	WAM C49580	JQ613140
Engaewa similis	WAM C49581	JQ613141
Engaewa similis	WAM C49582	JQ613142

Taxon	Sample Identification	GenBank Accession No.
Engaewa similis	WAM C49583	JQ613114
Engaewa similis	WAM C49586	JQ613115
Engaewa similis	WAM C49587	JQ613116
Engaewa similis	WAM C49588	JQ613122
Engaewa similis	WAM C49589	JQ613124
Engaewa Clade A	WAM C49676	JQ613144
Engaewa Clade A	WAM C49677	JQ613151
Engaewa Clade A	WAM C49678	JQ613107
Engaewa Clade A	WAM C49679	JQ613113
Engaeus cisternarius	Voucher Museum Victoria J45407	EF493110
Engaeus disjuncticus	Voucher Museum Victoria J45405	EF493102
Engaeus fossor	Voucher Museum Victoria J45510	EF493103
Engaeus fultoni	Voucher AIR2.1	EF493042
Engaeus hemicirratulus	Voucher Museum Victoria J14750	EF493104
Engaeus karnanga	Voucher Museum Victoria J45692	EF493105
Engaeus laevis	Voucher LEL1.1	EF493088
Engaeus lyelli	Voucher ENF1.2	EF493073
Engaeus lyelli	Voucher Museum Victoria J14710	EF493107
Engaeus lyelli	Museum Victoria J14711	EF493108
Engaeus lyelli	Voucher NRN2.1	EF493121
Engaeus mairener	Voucher Museum Victoria J45680	EF493109
Engaeus mallacoota	Voucher Museum Victoria J14713	EF493096
Engaeus martigener	Voucher Museum Victoria J45432	EF493111
Engaeus merosetosus	Voucher WPC2.1	EF493153
Engaeus nulloporius	Voucher Museum Victoria J4106	EF493112
Engaeus orientalis	Voucher Museum Victoria J14725	EF493113
Engaeus phyllocercus	Voucher Aus. Museum P67188	EF493041
Engaeus sericatus	Voucher PEN1.4	EF493125
Engaeus spinicaudatus	Voucher Museum Victoria J45696	EF493114
Engaeus strictifrons	Voucher TWH1.1	EF493149
Engaeus urostrictus	Voucher Museum Victoria J45681	EF493115
Engaeus yabbimunna	Voucher Museum Victoria J34475	EF493101