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Genomic analyses indicate two blue swimmer crab species in Australia, evidence for natural interspecific hybridization and genetic structure within species with implications for fisheries management and stock enhancement

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Highlights

- Genomic analyses indicated presence of two blue swimmer crab species in Australia.
- There is evidence for natural hybrids between *Portunus pelagicus* and *P. armatus*.
- We determined the population structure of *P. armatus* using SNPs.

- *P. armatus* in the west, north and east Australian coasts are genetically different.
- There were no significant genetic differences among eastern *P. armatus* populations.

Abstract

Blue swimmer crab species are widely distributed in Indian and west Pacific Oceans including the coastline around Australia and have been identified as potential candidates for stock enhancement. A recent taxonomic review revealed the presence of two blue swimmer crab species in Australia, namely Portunus armatus which is found around most of the Australian coastline, and Portunus pelagicus which is found in the northern coast where it is sympatric with *P. armatus*. It is important to understand population structure and genetic diversity of *Portunus* spp. to efficiently manage stocks. In the present study we investigated species classification and population structure of *Portunus* spp. in Australia using full and partial genomic information, namely mitochondrial COX1 gene sequences derived from whole genome sequencing and single nucleotide polymorphic (SNP) markers developed from partial genome sequencing. Blue swimmer crab samples were collected from the western ($n=52$), northern ($n=17$) and eastern ($n=110$) coasts of Australia. Genetic diversity and population genetic structure were assessed for within and between region variation. Our genomic results indicated the presence of two blue swimmer crab species in Australia. One, namely, *P. pelagicus* was found only in Northern Territory (Darwin), and other, namely *P. armatus* was found around Australia. There was evidence of natural hybridisation between two species in Northern Territory (Darwin) where they were sympatric. *P. armatus* populations from the western, northern and eastern regions were genetically different although there were no significant genetic differences observed between the Queensland and New South Wales populations nor between the two neighbouring populations in Western Australia. Results found in the present study supports the view that the *P. armatus* populations in western, northern and eastern regions of Australia should be treated as at least three different stocks. A lack of genetic structuring along the eastern coast suggests blue swimmer crabs can be considered as a single stock along the sampled range at least in relation to fisheries enhancement, release programs and broodstock management.

Introduction

Blue swimmer crabs *Portunus* spp. are a commercially important species complex distributed in the Indo-West Pacific region (Lai et al., 2010) and one of the most popular species targeted by fishers in Australia. Bryars and Adams (1999) reported the presence of two blue swimmer crab species in Australia based on an allozyme study. One species was found across Australia which Bryars and Adams (1999) named as *Portunus pelagicus*, while the other species was considered by the authors to be an undescribed 'cryptic' species or the east-Asian species *P. trituberculatus*, which was found in the northern regions of Australia. By contrast, using mitochondrial COX1, morphometric and morphological data Lai et al. (2010) considered samples from around Australia were mostly *P. armatus*. Only some *P. pelagicus* samples, defined by morphology, were found and they were restricted to Darwin in the Northern Territory (Lai et al., 2010). The *P. pelagicus* samples identified by Lai et al. (2010) which were sequenced were most closely related to the *P. segnis* (no Australian samples were sequenced). However, conclusions in Lai et al. (2010) with respect to the blue swimmer crab species found in Australia were not investigated and verified at genomic level. Thus, some level of uncertainty remains regarding the taxonomy of blue swimmer crabs in Australia, and this uncertainty may be addressed using genome-wide genetic analyses approaches. Such approaches have provided sufficient taxonomic resolution to unravel the genetic divergence, reproductive isolation and speciation among incipient species (Casas et al., 2021, Pineros et al., 2022, Premachandra et al., 2017, Seehausen et al., 2014). Resolution of the uncertainty regarding the blue swimmer crab taxonomy (and within population diversity - see following) is essential if the fisheries of this species group can be effectively managed by the different Australian state fisheries agencies that have responsibilities for such management.

Notwithstanding the issues surrounding taxonomy, there is also interest in the population biology and stock management of blue swimmer crabs. Johnston et al. (2011) and Marks et al. (2021) reported declines in commercial catches of blue swimmer crabs and concerns regarding the sustainability of the stock in Western Australia. Moreover, Johnson (2020) reported annual catches of blue swimmer crab in New South Wales have fluctuated, which may have been due to high levels of variability in juvenile recruitment (Junk et al., 2021). To stabilise recruitment variability and enhance fishing opportunities, in particular for recreational anglers, New South Wales Department of Primary Industries (NSW DPI) has identified the blue swimmer crab fishery as one which could benefit from a stocking program (Junk et al., 2021). Marine stock enhancement through release of hatchery bred animals is a well-established practice, however there are associated ecological and genetic risks, which can be managed with appropriate release strategies (Lorenzen et al., 2010). Because of potential permanent disturbances, the genetic consequences of marine stock enhancements must be carefully considered prior to any release program. Identifying any

distinct genetic population structure is fundamental in avoiding releasing animals which lack the similar genetic composition as wild conspecifics. Doing so can lead to outbreeding depression (Gilk et al., 2004), posing risks to the fitness of the supplemented population (Grant et al., 2017). Potential genetic impacts can be mitigated through fisheries management approaches such as sourcing broodstock within the boundaries of the target population or from genetically similar populations.

The genetic structure of blue swimmer crabs within Australia was earlier defined by a study using six microsatellite markers and a portion of the COX1 gene (Sezmiş, 2004), which reported some heterogeneity between samples from the west, north and east coasts of Australia. However, there are now reports that single nucleotide polymorphic (SNP) data can revealed population structure not evident from microsatellite data (Vendrami et al., 2017, Zimmerman et al., 2020). Further, genomic approaches have since been developed which can identify fine scale population structure, that are better suited to study the spatial and temporal units needed to manage fish stocks (Bernatchez et al., 2017). For example, a genomic level approach such as SNPs indicated a fine scale population structure of yellowfin tuna *Thunnus albacares* which was not detected previously (Grewe et al., 2015). The genetic structure of blue swimmer crab population defined by existing microsatellite data may not be adequate to resolve fine-scale genetic structure. Following due diligence (ensuring the existing genetic characteristics are maintained in any future stocking program) it is appropriate to re-examine the population structure of blue swimmer crabs in Australia using genomics.

There were two aims to the current study. Firstly, we reviewed the taxonomic status of blue swimmer crabs in Australia using full genomic and partial genomic sequencing, namely including SNPs generated from the partial genomic sequencing, together with assembled mitochondrial DNA from whole genome sequencing. Secondly, we analysed the genetic structure of blue swimmer crabs sampled from east, west and northern coastlines of Australia using SNPs. These findings are discussed in relation to potential stock enhancement initiatives. Resolution of these aims will better inform state fisheries agencies as to the sustainable management of blue swimmer crab species.

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Section snippets

Crab samples and data

Blue swimmer crab muscle tissue samples were collected into 85 % ethanol containing vials from 10 different locations across west (Western Australian locations), north (Darwin, Northern Territory) and east (NSW and Queensland locations) coasts of Australia (Fig. 1). The samples received in the current study from around Australia were already classified into two species groups by phenotype (based on Lai et al., 2010) with all samples outside the Northern Territory labelled as *P. armatus*, while...

Using COX1 region

The species status of blue swimmer crab samples used in the present study was initially determined by respective state government fisheries agencies based on morphology. COX1 mtDNA gene sequences were extracted from whole genomes of all 10 samples for which we conducted genome sequencing (samples were taken from Western Australia, Northern Territory, New South Wales and Queensland). For nine of the 10 samples, the COX1 gene showed 99 % homology with published *P. armatus* COX1 sequences...

Species level clarification

We found mtDNA COX1 sequence data from the present samples defined morphologically as *P. armatus* had very high (99 %) similarity to the published *P. armatus* COX1 data (Lai et al., 2010) and only moderate (93 %) similarity to the published *P. pelagicus* COX1 data (Lai et al., 2010). The corresponding values for the sample defined as *P. pelagicus* (PP05NT) were low for both published *P. armatus* and *P. pelagicus* COX1 data (89 % and 92 %, respectively) (Lai et al., 2010). However, we note that all...

Conclusion

Our genetic analyses using whole genome and partial genome sequencing data indicated the presence of two blue swimmer crab species in Australian waters. *P. armatus* were identified in east, west and northern coasts while *P. pelagicus* was only found in northern coast and are sympatric with *P. armatus*. There was evidence for natural hybridization between two species in this sympatric zone. *P. armatus* populations on the east, west and northern coasts are genetically different from each other and...

CRediT authorship contribution statement

Alistair Becker: Conceptualization. **Matthew Taylor:** Conceptualization. Alistair Becker involved in conceptualization, provided samples and funding, and participated in revisions and editing of the manuscript. Matthew Taylor contributed to conceptualization and NSW project supervision. Danielle Johnston assisted with the provision of samples from Western Australia, discussion of scientific content and the review and editing of manuscript. HKA Premachandra performed the laboratory work, data...

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper....

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