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Article *in* Aquatic Conservation Marine and Freshwater Ecosystems · June 2024

DOI: 10.1002/aqc.4172

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Received: 16 January 2024 | Revised: 2 April 2024 | Accepted: 22 April 2024

DOI: 10.1002/aqc.4172

ARTICLE

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Rediscovery, systematics and conservation of an enigmatic freshwater crayfish (Parastacidae) from the Australian monsoon tropics

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Funding information
The present study received donor funding from Janie Mason AM. The Museum and Art Gallery of the Northern Territory receives funding from the NT Government. Additional funding was received from the 2015–2020 State-Region Planning Contracts (CPER), European Regional Development Fund (FEDER), and intramural funds from the Centre National de la Recherche Scientifique and the University of Poitiers. All aspects were conducted, along with the decision to submit this manuscript for publication, by the authors independently of funding agencies.

Abstract

Freshwater wetlands are among the world's most valuable ecosystems, supporting diverse biota and critical ecological services, yet these habitats have suffered extensive and pervasive anthropogenic disturbance. Northern Australia represents a rare example of a relatively unmodified, vast wetland habitat. The freshwater crayfishes of the region are poorly documented, with one enigmatic species, the nutcracker yabby *Cherax nucifraga*, described from a single individual sampled opportunistically from the stomach of a predatory fish. Here we report on the rediscovery of *C. nucifraga* from a relatively limited distribution in semi-permanent coastal freshwater wetlands. Field studies were conducted to inform natural resource management and conservation. Genome skimming to recover mitogenomes, 18S–28S and histone sequences demonstrated a sister relationship with two congeners from the tropics, and moderate molecular genetic substructure was apparent within *C. nucifraga* between mainland and Melville Island locations. *Cherax nucifraga* is characterised by the presence of a strawberry-coloured soft patch on the outer margin of the claw, uniquely present in both mature males and females. Meristic and multivariate morphometric comparisons are made with the co-occurring redclaw *Cherax quadricarinatus* and the allopatric whiteclaw yabby *Cherax bicarinatus*, with a summary of diagnostic traits developed into a visual guide and key. Programs to further understand ecology, threats and traditional ecological knowledge will help to inform the future conservation management of the species in the face of increasing development and environmental change to northern Australian coastal freshwater wetlands. Specific conservation actions include identifying and protecting refuge habitats and preventing incursions by other *Cherax* species.

KEYWORDS

conservation, freshwater crayfish, genome skimming, molecular genetics, Parastacidae, rediscovery, wetlands

1 | INTRODUCTION

Freshwater crayfish (family Parastacidae) are considered a highly imperilled group of aquatic invertebrates attributed to over-exploitation, habitat degradation and destruction, alien species and disease and climate change (Bland, 2017; Hossain et al., 2018). Around one-third of global freshwater crayfish species are threatened with extinction, with the greatest number of threatened species recorded in Australia (Coughran & Furse, 2012; Richman et al., 2015). And there is a lot to lose, as Australia, despite being a largely arid continent, has an old, highly specious, distinctive and ecologically diverse crayfish fauna (Coughran & Furse, 2012). The fauna comprises some 10 genera and 167 species and counting (Burnham & Dawkins, 2013; Crandall & De Grave, 2017), and all are endemic other than the genus *Cherax* which is shared with the biogeographically adjoined New Guinea (Austin, 1996; Munasinghe et al., 2004). Functional diversity encompasses the world's largest growing freshwater species as cool-water perennial flow specialists (*Astacopsis*, *Euastacus*), small growing swamp specialists (*Tenuibranchiurus*), wetland species with symbiotic relationships (*Grammastacus*, *Geocharax*), generalists that have colonised large areas of the continent including the arid interior (*Cherax*), and to others that are adapted to a more terrestrial than aquatic lifestyle with elaborate burrows (*Engeus*, *Engaewa*) (Beatty et al., 2005; Horwitz & Richardson, 1986; Johnston & Robson, 2009).

Many species of Australian freshwater crayfish have adaptations suited to seasonal inundation in swamps or laterally connected riparian and floodplain habitats where they have significant roles as keystone species in aquatic trophic webs and as ecosystem engineers (Johnston & Robson, 2009; Reynolds et al., 2013). This occurrence and ecological importance within wetland ecosystems, a habitat type of global decline and concern (Fluet-Chouinard et al., 2023; Kingsford et al., 2016), implies the need to manage specific ecological and conservation requirements (Acosta & Perry, 2001; Bloomer et al., 2022). However, such conservation planning for freshwater crayfish is often inhibited by limited ecological knowledge, and taxonomic uncertainty (Coughran & Furse, 2012; Duffy et al., 2014; Whiterod et al., 2022). This is especially true for remote northern Australia where targeted surveys of freshwater crayfish species have been limited, with taxonomic descriptions restricted to opportunistically obtained samples, resulting in patchy distributional records (Austin, 1996; Pusey, 2011; Short, 1993).

The freshwater fauna of northern Australia includes eight valid species of *Cherax* (Figure 1). The most widespread and well-known species is the redclaw *Cherax quadricarinatus* (von Martens), a large crayfish prominent in recreational fisheries and traditional culture, that has been commercially grown for aquaculture since the mid-1980s (Austin, 1996; Rigg et al., 2020). Significant introductions to the wild have occurred as a consequence of this popularity, and the species is now widely established outside its natural range within Australia and in more than 20 other countries (Burrows, 2004; Doupé, 2007; Haubrock et al., 2021; King et al., 2022). Mature adult males have a conspicuous uncalcified bright red-coloured 'soft patch' on the distal outer margin of the first chelipeds (claws) (Karplus et al., 2003). This is a highly unusual trait in crayfish, unique to a subset of tropical *Cherax* species from northern Australia and New Guinea, varying in colour, size and position, and thought to be present only in mature males (Austin, 1996; Patoka, 2020). Many members of the genus bearing the soft patch are also popular in the aquarium trade (Faulkes, 2015).

At the other extreme, the most poorly known species from tropical Australia, and arguably Australia's most enigmatic crayfish species, is the nutcracker yabby *Cherax nucifraga* Short. The species is known only from a single individual discovered opportunistically inside the stomach of a large predatory fish, barramundi *Lates calcarifer*, in 1983 on the Reynolds River floodplain. The sample was deposited in the Museum and Art Gallery of the Northern Territory (NTM) collection, and then sometime later, noticed as distinct and described as new to western science (Short, 1991). The specimen, a large male, has claws with a wide spacing between the fingers, and an extended projection (large tubercle) on the inside of the small dactyl (finger) creating the appearance of a nutcracker device providing the genesis for the common name and etymology (Short, 1991). As a consequence of the unusual origin of this sample, virtually nothing is known regarding the distribution, ecology and live appearance (e.g. colour and sexual dimorphism) of the species. The recorded location of the ingested *C. nucifraga* specimen is in a remote coastal region with limited vehicle and boat access and comprising a large area of seasonally inundated floodplain, dense with aquatic vegetation and dominated by the apex predator, saltwater crocodile *Crocodylus porosus* — all factors that make field sampling of freshwater crayfish restrictive and challenging and that may have contributed to the lack of additional collection records. Unsurprisingly the species is listed as Data Deficient on the IUCN Red List of Threatened Species (IUCN, 2023) with no formal listing within local or national conservation and fisheries legislation.

Coastal catchments of northern Australia are unique by global standards in terms of having expansive wetlands in good habitat condition, currently having limited levels of hydrological disturbance and land reclamation, and few introduced alien fishes or crayfishes (Kingsford et al., 2016; Pusey et al., 2017). However, there are increasing pressures for water resource development, potentially alien fishes (which loom in adjoining regions and with some recent incursions), and much of the area is subjected to direct or indirect effects of agriculture, including grazing, feral herbivores and weeds (Finlayson et al., 2005; Hammer et al., 2019b; Pusey, 2011). Climate change will have an additive effect, with specific impacts on low-lying coastal wetlands (Karim et al., 2015; Nielsen et al., 2020). Proactive efforts to help conserve species and ecosystem function before major declines will be aided by detailed knowledge of species occurrence and ecology.

The present study reports on the rediscovery after 40 years of *C. nucifraga*, involving the first documented living specimens from multiple locations, including both males and females and a range of life history stages. Through the combination of field investigations, molecular genetic sequencing, morphological measurements and

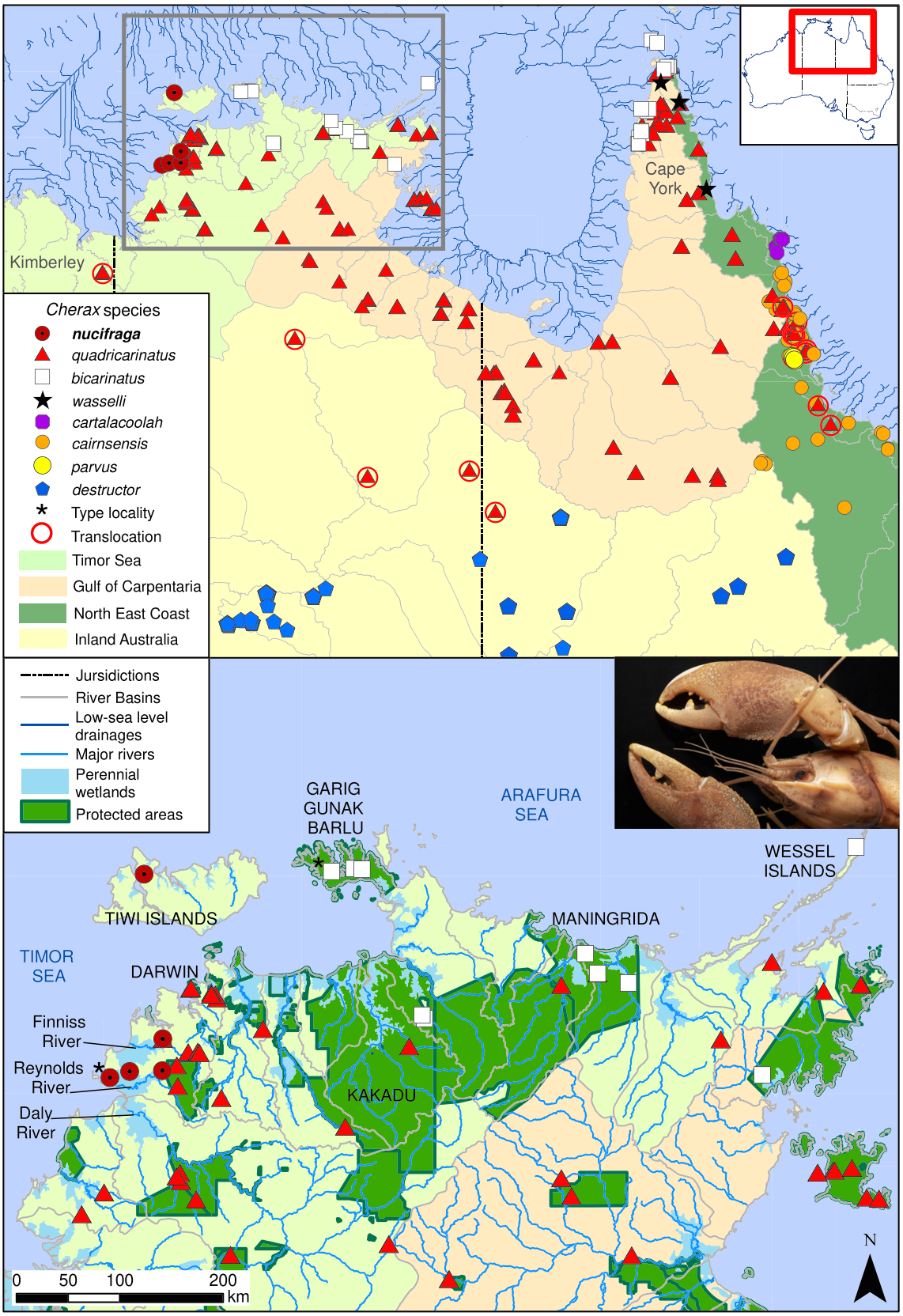


FIGURE 1 *Cherax* species in tropical Australia with a focus on *Cherax nucifraga* (darker red circles) showing original holotype sampled from a predatory fish stomach (*photo inset*). Crayfish distribution records based on physical examination of museum specimens over several decades, publicly available genetic sequences including those from this study, curated museum point data sourced from Atlas of Living Australia and literature review of translocations. Low-sea level drainages from Unmack (2001).

taxonomic comparisons, we aim to enhance knowledge on the recognition and identification of this previously poorly known species and begin to understand its ecology and environmental requirements as key information to input for land and resource management.

2 | METHODS

2.1 | Regional setting and taxonomic context

Northern Australia contains four distinctive drainage divisions, with the Timor Sea and Gulf of Carpentaria the primary focus of the current study (Figure 1). These divisions drain a large area of the costal catchment (~1,200,000 km^2) stretching from the Kimberley in the west across to the Cape York Peninsula in the east (Pusey et al., 2017). As a combination of relatively flat topography and a monsoon tropical climate, the region contains vast areas of wetlands (~30% of catchment area); wetland extent can fluctuate drastically in tune with prevailing climatic conditions where rain is concentrated in the 'wet season' from around December to April (Pusey, 2011, Hammer et al., 2019b; Figure 1).

The eight valid species of *Cherax* from northern Australia occupy differing geographic areas and habitats (Figure 1). The *C. nucifraga* holotype originated from the central part of the Timor Sea Drainage Division. This region is also inhabited by *C. quadricarinatus*, a species that prefers permanent freshwater bodies and is found in streams, billabongs and the margins of larger rivers (Austin, 1996; Baker et al., 2008), and a third species of *Cherax*, popularly known as the whiteclaw yabby (more of a wetland/swamp specialist), which has a particularly complex taxonomic and nomenclatural history. Here we refer to this species as *Cherax bicarinatus* (Gray), a position that is supported by the data presented in this study combined with an analysis of 16S rRNA sequences from GenBank, which indicates that this species is identical to *Cherax barretti* Clark described from a single specimen from the Wessel Islands, and *Cherax rhynchotus* Riek described from the tip of the Cape York Peninsula (Austin, 1996; Munasinghe et al., 2004; Riek, 1969). The older name *C. bicarinatus* has priority, and was described with a type locality of Port Essington settlement (King River, Garig Gunak Barlu National Park, Coburg Peninsula); topotypic samples of the three names were included in the current study. The nomenclatural history, taxonomy and genetics of *C. bicarinatus* will be dealt with more fully elsewhere.

Collectively *C. nucifraga*, *C. bicarinatus* and *C. quadricarinatus* are the Australian representatives of the soft patch-bearing group of *Cherax* and are the main focus of the study. Four tropical species from the Australian east coast, namely *Cherax cairnsensis* Riek, *Cherax cartalacoolah* Short, *Cherax parvus* Short & Davie and *Cherax wasselli* Riek are included for broader comparative purposes based on existing available genetic data (Austin, 1996, Munasinghe et al., 2004). *Cherax destructor* Clark also occurs in northern Australia (Figure 1) in inland drainages and is included in the genetic part of this study. Finally, for assessment of deeper relationships and for species benchmarking purposes representatives of the *Cherax* species from southwest Western Australia were included in the main genetic study, along with the outgroups *Euastacus armatus* (von Martens) and *Astacopsis gouldi* Clark.

2.2 | Field sampling approach

Given the uncertainty in where *C. nucifraga* may actually occur, a forensic-type approach was undertaken to investigate sources and adaptively inform field searches. Sampling focused on the early dry season following wetland inundation but at a point when locations became accessible by road after water recession. Methods included yabby nets (opera house style 0.7 m length with two ring openings; note this net type is being phased out in Australia in favour of pyramid styles to protect air-breathing fauna), bait traps (0.5 m length × 0.24 m width × 0.24 m height, 60 mm entrance), dip nets (0.3 m diameter square head, 4 mm mesh) and physical searches for animal remains such as claws around the water edge and nearby bird roosts (all where safe to do so with respect to the presence of *C. porosus*). The sampling included around 30 sites from the Daly River around to Darwin. Captured crayfish were returned live to the lab in individual containers, photographed, and then euthanised with an overdose of the anaesthetic AQUI-S® following manufacturer instructions, before being lodged in the NTM collection. Comparative material for genetic and morphological studies was sourced through fieldwork and the NTM collection. Environmental descriptors were recorded covering differing aspects of underwater cover, edge vegetation, water level, flow and water quality. The study was conducted in accordance with an NT Fisheries Permit (S17/3418) and following the Australian code for the care and use of animals for scientific purposes.

2.3 | Molecular data (genome skimming)

This study employed genome skimming to obtain molecular genetic data, which consists of the generation of low-coverage Illumina data (Grandjean et al., 2017; Tan et al., 2021). From the Illumina reads it was possible to recover complete mitochondrial genomes, 18S and 28S sequences and four nuclear histone sequences. Previous attempts to obtain molecular data from the *C. nucifraga* holotype failed, due most likely to its original preservation in formalin. Genome skimming to extract the mitochondrial genome, histone (H2A, H2B, H3, H4) and ribosomal RNA (18S, 28S) genes was performed largely as previously described (Grandjean et al., 2017, Tan et al., 2021). Briefly, the mitochondrial genome, the 18S and 28S genes and histone genes were recovered by mapping *de novo* assemblies to baits (sequences) of the same or related species for the target gene regions. In most cases, a single contig was recovered for each of the mitochondrial genomes, the 18S–28S contig and the four histone genes. SPAdes 3.15.5 (http://cab.spbu.ru/software/spades) was used to generate de novo assemblies in assemble-only mode using in the range of 3–5 million reads, with reads first trimmed using BBDuk (v38.84) (http://sourceforge.net/projects/bbmap). The mitogenome,

18S–28S contig and histone contig for each sample were mapped to the full sequence data set to check assembly accuracy and generate coverage statistics. All assemblies and analyses described above were carried out using Geneious Prime® 2023.2.1. Phylogenetic analyses were conducted using IQ-TREE essentially as described by Gan et al. (2018) using the IQ-TREE web server (Trifinopoulos et al., 2016). Individual trees were estimated for each set of genetic data (mitochondria, 18S–28S and histones) and the data were concatenated to generate a total evidence tree, with the data partitioned on the basis of the three gene types.

In addition to the main study, 16S rRNA sequences were downloaded from GenBank for all other northern Australian *Cherax* species and compared with the sequences generated from genome skimming also using the IQ-TREE web server.

2.4 | Morphological data

Direct morphological comparisons were made for the three soft patch species (*C. nucifraga*, *C. bicarinatus* and *C. quadricarinatus*) as a natural geographic, morphological and genetic grouping. Measurements and counts were made on the right-hand side of the body or chelipeds, unless damaged or regenerative. A total of 21 morphometric characters were assessed as detailed in Figure S1. Twelve of these consisted of general measurements to characterise variation as used previously for systematic studies of the genus *Cherax* (Austin & Knott, 1996), with additional measurements taken in relation to the position of the first rostral spine and the position of the soft outer patch on the propodus specific to the species under consideration. Measurements were made with digital callipers to the nearest 0.1 mm, with ratio data explored statistically using XLSTAT 2016.1.01 (Addinsoft™) employing Principal Components Analysis (PCA) (Pearson Correlation matrix with Varimax rotation). Qualitative or meristic data were obtained on the following characters: soft patch (presence or absence), number of rostral spines (RS) (right side and total, RST), the number of spines on the carpus (CS) and number of tubercles/spines along the mesial margin of propodus (PT), with a qualitative assessment of the development of proximal rostral spine (PS: rounded tubercle, blunt spine or sharp spine) and patterns of ridges on the dorsal surface of the carapace (cephalon), specifically the extent of the inner rostral carina relative to the anterior end or length of the postorbital ridge (RC: reaching, reaching beyond, reaching well beyond).

3 | RESULTS

3.1 | Rediscovery

The original registration data for the holotype male and only previously known specimen of *C. nucifraga* (NTM Cr007430; 44.6 mm occipital carapace length, OCL) indicated it was obtained from the stomach of a *L. calcarifer* caught at Palm Springs near Channel Point by Nimrod Safaris (NTM database; Short, 1991). Further lines of enquiry indicated Palm Springs can connect to a tidal creek just to the south of the small community at Channel Point in times of flood (matching to the wet season timing of collection of 19/3/1983) as part of runoff from the floodplain with wide connectivity/inundation. Moreover, around the time the specimen was collected, Hilton Graham, who was with Nimrod Safaris, was helping NT Fisheries catch samples of the highly mobile *L. calcarifer* (assumedly for biological data including stomach content) in the tidal creek (G. Webb pers. comm., 2021). This information helped to shift the search from a specific location/specialised habitat to a broader environment type (floodplain).

Physical searches in the early dry season of 2021 in proximity to the holotype location (Reynolds River floodplain) recovered a desiccated claw (NTM Cr019410) with physical characteristics matching *C. nucifraga*, from the margin of a drying wetland pool. The find was evidentially the remains of a predator's meal and was accompanied by three desiccated legs. A small vestigial piece of cartilage was found in one of the legs and successfully used for DNA extraction. The following year after the wet season and the recession of water levels, three live *C. nucifraga* were trapped in a roadside pool on the western Finniss River floodplain within the broader initial search area (the Reynolds and Finniss River floodplains interconnect during inundation). These consisted of two males (32.4–33.5 mm OCL) and a female (36.2 mm OCL) which were photographed and then preserved for morphological assessment (NTM Cr019501–3). Follow-up surveys at the same site later in the dry season when water was more concentrated recovered 10 discarded claws scattered around the pool edges, accompanied by bird droppings suggesting predation, most likely by cormorants (NTM Cr019506). By using a similar search image for bird roosting/feeding locations, a site on the eastern side of the Finniss floodplain was located, a small anabranch within a swampy habitat (three claws: NTM Cr019507). A return to this site in the mid-wet season between monsoon rain bursts in January 2023, revealed high (bank full) water levels including flooded lateral edges, and a number of juvenile crayfish were dip netted from the shallows at access points safe from crocodiles. After microscopic examination these were confirmed based on head ridge characteristics (see Morphological comparisons), as a mix of *C. nucifraga* (n = 9, 6.7–12.7 mm OCL) and *C. quadricarinatus* (n = 11, 4.6–9.3 mm OCL) (NTM Cr019532–3 and Cr019534–5 respectively). Checks for claws were also made on several visits to the lower Daly River area, but no signs of the species were evident.

A summary of environmental variables at field sampling sites is provided in Table 1. The site in the Reynolds River system was close to the coast in a broad flat seasonal floodplain habitat dominated by grasses, sedges and patches of *Pandanus* with variable levels of submerged aquatic macrophytes including the algae *Chara*; this is likely to be representative of the area, other than additional denser patches of *Melaleuca* near springs. The presence in deeper water in more perennial areas of the floodplain remains to be determined. The site on the western Finniss floodplain is further inland (50 km from the sea and 5 km below the Litchfield Escarpment) in a loosely

TABLE 1 Collated sampling records in tropical Australia for *Cherax nucifraga* including record type (adult or juvenile specimen, or claw only) and water quality (where known), holotype indicated (*).

Site	NTM reg.	River Basin	Location	Date sampled	Latitude	Longitude	Adults	Juveniles	Claws only	Temperature	pH	KH	GH	Salinityppm
N1	Cr007430*	Reynolds	Palm Springs, near Channel Point	19/03/1983	−13.1730	130.1660	1 M							
N2	Cr010290	Tiwi	Four Mile Swamp, Melville Island	28/03/1991	−11.4016	130.4658	1 M							
N3	Cr019410	Reynolds	Drying floodplain, Channel Point	15/07/2021	−13.1198	130.3404			1	30.5				183
N4	Cr019501–3	Finniss	Finniss River floodplain crossing	16/05/2022	−13.1135	130.6246	2 M, 1F			32.1	6.5	0	30	26
N5	Cr019506	Finniss	Finniss River floodplain crossing	2/06/2022	−13.1135	130.6246			10					Dry
N6	Cr019507	Finniss	Finniss River anabranch	3/06/2022	−12.8367	130.6290			3					Dry
N7	Cr019532–3	Finniss	Finniss River anabranch	26/01/2023	−12.8367	130.6290		9		30.2	6.5	0	0	20
N8	Cr019556–7	Finniss	As above, on-grown in aquarium	5/07/2023	−12.8367	130.6290	2F							

defined broad channel on the upper floodplain with interspersed ephemeral dry season pools, again thick with grasses, sedges and denser *Pandanus*. The site on the eastern Finniss River floodplain was at the terminus of the main river channel as it debouches and transitions to the floodplain, being on a small anabranch parallel with the main channel connected to littoral *Melaleuca* swamps. Crayfish burrows were observed in the dry season along the vertical banks of the anabranch channel and in *Acacia* root masses. In the wet season during high water levels, juveniles of both *C. nucifraga* and *C. quadricarinatus* were recorded in sympatry, dip netted together among leaf litter and small woody debris along shallower edges of the channel. Water chemistry at the time of collection for mainland *C. nucifraga* sampling sites was fresh (20–183 ppm), warm (>30 °C), slightly acidic (pH 6.5) and soft (carbonate and general hardness <30 ppm), with water tannin-stained (transparency ranged from 0.3–0.6 m).

The nutcracker-like claw of the holotype was suggested to be of use for "cracking" aquatic gastropod (snail) shells (Short, 1991), and while this cannot be ruled out, observations in aquaria indicated the 'fingers' of the second cheliped (walking legs) were deployed for this task. Moreover, the claw structure appeared to have a 'raking' function for gathering fine aquatic macrophytes for consumption (e.g. *Chara*). Fast growth was observed in captivity with two of the juveniles captured, both female, held for a period growing from 11–12 mm to 25–30 mm in 6.5 weeks, then to 39–41 mm after 5 months (at ∼28–32 °C) (NTM Cr019556–7). Soft patches developed in both females at post-moult lengths of 27.1 and 41.7 mm OCL, although the soft patch regressed on the smaller animal after a subsequent moult (but the specimens were still immature based on gonopore development). Captive animals showed strong intra- and inter-specific aggression.

Finally, in parallel to field activity, all NTM *Cherax* material was reviewed, provisionally confirming a second damaged museum specimen, a male 37.1 mm OCL collected from Melville Island (NTM Cr010290, lodged by NT Fisheries, March 1991, also provisionally identified to this taxon by J. Short, 1994: Table 1). This specimen was evidentially ethanol-fixed as it was successfully sequenced for DNA. This is the first verified record of any *Cherax* on the Tiwi Islands, however, a local language name is known for "yabbie/freshwater crayfish" which may apply to *C. nucifraga* (Puruntatameri et al., 2001). The recorded location on Melville Island consists of a series of smaller swamps and streams.

A summary of distribution records for *C. nucifraga* relative to river basins (n = 3), protected areas (n = 0), extent of occurrence (∼10,000km^2) and locations (n = 5) is provided in Figure 1.

3.2 | Molecular genetic data

Geographic location data and GenBank accession numbers of mitogenomes and nuclear contigs for all samples used in this study are summarised in Table S1. An average of 23,124,088 raw Illumina reads were obtained from three samples identified as *C. nucifraga*.

From each sample circularised mitogenomes were obtained along with a complete contig inclusive of the 18S, 5.8S, 28S, ITS1 and ITS2 genes and regions (referred to herein as the 18S–28S contig), and a contig comprising 4 histones genes (H2A, H2B, H3 and H4) together with their intervening intergenic regions (referred to herein as the histone contig). The average coverage for the mitogenomes was 91.7 bases, the 18S–28S contig 575.0 bases and the histone contig 191.8 bases for the three *C. nucifraga* specimens. Mitogenome length (15,924, 15,913 and 15,912 bp for samples PUL, CHP and FIR respectively) and gene order were consistent with other members of the genus *Cherax*. New mitogenomes were also generated for *C. bicarinatus* (GGS), *C. quadricarinatus* (RSS and REY) and *C. destructor* (FIN and UMB). Further, all samples yielded complete data (no gaps) for the mitogenomes, 18S–28S and histone contigs except for the histone contig for sample PUL with a string of 14 Ns in the second intergenic region. In addition, Illumina reads obtained for all other samples used in previous studies and retrieved from NCBI's Sequence Read Archive (SRA), also yielded 18S–28S and histone contigs of equivalent lengths without gaps. For completeness, mitogenomes were re-extracted from all data sets. The details of the raw data (read number and data in bp), the length of the assemblies (bp) and coverage (sequencing depth) for all samples are provided in Table S2 as well as Bioproject, Biosample, SRA and accession codes.

A summary of the relationships among samples for the complete data set is shown in Figure 2a. Trees generated separately from the mitogenome alignment (17,535 bp), 18S–28S alignment (12,819 bp) and the histone alignment (3,427 bp) are shown in Figure S2. The *C. nucifraga* samples from the Reynolds and Finniss floodplains are similar in all analyses and cluster together with Melville Island. The *C. nucifraga* samples cluster with the other soft patch species, *C. bicarinatus* and *C. quadricarinatus*, which clade together in the total evidence tree and the trees for each gene fragment. All three species form a lineage separate from the inland species *C. destructor* and the western Australian *Cherax* species. The analysis of the individual alignments shows the same relationships for the *C. nucifraga* samples, however, there is variation in the relationship among the three major groups (soft patch *Cherax*, *C. destructor* and western *Cherax*) and there is some variation in branch lengths (rates of molecular evolution), with the mitogenomes showing greater divergence in the soft patch lineages, the 18S–28S contig showing greater divergence for the *C. bicarinatus/C. quadricarinatus* lineage, and the histone contig showing some heterogeneity for certain samples (e.g. MAR, WOD).

A tree combing the above samples and those available for the other tropical Australian species on GenBank for the 16S rRNA is shown in Figure 2b, with full sample details in Table S3. This gene fragment separates the *C. nucifraga* samples in a similar manner to the total evidence tree with generally high nodal support (86 and 94), despite the short gene fragment length (mean 532 bp). The samples of *C. cairnsensis*, *C. cartalacoolah*, *C. parvus* and *C. wasselli* are all highly distinct, with each forming monophyletic groups with moderate to high support values (85–100). In contrast, the genetic samples topotypic for *C. rhynchotus* (northern Cape York) and *C. barretti* (Wessel Islands) all group with *C. bicarinatus* (Cobourg Peninsula) at very high similarity consistent with this cluster of samples belonging to a single species with support values of 99 and 98.

A spatial genetic comparison of the three focal species (Figures 1–2), identifies two major lineages in both *C. nucifraga* (i.e. mainland and Tiwi Islands lineages) and *C. quadricarinatus* (i.e. Reynolds/Darwin and Roper lineages), with divergence levels similar to that that observed between subspecies of *C. destructor*, and in contrast to *C. bicarinatus* which shows limited genetic substructure across tropical Australia (Table S4).

3.3 | Morphological comparisons

An initial analysis of the three soft patch *Cherax* comprising 24 individuals for 25 ratio combinations are shown in Figure 3a with dimensions 1 and 2 explaining 40% and 27% of variation (material examined and raw data is presented in Table S5). Three distinct clusters are evident matching to the nominal taxa, with sexual dimorphism apparent in each species. The first axis effectively separates *C. quadricarinatus* samples with positive scores from both the other species with neutral to negative scores. The second axis separates *C. nucifraga* (positive scores) and *C. bicarinatus* (negative scores). The holotype of *C. nucifraga* is slightly distinct in morphometric space from other *C. nucifraga*, perhaps owing to its larger size and being partly digested.

Strong correlations between factor variables and taxa are apparent, shown as vectors in Figure 3a and with raw values presented in Table S6. The most important shape characteristics separating *C. quadricarinatus* are the long narrow rostrum (RL/OCL, RW/RL), the larger head (HEW/OCL, HEW/CW), the wide areola (AW/OCL, AW/AL, AW/CW), the larger abdomen (ABL/OCL, ABW/OCL and ABD/OCL) and the narrower claw (PW/PL). The major morphometric differences separating *C. nucifraga* from *C. bicarinatus* are the broader rostrum (RW/OCL), longer rostrum 'tip' (RT/RL, RB/RW, RT/RW) and deeper abdomen (ABD/ABL). A simple way to distinguish *C. nucifraga* from *C. bicarinatus* is the ratio of rostral tip (RT – distance from first rostral spine to tip) to rostral length (RL – total rostrum length as the distance from tip to the base of the rostrum level with the posterior margin of the eye orbit) which is over 50% in *C. nucifraga* and under 35% in *C. bicarinatus*.

A second PCA analysis was conducted focused on determining differences among mature males of the three species based on the characteristics of the propodus, including the size and position of the soft patch (Figure 3b). The scores of the first two axes explain 49% and 36% of the variation, again with tight clusters corresponding to taxon and strong correlations to factor variables (Table S6). *Cherax quadricarinatus* is separated from the other two species on axis 1, on the basis of its narrower claw (PW/OCL, PW/PL) and the length of its soft patch relative to the width of the propodus (SPL/PW) and the length of the palm (SPL/PAL). The soft patch is longer than the proximal length (SPP/SPL) in *C. quadricarinatus* (extending posteriorly beyond the dactyl joint), whereas it is shorter in *C. bicarinatus* and *C. nucifraga* (extending only to about level or anterior to the dactyl

Figure 2

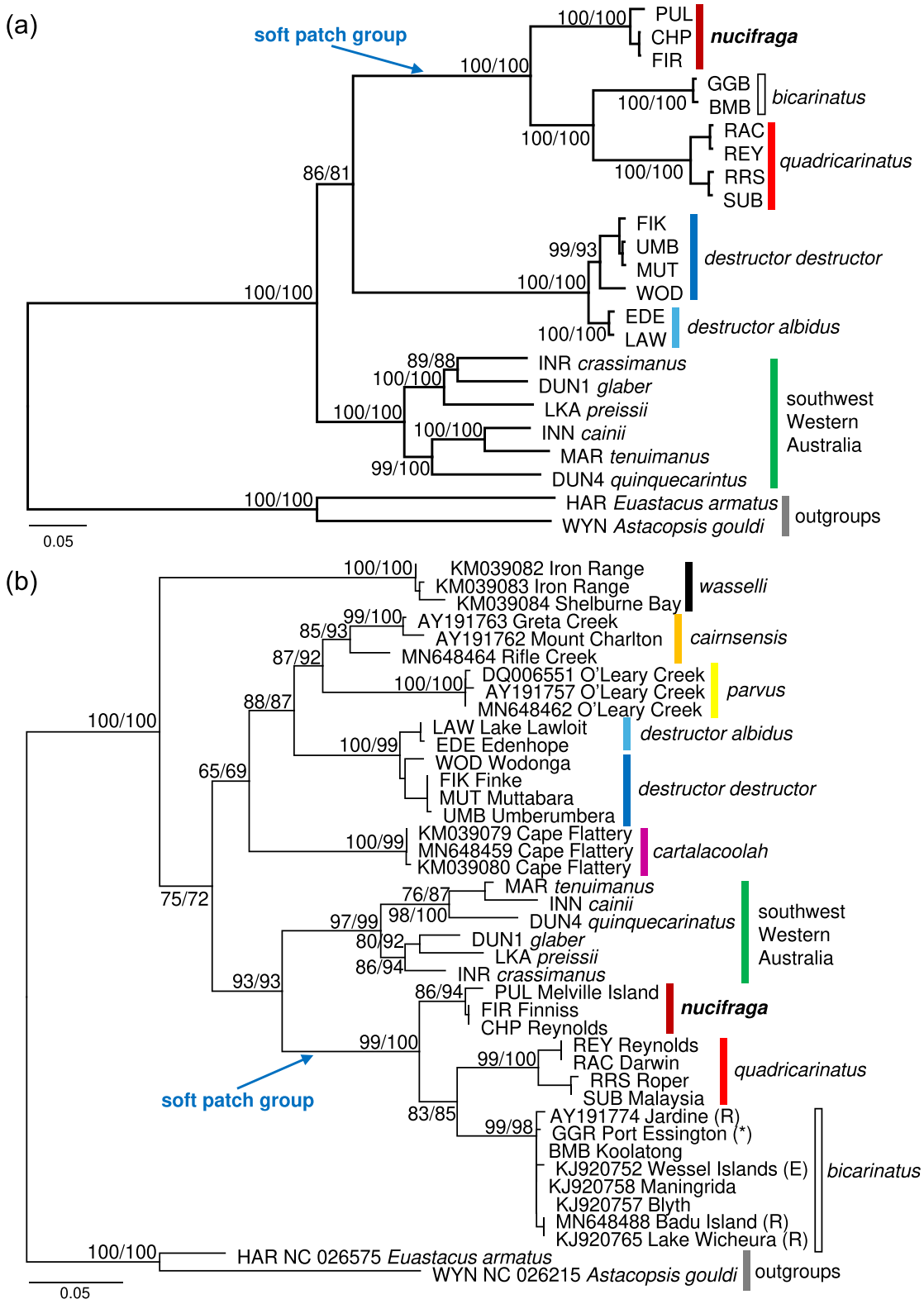


FIGURE 2 Visual summary of the molecular genetic relationships among samples for (A) new in-depth data sourced from genome skimming in this study for *Cherax nucifraga* and selected comparisons (see Table 2), based on concatenated data of the complete mitochondrial genomes, 18S and 28S sequences and four nuclear histone sequences; and (B) collated 16S data from this study and GenBank (see Table S1) for all tropical Australian *Cherax*; * = type locality of *Cherax bicarinatus*, E = population previously assigned to the synonym *Cherax barretti* (topotypic), R = populations previously assigned to the synonym *Cherax rhynchotus*. Phylogenetic analyses were conducted using the IQ tree.

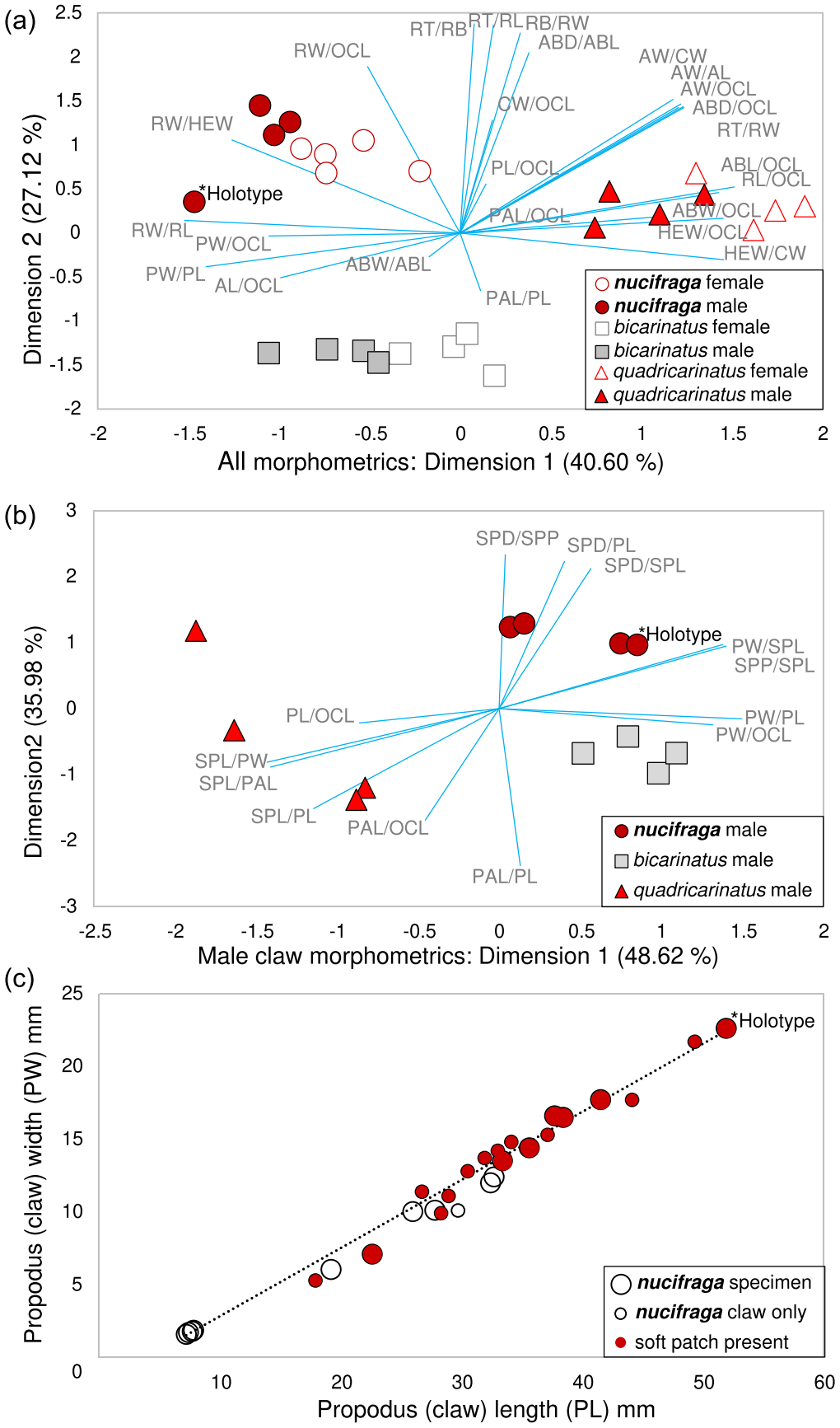


FIGURE 3 Legend on next page.

FIGURE 3 Morphological characteristics of *Cherax nucifraga* including comparison with co-occurring congeners *Cherax quadricarinatus and Cherax bicarinatus*: (A) Principal Components Analysis (PCA) as a combination of ratio data for 21 morphometric measurements; (B) PCA of selected claw ratio values of males only; and (C) claw morphology from a combination of whole specimens and individual discarded claws (Table 1) indicating the presence or absence of the soft patch.

joint). Thus, overall *C. nucifraga* and *C. bicarinatus* have stockier claws and a relatively shorter soft patch. Axis 2 separates the *C. nucifraga* samples with positive scores from the *C. bicarinatus* samples with negative scores. PAL/PL is smaller in *C. nucifraga*, indicating the species has a longer dactyl and the soft patch is more proximally positioned and does not extend to or close to the tip of the propodus as it does in *C. bicarinatus* (see SPD/SPP, SPD/SPL and SPD/PL).

A fully developed soft patch or partially de-calcified patch (in the case of *C. bicarinatus*) occurs on the chelipeds of larger males on the outer lateral margin, however, this trait is also observed in female *C. nucifraga* (Figures S1, S3). In addition to data from specimen material examined, the presence of the soft patch in *C. nucifraga* was noted in discarded claws from as small as 17.9 mm propodus length (PL), although the character does not seem to be reliably present until a size of ~30 mm PL (Figure 3c); some smaller claws could also be regenerative from mature individuals (e.g. as observed for the left cheliped on the Melville Island specimen; 26 mm PL with a soft patch).

The pattern of head ridges (rostral carinae) was variable among species, with *C. quadricarinatus* having very well-developed rostral carinae that reach beyond the posterior end of the postorbital carinae, giving the appearance of four parallel ridges across the middle of the cephalon (hence the name "quadricarinatus"), whereas in *C. nucifraga* the rostral carinae only extend to approximately one third the length of the postorbital ridges and in *C. bicarinatus* they are even shorter, terminating just beyond the anterior development of the postorbital ridge (thus giving the appearance of just two ridges across the mid-cephalon and also reflecting its specific epithet "bicarinatus"). This character appears to develop from early life stages, being observed readily microscopically in 4–6 mm OCL individuals of sympatric *C. nucifraga* and *C. quadricarinatus*. The number of primary spines on the carpus (CS) showed strong variation between *C. bicarinatus* (3–5) and *C. nucifraga/quadricarinatus* (1 or rarely 2). *Chera. nucifraga* additionally has a highly distinctive large secondary carpal spine, that is positioned just anteriorly and ventrally to the primary carpal spine and is absent in the other two species. The other spines or tubercles investigated (RS and PT) were uninformative for diagnosis (Table S5), and the proximal rostral spine (PS) in all three species formed a sharp or blunt spine.

Live adult colouration of *C. nucifraga* reveals a distinctive animal as shown in comparative Figures 4 and S3–S4. The previously unknown colour of the soft patch on the outer finger of the claw is strawberry red (grading to dark red on the dorsal surface of the claw), being possessed by both adult males and females (*vs* bright red in male *C. quadricarinatus* and white to light blue in male *C. bicarinatus*). The large tubercles on the inside of the fingers are blue along with spines and serration on the claw. The dorsal surface of the body is olive green to brown (similar to *C. bicarinatus* but which can grade to dark brown at times; *C. quadricarinatus* is a darker blue-green colour). The claws display a marbled appearance and there are distinct darker bands running laterally on the tail in adults and juveniles (also observed in *C. bicarinatus* but not *C. quadricarinatus*). The ventral surface is much lighter, grading from white on the claws to light brown on the body; the soft patch and blue markings are prominent from this view but are less distinct from the dorsal view.

An overall summary of morphometric, meristic and colour differences is provided in the visual guide and key of Figure 5. Additional taxonomic analysis is also included in Table S7.

4 | DISCUSSION

Despite major declines in aquatic biodiversity, there are still occasional positive stories where presumed extinct or very poorly known animals have been rediscovered, allowing unforeseen opportunities to focus on conservation management (Dooley et al., 2022; Scheffers et al., 2011). Multiple new records of *C. nucifraga* confirm the persistence of an apparently behaviourally cryptic species in expansive and difficult-to-sample wetland habitats in tropical Australia. This represents an exciting outcome for understanding regional aquatic biodiversity and for an otherwise highly threatened faunal group. The rediscovery highlights the general lack of dedicated survey efforts across remote tropical Australia and that there are still likely other unrealised ecological assets and taxonomic diversity in the region and in similar habitats more broadly. New data sheds important light on the environmental requirements, molecular genetic relationships and identification of this formerly enigmatic species.

4.1 | Distribution, life history and habitat

The study has boosted the number of site records of *C. nucifraga* from one to four during surveys over 2021–23, documenting live adult males, females and juveniles with temporal replication. Searching for discarded claws, best achieved when waters contract after the wet season, proved an effective rapid survey technique. Including the additional museum record for Melville Island, the distribution is now known to cover three different river basins, likely representing two independent locations: (a) mainland which has good connectivity across sites with continuous floodplain habitat of the Reynolds and Finniss rivers and (b) Tiwi Islands which is an isolated and spatially restricted population with a marine barrier. This initial assessment indicates an extent of occurrence of around 10,000 km^2, but the

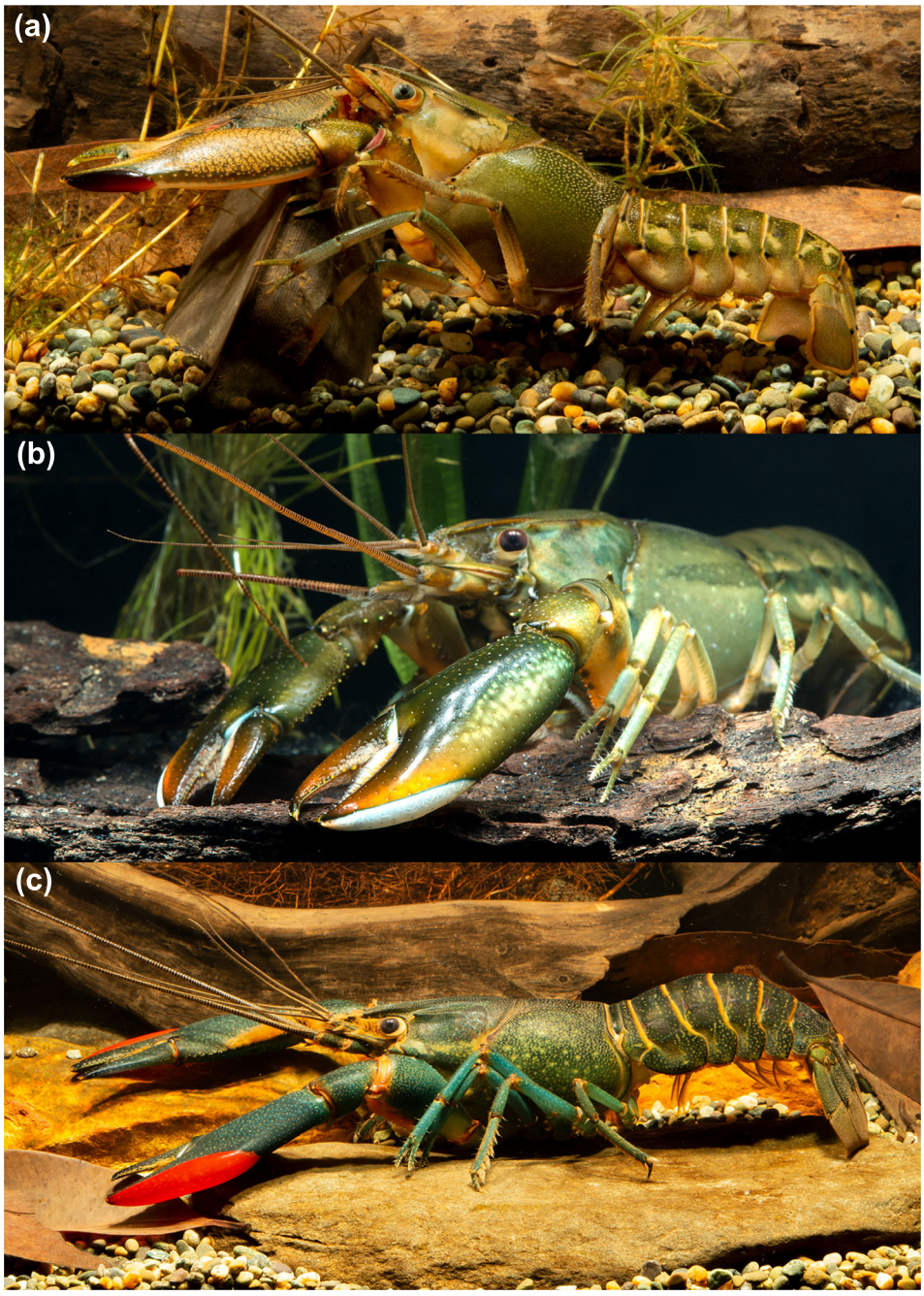
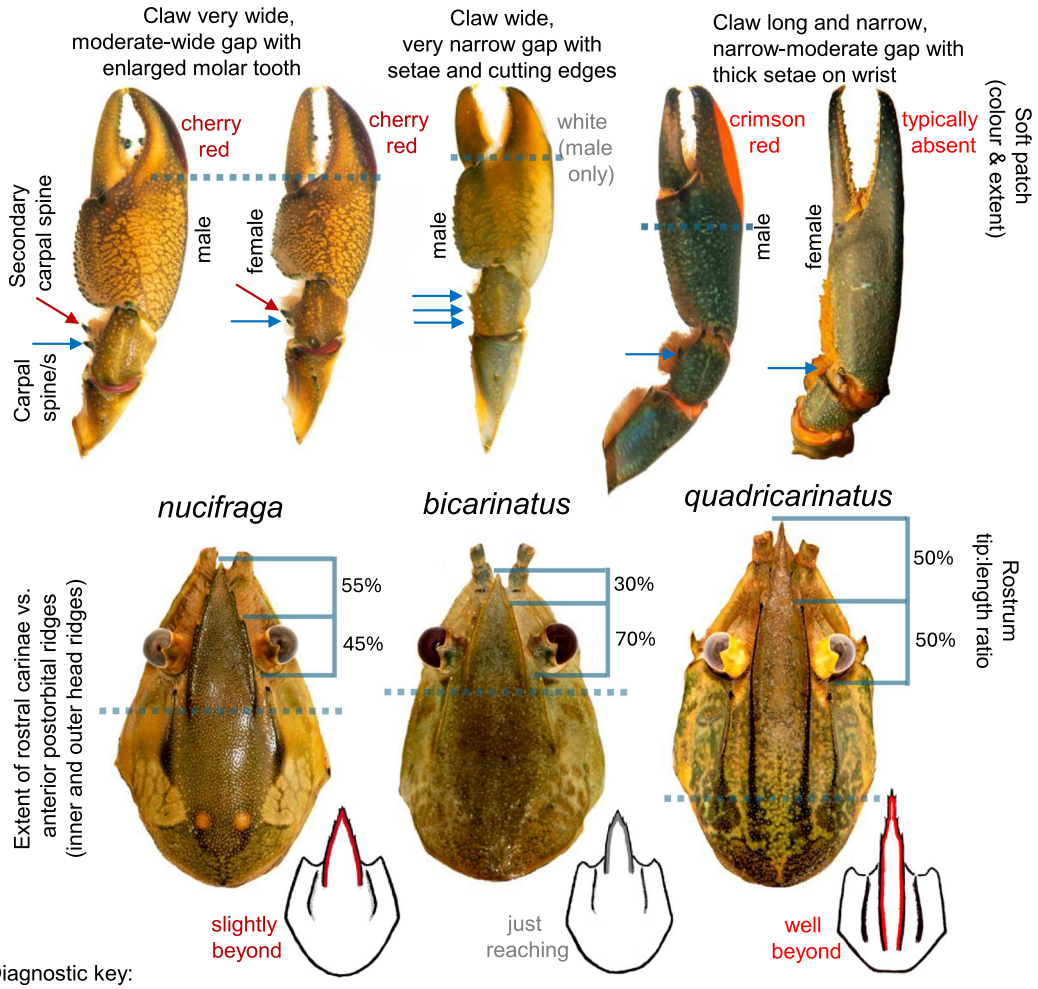


FIGURE 4 Aquarium images of male *Cherax nucifraga* including comparison with co-occurring congeners, Timor Sea drainage, Northern Territory: (A) *C. nucifraga*, Finniss River floodplain (NTM Cr019501, 33.5 mm OCL); (B) *Cherax bicarinatus*, Liverpool River system (NTM Cr018985, 31.5 mm OCL); and (C) *Cherax quadricarinatus*, Rapid Creek (NTM Cr019573, 43.9 mm OCL OCL). Photos M. Hammer.

range may double again if the distribution extends into connected floodplain habitats of the Daly and Moyle rivers for example. No current locations are recorded within the National reserve system, however Melville Island and parts of the Finniss River floodplain are Aboriginal Land with Land and Sea Ranger programs actively involved in land management.

Captured *C. nucifraga* ranged in size from 6.7 to 36.2 mm OCL, with the maximum natural size for the species being at least 44.9 mm OCL based on the holotype. Animals are coloured dorsally presumably for camouflage in floodplain habitats, but also show strong counter shadow, especially the pale claws interspersed with red and blue elements ventrally, which may allow colour or shape signalling to other individuals or threats. The robust claws are well suited to excavation or burrowing for shelter and persistence during dry periods. Diet is likely to be omnivorous including aquatic vegetation and invertebrates. Fast growth matches the dynamic, temporally variable (wet-dry) nature of monsoon tropical wetlands. A record of fish stomach content and the presence of discarded claws suggest predation upon *C. nucifraga* and a possible role in floodplain food chains.

Overall, new distribution data indicates that *C. nucifraga* may be ecologically differentiated with respect to *C. quadricarinatus* (minor overlap at riverine/floodplain transition zones) and allopatric with respect to *C. bicarinatus*; with the caveat that large areas remain to be fully surveyed as a future priority. The differences in observed



Diagnostic key:

1(a) Posterior extension of rostral carinae extending to near posterior of postorbital ridges giving the appearance of **four parallel ridges** across the middle of the cephalon; **claw narrow** and long especially in females (mean PW/PL= 31%, range 25–40%); male **soft patch long** occupying over half the claw length (mean SPL/PL= 64%, range 53–71%) and **bright red** in colour ……..……. *Cherax quadricarinatus*
(b) Posterior extension of rostral carinae terminating just or slightly beyond the anterior development of the postorbital ridges with the appearance of **two ridges** across the mid-cephalon; **claw wide to very wide** (mean PW/PL= 42%, range 35–47%); male **soft patch short** occupying less than half the claw length (mean SPL/PL=41%, range 35–47%) and not bright red ……..…….. 2

2(a) **Single carpal spine** along the lateral inner mesial margin of the claw, with distinctive large **secondary carpal spine** positioned just anteriorly and ventrally to the primary carpal spine; **rostrum tip long** relative to total rostrum length (mean RT/RL= 55%, range 50–58%); soft patch **dark red**, present in both males and females (at maturity) ……. *Cherax nucifraga*
(b) **Multiple primary carpal spines** (modally 3, range 3-5), with no secondary carpal spine; **rostrum tip short** (mean RT/RL= 32%, range 32–34%); male soft patch **white** to light blue ……..……. *Cherax bicarinatus*

FIGURE 5 Visual guide and key to Australian soft patch bearing freshwater crayfish with specific reference to *Cherax nucifraga*.

distribution could be a combination of intrinsic differences in biology and/or evolutionary histories.

4.2 | Molecular taxonomy, biogeography and phylogeny

Tropical Australia contains a group of eight *Cherax* species with varying phylogenetic affinities. Molecular genetic analysis indicates the *C. nucifraga* samples show levels of divergence typical of that for *Cherax* species. The species is most closely related to the other two soft patch-bearing species present (which are recovered as sister species), but with a distribution apparently restricted to the central Timor Sea drainage. This corresponds to biogeographic patterns witnessed in some other aquatic biota, including an endemic rainbowfish *Melanotaenia wilsoni*, a well-isolated population (~2000 km) of the terapontid *Pingalla lorentzi*, and general east–west break point in the terapontid genus *Syncomistes* (Hammer

et al., 2019a; Pusey et al., 2017; Shelley et al., 2020). During periods of lower sea levels (e.g. 130–30 ka), regional systems drained west to the Timor Sea compared to eastern connections through the Gulf of Carpentaria and New Guinea (Norman et al., 2024; Unmack, 2001), and this may in part help to explain the distributions. The apparent allopatric distribution with respect to *C. bicarinatus*, which is also known as a wetland burrowing species, might infer an additional biotic factor shaping distribution. Within-species genetic sub-structure noted across the two *C. nucifraga* locations (mainland and Tiwi Islands) seems to reflect more recent historic isolation with the marine barrier established following the recess of the last glacial maxima. Such spatial isolation and genetic divergence suggest two evolutionarily distinct units or stocks as the basis for separate management (Moritz et al., 2013). This would include maintaining separate broodstock for any captive breeding programs or aquaculture. Greater sampling of individuals for population genetic assessment will help to understand the size, dynamics, conservation actions and potential fisheries management of the two locations.

Analysis using the short 16S gene fragment allowed greater taxon sampling and gave consistent results to multi-gene data, regarding the distinctiveness of *C. nucifraga* and its relationships to a clade comprising *C. bicarinatus and C. quadricarinatus*. Further, this tree, as with the muti-gene data, indicates that these three species form a clade distinct from all other known tropical Australian *Cherax* species and *C. destructor* from inland Australia. The relationship among the deeper lineages within *Cherax* is less consistent based on the different analyses, but is similar to other studies (Munasinghe et al., 2004; Tan et al., 2018). Resolution of these older relationships will require greater taxon and gene sampling. In this regard, the substantial molecular genetic data obtained for this study using genome skimming can be expanded to help address the broader understanding of taxonomic, systematic and evolutionary relationships of the diverse *Cherax* genus (Munasinghe et al., 2004). It is noteworthy that this study significantly extends earlier genome skimming studies of crayfish (Grandjean et al., 2017; Tan et al., 2021) by demonstrating that these data sets can be usefully expanded to include longer contigs associated with nuclear gene clusters (i.e. a single 18S–28S contig and a single contig containing four histone genes).

4.3 | Identification

Accurate identification and taxonomy is a foundation for sound biological studies, fisheries management and conservation (Burnham & Dawkins, 2013; Crandall & De Grave, 2017). Assessment of morphological features and colour patterns verified three distinctive species in the study region. Recording of the first live representatives of *C. nucifraga* highlighted that the species has strawberry to dark red coloured soft patches on the outer finger, being present in both adult males and females which is unique across *Cherax*. The colour of the soft patch is somewhat similar to the more common and recreational targeted *C. quadricarinatus* (brighter red), with which previous captures may have been confused. There are also similarities with the general appearance of *C. bicarinatus* (e.g. wide claws, short inner head ridges, colour patterns), however, its white male soft patch is distinctive, albeit the presence of this trait generally is apparently plastic and labile relating to sexual maturity and possibly other environmental/biotic factors. Some more reliable diagnostic traits to separate each species include the patterns of carinae/ridges across the cephalon, shape of claws, soft patch characteristics, number and position of carpal spines on the claws and placement of spines along the rostrum. Information has been distilled as a taxonomic key and visual guide to allow for laboratory verification and identification in the field.

4.4 | Conservation and management

Rediscovery of *C. nucifraga* allows a spotlight to be cast on conservation and management implications including the identification of potential threats. As a narrow-range endemic species with few locations, and given all known habitat is on low-lying floodplains such that projected declines in the extent and quality of habitat could easily be inferred as a result of climate change and sea level rise, *C. nucifraga* is likely to qualify as Vulnerable on the IUCN Red List for Threatened Species (B2a,biii: IUCN, 2023), and should thus be considered for management protection in national and state legislation.

The popularity of Australian crayfish species in aquaculture, both intensive and *ex situ* (i.e. hatcheries to farm dams), as bait for predatory fishes, and as subjects in the aquarium trade, provides multiple and well-realised introduction pathways (Faulkes, 2015; Haubrock et al., 2021; Lintermans, 2004). Globally, the introduction of exotic crayfish species (including Australian region endemics) has led to major threatening processes such as disease and competitive exclusion (Lodge et al., 2012). Equally, the introduction of native species outside of the natural range can pose similar threats (James et al., 2015), with this particularly apparent in Australia with major established invasions of *Cherax cainii* Austin, *C. destructor* and *C. quadricarinatus* either in large areas naturally naïve to freshwater crayfish (Kimberley, Pilbara, Kangaroo Island) or where strong interaction has been noted with local native species including narrow range endemics (Coughran & Daly, 2012; Horwitz, 1990; Nguyen et al., 2002). The implications for *C. nucifraga* are thus two-fold: (a) proactive management is required to prevent incursions that may lead to negative biological interactions (e.g. the floodplain species *C. destructor/C. bicarinatus* within the mainland range or any non-native species of *Cherax* in more restricted habitat on Melville Island) and (b) the species itself (e.g. via aquarium trade, aquaculture), with rapid growth and likely wide environmental tolerances (tropical floodplain extremes), could become established and invasive elsewhere.

Managing wetlands at scale is a complex and challenging, long-term commitment. River catchment storage structures (extractive use) or lowland regulation (e.g. levees) may act to deprive or artificially increase periods of floodplain inundation and should be mindful to

maintain seasonal variability and a heterogeneous mosaic of floodplain habitat/vegetation types and ecological processes that *C. nucifraga* is likely adapted to (e.g. as a competitive advantage over *C. quadricarinatus*) (Acosta & Perry, 2001; Adams et al., 2021). Building resilience in crayfish populations including specific refuge areas through habitat protection, exclusion of herbivores and restoration (Bubb et al., 2008; Hill et al., 1987; Kozák et al., 2011) will be a key to buffering the impacts of climate change that may, for example, reduce areas of suitable habitat seasonally or permanently (e.g. marine transgression with sea-level rise: Mulrennan & Woodroffe, 1998) and lead to greater variability in the frequency and severity of dry periods and floods (Karim et al., 2015).

Clearly, there is still much to be uncovered about *C. nucifraga*, with this study providing a sound foundation for future studies with respect to geographic locations and methods for more detailed distribution mapping, and assessing seasonal and interannual recruitment dynamics linked to prevailing environmental conditions (Bubb et al., 2008; van der Heiden & Dorn, 2017). New molecular genetic resources (~30,000 bp) provide a suite of markers available for eDNA sampling that may help to speed up many aspects (Atkinson et al., 2019; Baudry et al., 2021). Education and awareness campaigns to foster citizen science reports are also likely to be a valuable contribution to spatial and temporal mapping (Callaghan et al., 2021), and traditional ecological knowledge will help to inform the future conservation management of *C. nucifraga* and its habitat in view of increasing development and environmental change to northern Australian coastal wetland habitats (Bangalang et al., 2022).

AUTHOR CONTRIBUTIONS

MH – conceptualised and developed the study, sourced funding, and led field sampling, manuscript preparation, visualisation and figure preparation.

NW – contributed to conceptualisation, conservation expertise and manuscript preparation.

FG – undertook molecular analyses and contributed to critical review of the manuscript.

JT – undertook molecular analyses and contributed to critical review of the manuscript.

SH – provided collections support and contributed to manuscript preparation.

CA – provided guidance over the duration of the study, sourced funding, participated in field research, undertook molecular, morphological and taxonomic analyses, and manuscript preparation.

ACKNOWLEDGMENTS

We acknowledge the traditional owners across tropical Australia and New Guinea, recognise their ongoing connection to country, and pay respects to Elders past, present and emerging. This project benefited from support in the field from Dan Dority, Matthew and Edward Hammer, Denham Crocker and David Wilson and from the help of NT Fisheries and the NTM Natural Sciences collections team. Grahame Webb provided valuable information on Northern Territory history. Janie Mason AM is thanked for her support of the project. The Museum and Art Gallery of the Northern Territory receives funding from the NT Government. Additional funding was received from the 2015–2020 State-Region Planning Contracts (CPER), European Regional Development Fund (FEDER), and intramural funds from the Centre National de la Recherche Scientifique and the University of Poitiers.

CONFLICT OF INTEREST/COMPETING INTERESTS

The authors do not have any conflict of interest or competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/.

ETHICS STATEMENT

The study was conducted in accordance with all necessary permits and approvals. This included NT Fisheries Collection Permit (S17/3418) and following the Australian code for the care and use of animals for scientific purposes.

CONSENT FOR PUBLICATION

All authors and their organisations consent for the manuscript to be published.

AVAILABILITY OF DATA AND MATERIAL

All data will be provided upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hammer, M.P., Whiterod, N.S., Grandjean, F., Tromp, J.J., Horner, S.K. & Austin, C.M. (2024). Rediscovery, systematics and conservation of an enigmatic freshwater crayfish (Parastacidae) from the Australian monsoon tropics. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 34(6), e4172. https://doi.org/10.1002/aqc.4172

SUPPLEMENT

Aquatic Conservation: Marine and Freshwater Ecosystems

Rediscovery, systematics and conservation of an enigmatic freshwater crayfish (Parastacidae) from the Australian monsoon tropics

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Table S1

Location details and GenBank accessions for freshwater crayfish genetic data obtained through genome skimming in the current study (^= new Illumina sequencing data for this study, otherwise reads retrieved from NCBI's Sequence Read Archive; *= type locality). Australian provinces: NT=Northern Territory, WA=Western Australia, QLD=Queensland, NSW=New South Wales, VIC=Victoria, TAS=Tasmania.

Species	Code	Voucher	Locality	Province	Latitude	Longitude	Mitogenome	18S-28S contig	Histone contig
Cherax nucifraga^	PUL	NTM Cr010290	Four Mile Swamp, Melville Island	NT	-11.4016	130.4658	MN648461	OQ991221	OR004539
C. nucifraga^	CHP	NTM Cr019410	Reynolds River floodplain	NT	-13.1199	130.3403	OQ868369	OQ873316	OQ870571
C. nucifraga^	FIR	NTM Cr019501	Finniss River floodplain	NT	-13.1136	130.6246	OQ935536	OQ991226	OR004534
C. bicarinatus	BMB	NTM Cr014409	Koolatong River system, Blue Mud Bay	NT	-13.1500	135.8500	KM501041	OQ999415	OR004540
C. bicarinatus^*	GGB	NTM Cr019389	Port Essington, Garig Gunak Barlu NP	NT	-11.3783	132.0970	OQ955828	OQ991222	OR004535
C. quadricarinatus^	REY	NTM Cr019536	Southern tributary Reynolds River	NT	-12.9575	130.9433	OQ915513	OQ991223	OR004536
C. quadricarinatus	RAC		Rapid Creek, Darwin	NT	-12.3969	130.8733	HG942364	OQ999416	OR004541
C. quadricarinatus^	RRS	NTM Cr019465	Southern tributary Roper River	NT	-15.5078	133.7957	OQ868368	OQ862823	OQ863205
C. quadricarinatus	SUB		Subang Ria, Kuala Lumpur (introduced)	Malaysia	3.0815	101.5960	NC_022937	OQ999417	OR032574
C. destructor^	FIK	NTM Cr017990	Running Waters, Finke River	NT	-24.3081	132.9030	OQ927376	OQ991224	OR004537
C. destructor^	UMB	NTM Cr019238	Umberumbera Waterhole, Toko Ranges	NT	-22.7843	137.9385	OQ935535	OQ991225	OR004538
C. destructor	WOD		Murray River, Wodonga	VIC	-36.0761	146.8688	HG942177	OQ999418	OR032575
C. destructor	MUT		Thomson River, Muttaburra	QLD	-22.5830	144.5680	HG942178	OQ999418	OR032576
C. destructor	EDE		Swamp southwest of Edenhope	VIC	-37.0497	141.3052	HG799092	OQ999420	OR032577
C. destructor	LAW		Lake Lawloit, northwest of Horsham	VIC	-36.4183	141.4626	MN913555	OQ999421	OR032578
C. tenuimanus	MAR		Margaret River, northeast of township	WA	-33.9337	115.1468	KP205429	OQ999422	OR032579
C. cainii	INN		Seafood market, Innaloo, Perth	WA	-31.9029	115.7995	HG942366	OQ999423	OR032580
C. crassimanus	INR		Inlet River, northwest of Walpole	WA	-34.9178	116.5699	HG942365	OQ999424	OR032581
C. quinquecarinatus	DUN4		Marri Conservation Res., Dunsborough	WA	-33.6111	115.1029	HG799091	OQ999425	OR032582
C. glaber	DUN1		Marri Conservation Res., Dunsborough	WA	-33.6111	115.1029	KF649852	OQ999426	OR032583
C. preissii	LKA		Lower Kalgan River, northeast Albany	WA	-34.8957	118.0017	HG799097	OQ862822	OQ863204
Euastacus armatus	HAR		Ovens River, Harrietville	VIC	-36.8851	147.0628	KP294310	OQ999427	OR032584
Astacopsis gouldi	WYN		Big Creek, Wynyard	TAS	-41.0529	145.6929	KM458973	OQ999428	OR032585

Table S2

Assembly statistics for all samples sourced through genome skimming in the current study, sample code location data provided in Table S1 (SRA= NCBI's Sequence Read Archive, Total reads= trimmed values, RL= read length, contig lengths given in base pairs, Accession= GenBank).

								Mitogenome			18-28S contig			Histone contig		
Species	Code	BioProject	BioSample	SRA	Total reads	RL	Data volume	Depth	Length	Accession	Depth	Length	Accession	Depth	Length	Accession
Cherax nucifraga	PUL	PRJNA580037	SAMN13741226	SRR10854504	11,108,354	150	1,666,253,100	48.8	15924	MN648461	77.5	7321	OQ991221	14.5	2926	OR004539
C. nucifraga	CHP	PRJNA975740	SAMN35342593	SRR24735243	27,254,992	150	4,088,248,800	182.4	15913	OQ868369	345.8	7335	OQ873316	34.5	2933	OQ870571
C. nucifraga	FIR	PRJNA975740	SAMN35342704	SRR24735242	31,008,918	150	4,651,337,700	44.0	15912	OQ935536	1303.0	7335	OQ991226	526.3	2909	OR004534
C. bicarinatus	BMB	PRJNA485382	SAMN09789081	SRR7698970	6,369,414	150	955,412,100	22.0	16099	KM501041	121.2	7469	OQ999415	177.8	2879	OR004540
C. bicarinatus	GGB	PRJNA975740	SAMN35342713	SRR24735241	16,291,862	250	4,072,965,500	87.6	15890	OQ955828	1564.0	7477	OQ991222	700.9	2878	OR004535
C. quadricarinatus	REY	PRJNA975740	SAMN35342799	SRR24735240	14,036,296	150	2,105,444,400	16.8	15868	OQ915513	214.4	7554	OQ991223	103.9	2904	OR004536
C. quadricarinatus	RAC	PRJNA975740	SAMN35523790	SRR24759531	2,306,370	150	345,955,500	28.6	15871	HG942364	86.5	7554	OQ999416	15.5	2913	OR004541
C. quadricarinatus	RRS	PRJNA975740	SAMN35342807	SRR24735239	16,944,998	250	4,236,249,500	487.8	15865	OQ868368	1225	7567	OQ862823	334.6	2905	OQ863205
C. quadricarinatus	SUB	PRJNA485382	SAMN09789087	SRR7698967	1,599,092	250	399,773,000	60.0	15869	NC_022937	135.6	7538	OQ999417	33.8	2898	OR032574
C. quadricarinatus	SUB	PRJNA326564	SAMN05285266	SRR3714356	1,271,712	250	317,928,000									
C. destructor	FIK	PRJNA975740	SAMN35344616	SRR24735238	28,044,832	150	4,206,724,800	153.3	15718	OQ927376	4458.0	7254	OQ991224	339.9	2831	OR004537
C. destructor	UMB	PRJNA975740	SAMN35344762	SRR24735237	24,561,530	150	3,684,229,500	79.6	15716	OQ935535	1001.0	7239	OQ991225	116.4	2825	OR004538
C. destructor	WOD	PRJNA977456	SAMN35525487	SRR24761725	3,800,294	250	950,073,500	43.3	15713	HG942177	172.0	7258	OQ999418	88.5	2790	OR032575
C. destructor	MUT	PRJNA977456	SAMN35525488	SRR24761724	2,744,254	250	686,063,500	39.0	15707	HG942178	282.6	7241	OQ999418	48.1	2805	OR032576
C. destructor	EDE	PRJNA485382	SAMN09789082	SRR7698971	2,897,370	250	724,342,500	32.7	15897	HG799092	239.7	7257	OQ999420	101.7	2788	OR032577
C. destructor	LAW	PRJNA588861	SAMN13258587	SRR10467055	8,653,518	150	1,298,027,700	44.9	15936	MN913555	852.5	7257	OQ999421	96.0	2787	OR032578
C. tenuimanus	MAR	PRJNA485382	SAMN09789090	SRR7698964	3,562,226	150	534,333,900	15.5	15797	KP205429	158.6	7320	OQ999422	38.3	2845	OR032579
C. cainii	INN	PRJNA977459	SAMN35525485	SRR24767647	3,074,878	250	768,719,500	35.1	15801	HG942366	277.7	7321	OQ999423	81.5	2874	OR032580
C. crassimanus	INR	PRJNA977459	SAMN35525486	SRR24767646	3,095,762	150	464,364,300	110.9	15807	HG942365	117.0	7302	OQ999424	556.6	2836	OR032581
C. quinquecarinatus	DUN4	PRJNA485382	SAMN09789088	SRR7698966	1,522,058	250	380,514,500	34.1	15810	HG799091	97.3	7364	OQ999425	114.2	2711	OR032582
C. glaber	DUN1	PRJNA485382	SAMN09789084	SRR7698962	33,333,172	100	3,333,317,200	260.1	15806	KF649852	718.0	7306	OQ999426	958.1	2807	OR032583
C. preissii	LKA	PRJNA485382	SAMN09789086	SRR7698960	1,693,320	250	423,330,000	19.3	15809	HG799097	74.4	7297	OQ862822	28.1	2855	OQ863204
Euastacus armatus	HAR	PRJNA977846	SAMN35537888	SRR24771099	3,757,044	150	563,556,600	17.1	15555	KP294310	495.9	12023	OQ999427	98.8	2632	OR032584
		PRJNA977846	SAMN35537888	SRR24771098	2,946,148	250	736,537,000									
Astacopsis gouldi	WYN	PRJNA485382	SAMN09789071	SRR7698891	5,690,724	250	1,422,681,000	52.7	16678	KM458973	553.3	7558	OQ999428	79.6	2715	OR032585
		PRJNA326564	SAMN05285265	SRR3714355	5,588,278	250	1,397,069,500									

Table S3

Detailed location data for 16S rRNA sequences from tropical Australian species of *Cherax* data available on GenBank used as comparative material to supplement data generated in this study (NT= Northern Territory, QLD= Queensland; E= topotypic for the synonym *Cherax barretti*, R= topotypic for the synonym *Cherax rhynchotus*).

Species	Code	Voucher	GenBank Accession	Locality	Province	Lat	Long
Cherax bicarinatus	BLY	NTM Cr008219	KJ920757	Billabong near Blyth River	NT	-12.4145	134.6985
C. bicarinatus	MAN1		KJ920758	Mann River billabong, Maningrida	NT	-12.2874	134.0937
C. bicarinatus [E]	WES		KJ920752	Wessel Islands	NT	-11.1667	136.6667
C. bicarinatus [R]	JAR	NTM Cr019577	AY191774	Jardine River, Cape York	QLD	-11.1055	142.2833
C. bicarinatus	BAI	QM W12023	MN648488	Badu Island, Torres Straight	QLD	-10.1555	142.1573
C. bicarinatus	WIC	QM W16799	KJ920765	Lake Wicheura, Cape York	QLD	-10.7690	142.5634
C. cairnsensis	GRE		AY191763	Greta Creek, north of Proserpine	QLD	-20.2237	148.3970
C. cairnsensis	MTC		AY191762	north-east of Mount Charlton	QLD	-20.8799	148.6707
C. cairnsensis	RIF	QM W24648	MN648464	Rifle Creek, Mitchell River	QLD	-16.6350	145.4014
C. parvus	OLE1	QM W26639	AY191757	O'Leary Creek, Upper Tully	QLD	-17.9353	145.6186
C. parvus	OLE2		DQ006551	O'Leary Creek, Upper Tully	QLD	-17.9353	145.6186
C. parvus	OLE3	QM W26640	MN648462	O'Leary Creek, Upper Tully	QLD	-17.9353	145.6186
C. cartalacoolah	CAF1	QM W18228	KM039079	Cape Flattery, Cape York	QLD	-14.9839	145.3339
C. cartalacoolah	CAF2	QM W18228	KM039080	Cape Flattery, Cape York	QLD	-14.9839	145.3339
C. cartalacoolah	CAF3	QM W18238	MN648459	Cape Flattery, Cape York	QLD	-14.9839	145.3339
C. wasselli	IRA1	QM W16472	KM039082	Iron Range, Cape York	QLD	-12.6276	143.3835
C. wasselli	IRA2	QM W16472	KM039083	Iron Range, Cape York	QLD	-12.6276	143.3835
C. wasselli	SHEL	QM W11852	KM039084	Shelburne Bay, Cape York	QLD	-11.7376	142.6364

Table S4

Genetic similarity matrix for 16S rRNA sequences from tropical Australian species of *Cherax* as a combination of new data from this study and reference material on GenBank. Codes for sample location and source match Tables S1–S3, data displayed visually in Figure 3b.

Taxon	Code	PUL	FIR	CHP	REY	RAC	RRS	SUB	GGR	BMB	BLY	MAN	WES	JAR	BAI	WIC	LAW	EDE	WOD	FIK	MUT	UMB	OLE2	OLE3	OLE1	CAF1	CAF2	CAF3	IRA1	IRA2	SHE	GRE	MTC	RIF	MAR	INN	DUN4	DUN1	INR	LKA	WYN	HAR
nucifraga	PUL		98.8	98.8	92.0	92.0	91.8	92.0	93.0	93.2	92.0	92.6	92.6	91.9	92.3	92.7	86.3	86.1	86.3	85.9	85.9	85.8	87.5	86.6	86.3	85.1	84.9	87.3	83.7	83.2	84.4	86.8	85.9	86.5	87.5	87.8	86.2	87.6	88.0	88.0	82.3	82.0
nucifraga	FIR	98.8		100.0	92.8	92.8	92.5	92.7	92.7	92.8	91.5	92.3	92.2	91.4	91.6	92.3	85.8	85.6	85.9	85.4	85.4	85.2	87.3	86.5	86.1	84.4	84.3	86.8	83.7	83.2	84.4	86.7	85.7	86.3	87.5	87.8	86.4	87.4	88.0	87.6	81.9	82.4
nucifraga	CHP	98.8	100.0		92.8	92.8	92.5	92.7	92.7	92.8	91.5	92.3	92.2	91.4	91.6	92.3	85.8	85.6	85.9	85.4	85.4	85.2	87.3	86.5	86.1	84.4	84.3	86.8	83.7	83.2	84.4	86.7	85.7	86.3	87.5	87.8	86.4	87.4	88.0	87.6	81.9	82.4
quadricarinatus	REY	92.0	92.8	92.8		100.0	97.0	97.4	92.8	93.0	92.2	92.8	92.4	92.3	91.6	92.5	84.6	84.4	84.6	84.6	84.6	84.4	85.9	85.1	84.4	84.1	84.0	85.9	81.8	81.4	82.5	85.2	84.2	85.3	86.8	87.0	86.6	87.3	87.6	85.5	81.1	82.4
quadricarinatus	RAC	92.0	92.8	92.8	100.0		97.0	97.4	92.8	93.0	92.2	92.8	92.4	92.3	91.6	92.5	84.6	84.4	84.6	84.6	84.6	84.4	85.9	85.1	84.4	84.1	84.0	85.9	81.8	81.4	82.5	85.2	84.2	85.3	86.8	87.0	86.6	87.3	87.6	85.5	81.1	82.4
quadricarinatus	RRS	91.8	92.5	92.5	97.0	97.0		99.7	92.0	92.1	90.9	91.9	91.5	91.2	90.4	91.5	84.7	84.6	85.2	84.6	84.6	84.4	85.5	84.7	84.1	83.0	82.9	85.0	80.5	80.0	81.2	85.2	84.6	85.5	86.5	87.0	86.6	86.9	87.1	85.2	81.3	81.9
quadricarinatus	SUB	92.0	92.7	92.7	97.4	97.4	99.7		92.3	92.5	91.3	92.3	91.9	91.7	91.0	91.9	85.1	84.9	85.1	84.7	84.7	84.6	85.9	85.1	84.4	83.5	83.3	85.4	80.7	80.2	81.4	85.5	84.6	85.9	86.6	87.1	86.8	87.1	87.3	85.4	81.7	82.2
bicarinatus	GGR	93.0	92.7	92.7	92.8	92.8	92.0	92.3		99.8	99.8	99.8	99.5	99.3	99.7	99.4	84.9	84.7	84.2	84.2	84.2	84.2	86.1	85.1	84.6	85.5	85.4	87.1	81.6	81.2	82.3	85.5	84.6	85.5	87.5	87.7	86.4	87.8	88.0	86.6	81.3	82.4
bicarinatus	BMB	93.2	92.8	92.8	93.0	93.0	92.1	92.5	99.8		100.0	100.0	99.6	99.6	99.7	99.6	84.7	84.6	84.4	84.4	84.4	84.4	86.1	85.2	84.8	85.3	85.2	87.0	81.8	81.4	82.5	85.7	84.8	85.7	87.7	87.8	86.6	88.0	88.1	86.8	81.3	82.4
bicarinatus	BLY	92.0	91.5	91.5	92.2	92.2	90.9	91.3	99.8	100.0		100.0	99.8	99.5	99.7	99.8	82.9	82.7	82.5	82.5	82.5	82.5	84.3	84.8	84.8	85.3	85.2	85.4	81.8	81.4	82.5	85.0	83.8	85.2	86.3	86.3	84.1	85.8	85.8	84.3	78.8	79.9
bicarinatus	MAN	92.6	92.3	92.3	92.8	92.8	91.9	92.3	99.8	100.0	100.0		99.6	99.6	99.7	99.8	83.7	83.5	83.3	83.3	83.3	83.3	85.3	84.5	84.2	85.3	85.2	85.9	81.8	81.4	82.5	85.2	84.2	85.2	86.9	87.0	85.7	87.2	87.6	85.9	80.8	81.8
bicarinatus	WES	92.6	92.2	92.2	92.4	92.4	91.5	91.9	99.5	99.6	99.8	99.6		99.3	99.4	99.4	84.1	83.9	83.7	83.7	83.7	83.7	86.0	84.8	84.6	85.1	84.9	86.2	81.6	81.2	82.3	85.5	84.6	85.6	86.9	87.1	85.8	87.2	87.4	86.0	81.3	82.2
bicarinatus	JAR	91.9	91.4	91.4	92.3	92.3	91.2	91.7	99.3	99.6	99.5	99.6	99.3		99.3	99.3	82.5	82.3	82.0	82.0	82.0	82.0	84.1	84.1	84.1	85.1	84.9	84.7	81.6	81.2	82.3	84.7	83.6	84.8	86.1	86.0	83.8	85.6	85.6	84.0	78.9	80.4
bicarinatus	BAI	92.3	91.6	91.6	91.6	91.6	90.4	91.0	99.7	99.7	99.7	99.7	99.4	99.3		100.0	83.3	83.0	82.1	82.4	82.4	82.4	84.9	84.9	84.9	82.9	82.8	84.6	78.2	77.5	78.8	85.5	84.0	86.2	84.9	85.2	83.1	84.6	85.2	83.0	77.9	78.4
bicarinatus	WIC	92.7	92.3	92.3	92.5	92.5	91.5	91.9	99.4	99.6	99.8	99.8	99.4	99.3	100.0		83.6	83.4	83.2	83.2	83.2	83.2	84.8	84.1	83.7	85.1	84.9	85.6	81.8	81.4	82.5	84.7	83.7	84.7	86.5	86.7	85.4	86.8	87.2	85.9	80.2	81.2
destructor	LAW	86.3	85.8	85.8	84.6	84.6	84.7	85.1	84.9	84.7	82.9	83.7	84.1	82.5	83.3	83.6		99.8	97.9	97.7	97.7	97.6	90.1	90.3	89.7	86.4	86.3	88.0	84.6	84.1	83.5	90.8	90.4	91.6	85.3	84.9	84.6	86.5	86.7	85.8	83.1	85.1
destructor	EDE	86.1	85.6	85.6	84.4	84.4	84.6	84.9	84.7	84.6	82.7	83.5	83.9	82.3	83.0	83.4	99.8		97.7	97.6	97.6	97.4	89.9	90.1	89.5	86.2	86.1	87.9	84.8	84.4	83.7	90.6	90.2	91.5	85.5	85.1	84.8	86.7	86.8	86.0	83.1	85.1
destructor	WOD	86.3	85.9	85.9	84.6	84.6	85.2	85.1	84.2	84.4	82.5	83.3	83.7	82.0	82.1	83.2	97.9	97.7		98.1	98.1	97.9	89.3	89.8	89.1	86.2	86.1	87.9	84.8	84.4	83.7	90.6	90.6	91.3	85.1	84.6	83.9	86.7	87.2	85.6	81.8	83.8
destructor	FIK	85.9	85.4	85.4	84.6	84.6	84.6	84.7	84.2	84.4	82.5	83.3	83.7	82.0	82.4	83.2	97.7	97.6	98.1		100.0	99.8	89.3	89.6	89.1	86.2	86.1	87.9	84.1	83.7	83.0	91.0	90.6	91.6	85.0	84.8	84.1	86.5	86.7	85.3	82.0	84.4
destructor	MUT	85.9	85.4	85.4	84.6	84.6	84.6	84.7	84.2	84.4	82.5	83.3	83.7	82.0	82.4	83.2	97.7	97.6	98.1	100.0		99.8	89.3	89.6	89.1	86.2	86.1	87.9	84.1	83.7	83.0	91.0	90.6	91.6	85.0	84.8	84.1	86.5	86.7	85.3	82.0	84.4
destructor	UMB	85.8	85.2	85.2	84.4	84.4	84.4	84.6	84.2	84.4	82.5	83.3	83.7	82.0	82.4	83.2	97.6	97.4	97.9	99.8	99.8		89.3	89.4	89.1	86.2	86.1	87.7	84.1	83.7	83.0	91.0	90.6	91.6	84.8	84.6	84.1	86.5	86.7	85.3	82.0	84.4
parvus	OLE2	87.5	87.3	87.3	85.9	85.9	85.5	85.9	86.1	86.1	84.3	85.3	86.0	84.1	84.9	84.8	90.1	89.9	89.3	89.3	89.3	89.3		99.8	99.6	87.6	87.5	88.9	83.3	82.9	82.9	91.6	90.8	91.1	84.5	84.7	84.7	85.7	85.3	84.1	80.6	83.2
parvus	OLE3	86.6	86.5	86.5	85.1	85.1	84.7	85.1	85.1	85.2	84.8	84.5	84.8	84.1	84.9	84.1	90.3	90.1	89.8	89.6	89.6	89.4	99.8		99.6	87.6	87.5	88.4	83.7	83.2	83.2	91.0	90.2	90.7	84.4	84.4	84.1	85.6	84.9	83.7	79.8	82.2
parvus	OLE1	86.3	86.1	86.1	84.4	84.4	84.1	84.4	84.6	84.8	84.8	84.2	84.6	84.1	84.9	83.7	89.7	89.5	89.1	89.1	89.1	89.1	99.6	99.6		87.6	87.5	88.2	83.7	83.2	83.2	91.2	90.4	90.4	83.9	83.9	83.6	85.0	84.3	83.2	79.8	82.2
cartalacoolah	CAF1	85.1	84.4	84.4	84.1	84.1	83.0	83.5	85.5	85.3	85.3	85.3	85.1	85.1	82.9	85.1	86.4	86.2	86.2	86.2	86.2	86.2	87.6	87.6	87.6		99.7	100.0	86.0	85.5	86.0	88.1	87.1	88.7	85.3	84.2	84.4	83.9	84.6	83.5	79.4	82.5
cartalacoolah	CAF2	84.9	84.3	84.3	84.0	84.0	82.9	83.3	85.4	85.2	85.2	85.2	84.9	84.9	82.8	84.9	86.3	86.1	86.1	86.1	86.1	86.1	87.5	87.5	87.5	99.7		99.7	85.9	85.4	85.9	87.9	87.0	88.6	85.2	84.1	84.3	83.8	84.5	83.4	79.2	82.4
cartalacoolah	CAF3	87.3	86.8	86.8	85.9	85.9	85.0	85.4	87.1	87.0	85.4	85.9	86.2	84.7	84.6	85.6	88.0	87.9	87.9	87.9	87.9	87.7	88.9	88.4	88.2	100.0	99.7		86.0	85.5	86.0	88.4	87.6	89.1	86.5	85.3	85.8	85.4	86.1	85.1	81.7	84.5
wasselli	IRA1	83.7	83.7	83.7	81.8	81.8	80.5	80.7	81.6	81.8	81.8	81.8	81.6	81.6	78.2	81.8	84.6	84.8	84.8	84.1	84.1	84.1	83.3	83.7	83.7	86.0	85.9	86.0		99.5	98.4	85.1	84.4	84.6	83.5	83.0	82.6	84.6	84.6	83.5	78.4	81.4
wasselli	IRA2	83.2	83.2	83.2	81.4	81.4	80.0	80.2	81.2	81.4	81.4	81.4	81.2	81.2	77.5	81.4	84.1	84.4	84.4	83.7	83.7	83.7	82.9	83.2	83.2	85.5	85.4	85.5	99.5		98.4	84.6	83.9	84.2	83.1	82.6	82.2	84.2	84.2	83.0	78.0	80.9
wasselli	SHE	84.4	84.4	84.4	82.5	82.5	81.2	81.4	82.3	82.5	82.5	82.5	82.3	82.3	78.8	82.5	83.5	83.7	83.7	83.0	83.0	83.0	82.9	83.2	83.2	86.0	85.9	86.0	98.4	98.4		84.6	83.9	84.2	83.1	83.5	82.2	83.5	84.2	82.6	78.7	79.8
cairnsensis	GRE	86.8	86.7	86.7	85.2	85.2	85.2	85.5	85.5	85.7	85.0	85.2	85.5	84.7	85.5	84.7	90.8	90.6	90.6	91.0	91.0	91.0	91.6	91.0	91.2	88.1	87.9	88.4	85.1	84.6	84.6		98.9	95.3	85.8	85.7	84.5	86.1	86.3	84.5	81.1	83.1
cairnsensis	MTC	85.9	85.7	85.7	84.2	84.2	84.6	84.6	84.6	84.8	83.8	84.2	84.6	83.6	84.0	83.7	90.4	90.2	90.6	90.6	90.6	90.6	90.8	90.2	90.4	87.1	87.0	87.6	84.4	83.9	83.9	98.9		94.5	85.4	85.4	84.1	85.7	85.9	84.1	80.5	82.1
cairnsensis	RIF	86.5	86.3	86.3	85.3	85.3	85.5	85.9	85.5	85.7	85.2	85.2	85.6	84.8	86.2	84.7	91.6	91.5	91.3	91.6	91.6	91.6	91.1	90.7	90.4	88.7	88.6	89.1	84.6	84.2	84.2	95.3	94.5		86.1	86.1	84.8	86.7	88.0	85.2	81.7	83.5
tenuimanus	MAR	87.5	87.5	87.5	86.8	86.8	86.5	86.6	87.5	87.7	86.3	86.9	86.9	86.1	84.9	86.5	85.3	85.5	85.1	85.0	85.0	84.8	84.5	84.4	83.9	85.3	85.2	86.5	83.5	83.1	83.1	85.8	85.4	86.1		97.1	94.6	92.4	92.5	91.7	80.2	81.8
cainii	INN	87.8	87.8	87.8	87.0	87.0	87.0	87.1	87.7	87.8	86.3	87.0	87.1	86.0	85.2	86.7	84.9	85.1	84.6	84.8	84.8	84.6	84.7	84.4	83.9	84.2	84.1	85.3	83.0	82.6	83.5	85.7	85.4	86.1	97.1		93.4	91.7	92.2	90.8	80.9	82.1
quinquecarinatus	DUN4	86.2	86.4	86.4	86.6	86.6	86.6	86.8	86.4	86.6	84.1	85.7	85.8	83.8	83.1	85.4	84.6	84.8	83.9	84.1	84.1	84.1	84.7	84.1	83.6	84.4	84.3	85.8	82.6	82.2	82.2	84.5	84.1	84.8	94.6	93.4		92.3	92.0	91.5	79.7	82.1
glaber	DUN1	87.6	87.4	87.4	87.3	87.3	86.9	87.1	87.8	88.0	85.8	87.2	87.2	85.6	84.6	86.8	86.5	86.7	86.7	86.5	86.5	86.5	85.7	85.6	85.0	83.9	83.8	85.4	84.6	84.2	83.5	86.1	85.7	86.7	92.4	91.7	92.3		95.8	95.3	80.6	83.4
crassimanus	INR	88.0	88.0	88.0	87.6	87.6	87.1	87.3	88.0	88.1	85.8	87.6	87.4	85.6	85.2	87.2	86.7	86.8	87.2	86.7	86.7	86.7	85.3	84.9	84.3	84.6	84.5	86.1	84.6	84.2	84.2	86.3	85.9	88.0	92.5	92.2	92.0	95.8		94.8	81.4	83.0
preissii	LKA	88.0	87.6	87.6	85.5	85.5	85.2	85.4	86.6	86.8	84.3	85.9	86.0	84.0	83.0	85.9	85.8	86.0	85.6	85.3	85.3	85.3	84.1	83.7	83.2	83.5	83.4	85.1	83.5	83.0	82.6	84.5	84.1	85.2	91.7	90.8	91.5	95.3	94.8		79.5	82.6
Astacopsis	WYN	82.3	81.9	81.9	81.1	81.1	81.3	81.7	81.3	81.3	78.8	80.8	81.3	78.9	77.9	80.2	83.1	83.1	81.8	82.0	82.0	82.0	80.6	79.8	79.8	79.4	79.2	81.7	78.4	78.0	78.7	81.1	80.5	81.7	80.2	80.9	79.7	80.6	81.4	79.5		89.0
Euastacus	HAR	82.0	82.4	82.4	82.4	82.4	81.9	82.2	82.4	82.4	79.9	81.8	82.2	80.4	78.4	81.2	85.1	85.1	83.8	84.4	84.4	84.4	83.2	82.2	82.2	82.5	82.4	84.5	81.4	80.9	79.8	83.1	82.1	83.5	81.8	82.1	82.1	83.4	83.0	82.6	89.0	

Table S5

Raw morphometric data for Top End *Cherax* species. Character codes match Figure 2. Meristic character codes (blue highlight): RS= number of rostral spines, RST= total rostral spines (left and right side), PT= number of tubercles/spines along mesial margin of propodus, CS= the number of spines on the carpus, SP= soft patch present or absent. * = holotype. Note the measurements of CW for Cr007430 & Cr010290 were estimated due to carapace damage. Material examined: *C. nucifraga*, four male & three female specimens (32.4–44.6 mm OCL: see 'adults column', Table 1, the two on-grown females were measured on two occasions, two months and five months after capture, to boost representative data); *C. bicarinatus*, four male & four female specimens (26.5–32.4 mm OCL: NTM Cr014409 Koolatong River); and *C. quadricarinatus*, four male & four female specimens (32.8–48.4 mm OCL: NTM Cr017932 Finniss River, NTM Cr019536 Reynolds River).

NTM	Species	River	Sex	OCL	TCL	FSL	RT	RB	RL	RW	HEW	CW	AL	AW	ABL	ABD	ABW	PL	PW	PAL	SPL	SPD	SPP	RS	RST	PT	CS	SP
Cr007430*	*nucifraga*	Reynolds	M	44.6	56.5	50.5	6.0	5.9	11.9	7.6	17.9	31.0	21.3	5.5	41.6	15.3	24.3	47.6	21.7	22.8	16.6	5.2	33.9	2	4	20	1	1
Cr010290	*nucifraga*	Tiwi	M	37.1	49.2	42.2	7.0	5.1	12.1	7.1	16.2	25.2	17.1	5.2	35.8	13.6	20.8	37.3	17.6	17.9	14.4	4.2	26.3	4	8	21	1	1
Cr019501	*nucifraga*	Finniss	M	33.5	43.4	37.8	5.6	4.3	9.9	6.0	13.8	22.2	14.8	4.5	33.4	12.9	18.9	35.3	15.1	16.3	14.1	3.7	22.8	3	6	19	1	1
Cr019503	*nucifraga*	Finniss	M	32.4	41.7	36.5	5.2	4.1	9.3	5.9	13.4	21.2	14.1	4.2	32.1	12.0	17.8	33.1	14.3	15.1	12.7	3.2	19.8	3	6	16	1	1
Cr019502	*nucifraga*	Finniss	F	36.2	45.9	40.4	5.5	4.2	9.7	6.4	14.5	23.4	16.4	4.8	36.6	13.5	22.3	31.4	13.1	12.9	13.5	4.0	18.5	4	7	17	1	1
Cr019556	*nucifraga*	Finniss	F	39.9	52.3	45.6	6.7	5.7	12.4	7.2	16.1	25.4	19.0	5.3	40.5	15.6	21.8	32.7	12.4	12.3	0.0	0.0	0.0	2	5	16	1	0
Cr019556	*nucifraga*	Finniss	F	41.7	55.4	48.1	7.3	6.4	13.7	7.5	17.0	26.8	19.7	5.4	42.2	16.0	25.0	37.9	16.6	16.1	16.4	5.2	26.0	3	6	18	1	1
Cr019557	*nucifraga*	Finniss	F	34.4	45.1	39.2	5.9	4.8	10.7	6.4	14.6	21.5	16.1	4.1	35.6	14.1	17.4	27.8	10.1	11.2	0.0	0.0	0.0	4	8	19	1	1
Cr019557	*nucifraga*	Finniss	F	39.4	52.2	45.1	7.1	5.7	12.8	7.2	16.9	24.9	18.2	5.2	41.8	15.8	22.8	32.4	12.0	13.3	0.0	0.0	0.0	4	8	21	1	0
Cr014409	*bicarinatus*	Koolatong	F	26.5	35.2	32.3	2.9	5.7	8.6	4.5	11.5	17.0	11.9	3.2	27.9	9.7	16.3	21.5	8.6	9.2	0.0	0.0	0.0	2	4	17	3	0
Cr014409	*bicarinatus*	Koolatong	F	27.1	35.7	32.8	2.9	5.7	8.6	4.5	11.6	17.1	12.7	2.9	28.0	9.8	16.1	21.2	7.9	9.0	0.0	0.0	0.0	3	5	17	3	0
Cr014409	*bicarinatus*	Koolatong	F	28.2	37.2	34.4	2.8	6.2	9.0	4.5	12.1	17.8	12.9	3.0	29.4	10.1	16.9	23.8	8.4	10.5	0.0	0.0	0.0	2	4	15	3	0
Cr014409	*bicarinatus*	Koolatong	F	30.1	38.8	35.9	2.9	5.8	8.7	4.9	12.4	18.8	14.3	3.0	31.0	10.7	18.0	24.6	9.5	10.7	0.0	0.0	0.0	2	4	17	3	0
Cr014409	*bicarinatus*	Koolatong	M	28.1	36.4	33.7	2.7	5.7	8.4	4.4	12.0	18.0	12.6	2.9	27.7	9.5	15.4	26.6	12.5	13.1	11.6	1.0	19.0	2	4	16	4	1
Cr014409	*bicarinatus*	Koolatong	M	30.9	40.2	37.2	3.0	6.3	9.3	4.9	13.0	19.6	14.1	2.8	29.5	10.7	16.4	29.9	14.2	15.3	12.3	1.2	21.5	3	6	17	5	1
Cr014409	*bicarinatus*	Koolatong	M	31.9	40.5	37.8	2.7	5.9	8.6	5.0	13.1	20.1	15.3	3.1	30.0	10.5	16.3	32.8	15.2	16.9	13.8	1.0	23.5	3	6	21	4	1
Cr014409	*bicarinatus*	Koolatong	M	32.4	42.1	39.2	2.9	6.8	9.7	5.3	14.4	20.4	15.0	3.2	30.7	11.3	17.0	31.3	14.1	15.7	14.6	0.9	22.1	3	6	18	3	1
Cr017932	*quadricarinatus*	Finniss	F	32.8	48.9	40.7	8.2	7.9	16.1	5.6	16.1	21.0	13.7	5.7	36.2	13.8	20.8	30.8	7.8	13.7	0.0	0.0	0.0	3	6	17	1	0
Cr017932	*quadricarinatus*	Finniss	F	35.3	51.9	43.2	8.7	7.9	16.6	6.0	16.8	22.2	15.2	6.1	39.4	14.9	22.0	32.4	8.2	14.6	0.0	0.0	0.0	3	6	17	1	0
Cr017932	*quadricarinatus*	Finniss	M	35.6	51.6	43.4	8.2	7.8	16.0	5.8	17.1	23.2	15.6	6.2	38.7	15.0	20.8	37.3	11.9	16.8	19.8	2.4	17.9	3	5	18	2	1
Cr017932	*quadricarinatus*	Finniss	F	45.7	66.2	55.9	10.3	10.2	20.5	7.7	21.8	28.9	20.4	7.5	51.2	19.2	29.3	43.2	10.9	19.5	0.0	0.0	0.0	3	6	17	1	0
Cr017932	*quadricarinatus*	Finniss	M	43.8	62.3	52.7	9.6	8.9	18.5	6.8	20.5	28.6	19.3	7.2	46.4	17.9	25.3	45.8	15.4	22.6	28.5	1.1	21.1	3	6	20	1	1
Cr019536	*quadricarinatus*	Reynolds	F	34.5	50.6	42.3	8.3	7.8	16.1	5.8	16.2	23.4	15.9	6.5	38.4	14.6	21.5	32.6	9.9	14.9	0.0	0.0	0.0	3	7	15	1	0
Cr019536	*quadricarinatus*	Reynolds	M	48.4	68.4	58.3	10.1	9.9	20.0	8.0	23.1	31.5	22.0	7.8	51.5	18.9	28.3	55.2	21.5	29.6	34.4	1.5	26.2	2	4	19	1	1
Cr019536	*quadricarinatus*	Reynolds	M	35.9	51.4	43.7	7.7	7.8	15.5	6.1	17.5	23.8	16.0	6.3	37.5	14.5	20.7	39.1	15.7	20.7	24.9	1.1	18.1	2	4	16	1	1

Table S6

Raw factor variable loading scores for Principal Coordinates Analysis (PCA) comparisons of *Cherax nucifraga* and co-occurring *Cherax quadricarinatus* and *Cherax bicarinatus*: (A) as a combination of ratio data for 20 morphometric measurements; and (B) selected claw ratio values of males only (shown visually in Fig. 3). Large positive/negative correlations are indicated with darker highlight. See Figure S1 for character codes.

	Factor loadings (A)		
Ratio	D1	D2	D3
RL/OCL	0.951	0.218	0.142
RW/OCL	-0.321	0.784	-0.330
HEW/OCL	0.913	0.070	0.315
CW/OCL	0.112	0.532	0.452
AL/OCL	-0.624	-0.210	-0.185
AW/OCL	0.739	0.630	0.167
ABL/OCL	0.896	0.191	-0.322
ABD/OCL	0.758	0.591	-0.164
ABW/OCL	0.672	0.075	-0.353
PL/OCL	0.089	0.230	0.936
PW/OCL	-0.664	-0.015	0.733
PAL/OCL	0.081	0.006	0.984
PW/PL	-0.881	-0.159	0.366
PAL/PL	0.071	-0.272	0.936
RW/RL	-0.957	0.059	-0.215
RT/RL	0.115	0.981	-0.023
RT/RW	0.777	0.591	0.151
RB/RW	0.208	0.942	0.053
RW/HEW	-0.792	0.439	-0.402
ABD/ABL	0.239	0.850	0.100
ABW/ABL	-0.108	-0.112	-0.135
RT/RB	0.048	0.986	-0.037
HEW/CW	0.914	-0.125	0.109
AW/CW	0.765	0.608	0.117
AW/AL	0.773	0.596	0.177

	Factor loadings (B)	
Ratio	D1	D2
PL/OCL	-0.567	-0.087
PW/OCL	0.866	-0.097
PAL/OCL	-0.299	-0.676
PW/PL	0.984	-0.062
PAL/PL	0.086	-0.952
SPL/PL	-0.752	-0.605
SPL/PW	-0.941	-0.324
SPL/PAL	-0.926	-0.353
SPD/SPP	0.025	0.935
SPD/SPL	0.372	0.851
SPP/SPL	0.906	0.389
SPD/PL	0.265	0.895
PW/SPL	0.920	0.380

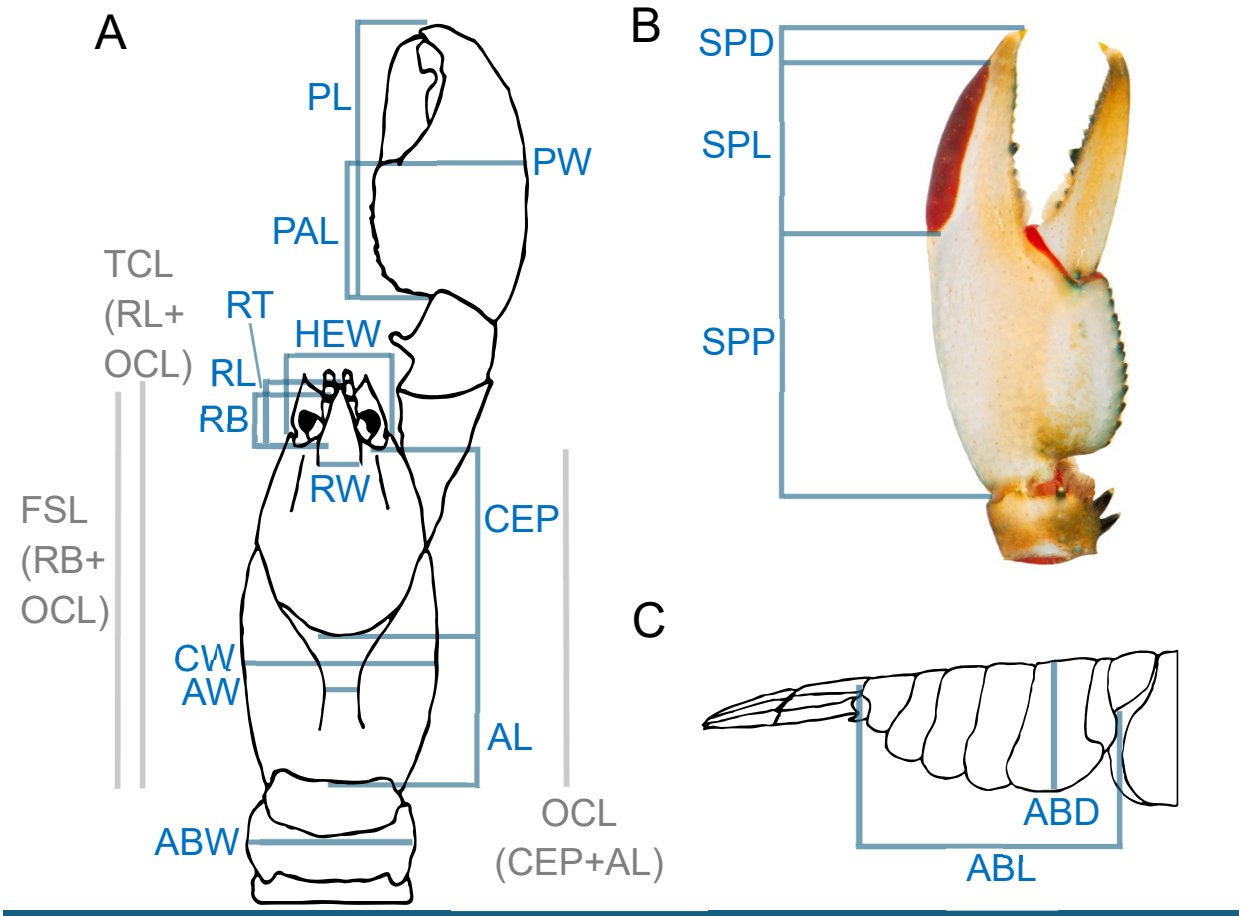
Table S7
Comparison of diagnostic characters for *Cherax nucifraga* and *Cherax bicarinatus* between Short (1991) and the current study based on greater specimen availability and condition. See text for material examined. Explored more fully in text below.

Trait	**Holotype**	***Cherax nucifraga***	***Cherax bicarinatus***
After Short (1991)			
1a. Molar tooth on moveable finger (dactyl)	Yes	Small, moderate and large	Small or absent
1b. Gape between cheliped "fingers"	Strongly gaping	Slightly to moderately gaping	Moderately gaping in males, absent in females
2. Row of tubercles on dorsal surface of carpus of principal chelipeds	Absent	None or occasional single tubercle	3 or more tubercles present
3a. Wide rostrum (as a % of rostrum length)	64%	55–66%	50–58%
3b. Rostrum extending beyond the beginning of the post orbital carinae	Yes	Yes (to approximately 1/3 the length of the post orbital carinae)	Yes (to just beyond the commencement of the post orbital carinae)
After this study			
4. Rostral spines/tubercles	5 (blunt tubercles)	6–8	4–6
5. Primary spines on inner mesial margin of carpus of first cheliped	1	1	3–5
6. Secondary spine on lower inner mesial margin of carpus of first cheliped	1	1	0
7. First spine on rostrum half-way along (RB/RL)	50%	42–47%	66–70%
8. First spine on rostrum half-way along (RT/RB)	102%	114–137%	43–51%
9a. Uncalcified patch on first chelipeds: adult males	1/1	4/4	4/4
9b. Uncalcified patch on first chelipeds: adult females	NA	3/3	0/4

Taxonomic analysis

Given the species treatment of Short (1991) was based on very limited available material, namely a single partially digested specimen of *C. nucifraga* and a literature account of another single specimen for *C. bicarinatus* (as *C. barretti*), it is worth revisiting diagnostic characters based on the now greater availability of fresh material. It is apparent the two key traits relating to the molar tooth and the gape emphasised by Short are more variable and not diagnostic based on the viewing of a greater series of representative specimens of both sexes. The presence of a row of small tubercles on the dorsal surface of carpus of the principal chelipeds of *C. bicarinatus* was also determined to be present in this species and absent from *C. nucifraga*. However, these tubercles are small and difficult to detect and are best determined with a fine needle 'scraped' across the surface of the carpus. The third character emphasised by Short was the width of the rostrum, which is confirmed as generally broader in *C. nucifraga*, but the range of values overlap with those observed for *C. bicarinatus* (Tables S4 and S6). Nevertheless, five additional traits are highlighted in this study as either diagnostic or expanding on the description by Short for traits of taxonomic importance. The total number of rostral spines/tubercles was found to range from 6–8 among the new samples (holotype= 5), are better characterised as blunt spines rather than as "blunt tubercles" and are generally more numerous than the number found in *C. bicarinatus* (4–6). A distinctive feature separating *C. nucifraga* and *C. bicarinatus* is the number of spines on the inner mesial margin of the carpus: *C. nucifraga* is characterised by a single spine, typical for the genus, whereas in *C. bicarinatus* the number varies from 3–5. These single or multiple spines are referred to as primary mesial carpal spines and occur along the lateral inner mesial margin, with *C. nucifraga* having an additional secondary mesial spine (as described above), an atypical condition for *Cherax* more generally. This secondary spine 'points' in an anterio-ventral direction and is usually distinctly larger than the typical mesial carpal spine which is directed anterio-laterally (Fig. 8). Short (1991) noted that that first rostral spine is positioned approximately halfway along the rostrum. This observation is essentially confirmed as can be seen by the ratios in Table S3 (RB/RL and RT/RB) and it is apparent each of these ratios do not overlap with the *C. bicarinatus* samples, in which the first rostral spine occurs much closer to the tip of the rostrum.

Figure S1
Details of *Cherax* morphometric characters used for comparisons in this study, adapted from Austin and Knott (1996) including: (A) body measurements in dorsal view, (B) cheliped measurements for species with a soft patch present (*Cherax nucifraga* female pictured), and (C) abdomen measurements in lateral view.



LEGEND

	Claw (first cheliped)		**Abdomen**
PL	Propodus length	ABL	Abdomen length
PW	Propodus width	ABD	Abdomen depth
PAL	Palm length	ABW	Abdomen width
SPL	Soft patch length		**Cephalothorax**
SPD	Soft patch distal length	CEP	Cephalon length
SPP	Soft patch proximal length	AL	Areolar length
	Head	CW	Carapace width
HEW	Head width	AW	Areolar width
RL	Rostrum length		**Combined**
RB	Rostrum base length (=FSL-OCL)	OCL	Occipital carapace length
RT	Rostrum tip length (=TCL-FSL)	FSL	First spine carapace length
RW	Rostrum width	TCL	Total carapace length

Figure S2

Visual summary of the molecular genetic relationships among samples for new in-depth data sourced from genome skimming in this study for *Cherax nucifraga* and selected comparisons (see Table 2 for taxon/location codes, and Table S2 for assembly information), based on: (A) complete mitogenomes, (B) 18S and 28S contig sequences; and (C) histone contig sequences. Phylogenetic analyses were conducted using IQ-TREE.

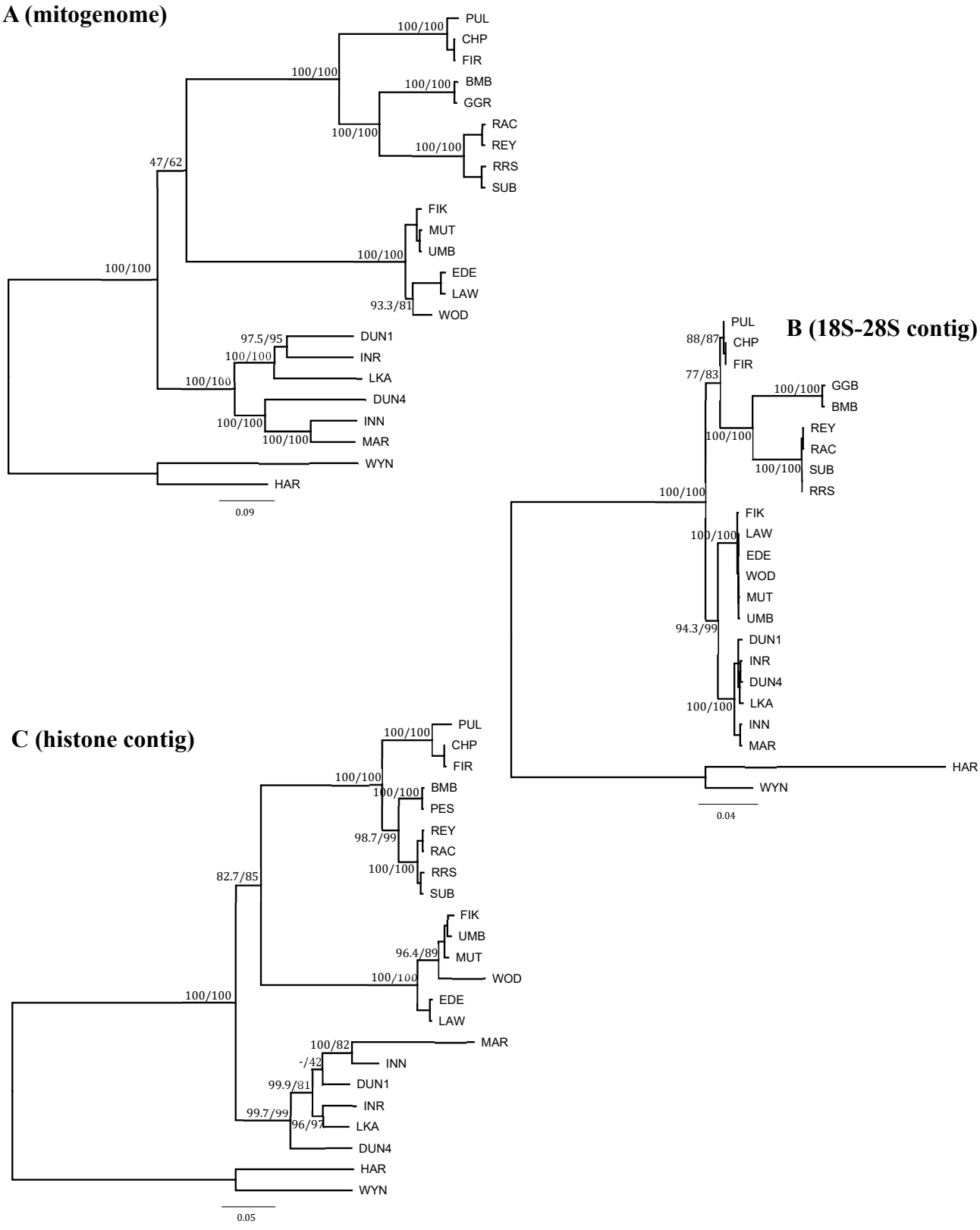


Figure S3
Aquarium images comparing *Cherax nucifraga* (A–C) and *Cherax quadricarinatus* (D–F) respectively: (A + D) adult males showing contrasting head ridge patterns (NTM Cr019501 + NTM Cr019573); (B + E) adult females, note soft patch present only in *C, nucifraga* (NTM Cr019502, 36.2 mm OCL + NTM Cr019574, 41.4 mm OCL); and (C + F) juveniles less than 12 mm OCL (NTM Cr Cr019532–3 + Cr019534–5). Photos M. Hammer.

Figure S4
Specimen images of a male *Cherax nucifraga* in lateral, dorsal and ventral view, Finniss River floodplain, 33.5 mm OCL (NTM Cr019501). Photos M. Hammer.