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Short Communication

Genetic studies of Australian *Trichomya hirsuta* (Bivalvia: Mytilidae) suggest antitropical divergence of this species

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ABSTRACT

The hairy mussel *Trichomya hirsuta* (Lamarck, 1819) has disjunct known ranges in northeast Asia and Australia. There are substantial DNA sequence divergences for mitochondrial cytochrome *c* oxidase subunit I and 16S ribosomal RNA between specimens from these ranges showing that neither is likely to derive from a recent colonization. The most recent common ancestor of the observed haplotypes may have lived as long ago as the early Pliocene. It is, however, suggested here that the mussels from the two regions continue to be regarded, tentatively, as conspecific because intraspecific divergence of mitochondrial DNA sequences can be very high in Mytilidae. The present knowledge of fossil history suggests that the direction of colonization in *Trichomya* may have been from the Southern to the Northern Hemisphere in contrast with migrations of other genera of Mytilidae.

Introduction

Antitropical distributions of sister group taxa are not uncommon at higher taxonomic levels (Lindberg 1991; Nakano and Ozawa 2007). However, disjunct antitropical ranges are unusual within a single species. Indeed, where no morphological differentiation between specimens is observed, it is reasonable to question whether one or the other of the ranges represents an introduction. This is particularly the case for the Mytilidae, a family of bivalves that contains a number of widely invasive taxa including *Mytilus* (Gérard et al 2008), *Limnoperna fortunei* (Endo et al 2009), and *Xenostrobus securis* (Kimura et al 1999; Pascual et al 2010).

Trichomya hirsuta (Lamarck, 1819) is found in eastern and southeastern Australia [including northern Queensland (Lamprell and Healy 1998)] and in eastern Asia (Bernard et al 1993—in which it is recorded as *Dentimodiolus hirsutua*; Higo et al 1999). Its range in eastern Asia extends from Japan (Habe 1977; Higo et al 1999) to the Gulf of Tonkin (Dautzenberg and Fischer 1905; Nguyen 2001). Nguyen (2001) found it at Bỉm Sơn in Thanh Hóa Province although it was not reported in a recent survey of Hainan (Hasegawa et al 2001). In China, the species' range is apparently limited to southern provinces, especially Guangdong and Guangxi and extending to Fujian (Wang, 2004) being absent from more northern areas such as Qingdao (Morton 1990). Even within the southern provinces the distribution is sporadic. It is not found in Hong Kong, for example (Morton and Morton 1983). With the exception of a report from Singapore (Morris and Purchon 1981), it is not definitely known between eastern Asia and eastern Australia from areas such as the Philippines or Indonesia although Bernard et al (1993) suggested without detail that it is found in the latter country. Specimens of the species from the two hemispheres are conchologically similar. There has, however, been no detailed conchological or anatomical analysis to examine relationships between nominal *T. hirsuta* from the two regions.

The contemporary distribution of *T. hirsuta* is unlikely to represent very recent colonization as the species was recorded from Japan in the 1860's (Lischke 1869) and Australia in the early 19th century (Lamarck 1819). There is a possibility that the distribution may have resulted from earlier anthropogenic introductions. Presumed specimens of the species have been found as fossils in both the Southern and the Northern hemispheres although as noted above, detailed morphological analyses are lacking. The species has been recorded in the Northern Hemisphere from the Ryûkyû Group, Japan (Nomura and Zinbo 1934) from a formation probably of Late Pleistocene age (Hayami 1984). It has been recorded from the Pliocene (Ludbrook 1955) and the Pleistocene in Australia (Thorn and Murray-Wallace 1988).

Mitochondrial DNA (mtDNA) sequences for parts of the cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA)

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have recently become available from a *T. hirsuta* specimen from China (Liu et al 2011). DNA sequences of these genes were collected from Australian specimens to investigate whether this disjunct population is genetically differentiated from the Northern Hemisphere specimen. The intron in ATP synthetase subunit α (ATPSα) was also sequenced to provide information on variation in a nuclear gene in case the mitochondrial diversity suggested the presence of multiple cryptic species among Australian specimens.

Materials and methods

Specimen and sequence details

Specimens were obtained for this study by hand collection at localities A, B, and C in the following list and from museum specimens from location D (see Figure 1). GenBank data were included from a specimen from an unspecified location in Queensland (COI: KX713503; 16S rRNA: KX713260, Combosch et al 2017) and the Guigang Peninsula in Guangxi Province China (COI: GQ480305; 16S rRNA; GQ472163, Liu et al 2011).

Locality A: South Australia, Kangaroo Island, Kingscote, E of Wharf, S35°39′19″ E137°38′32″, 8 vi 2012 (R. Golding) (70% ethanol). Specimens C. 553315, C.553316, C.553317 and C.553318 were all sequenced for each of COI, 16S rRNA, and the ATPSα intron except that C.553317 was not sequenced for ATPSα.
Locality B: Southern New South Wales: Green Cape, southern side, S37°15′45″ E150°03′3″, 18 viii 2009 (P. Middelfart, D. Colgan) (frozen at −80°C). Six specimens (C.553320–C.553325), all sequenced for COI. C.553320 was also sequenced for 16S rRNA and the ATPSα intron.
Locality C: Sydney Harbour, Bottle and Glass Rocks, S33°50′51″ E151°16′12″, 28 iii 2007 (D. Colgan, T. Reutelshöfer) (Frozen at −80°C). One specimen (C.553326) was sequenced for COI.
Locality D: Northern Queensland Port Clinton, beach to the S of Holtness Point, S22°32′30″ E150°45′20″, 1 ix 2002 (I. Loch, D.L. Beechey, A.C. Miller) (70% ethanol). Two specimens (C.532860, C.532861), both sequenced for the ATPSα intron. C.532860 was also sequenced for COI and C.532861 for 16S rRNA.

GenBank accession numbers for the sequences collected here are MF320272–MF320283 for COI, MF320286–MF320291 for 16S rRNA, and MF320292–MF320297 for ATPSα.

Molecular methods and analyses

The techniques for DNA extraction, fragment amplification of COI by the polymerase chain reaction, and DNA sequencing generally follow the methods of Colgan and Da Costa (2009). 16S rRNA was amplified using the 16Sar/16Sbr primers (Palumbi 1996) with an annealing temperature between 46°C and 48°C. The ATPSα intron was amplified using the primers in Jarman et al (2002), usually with an annealing temperature of 53°C. After examination of polymerase chain reaction products on agarose gels, single-banded products of the appropriate size were sequenced commercially by Macrogen Inc. (Seoul, Korea) using the original primers.

Chromatogram examination and contig assembly were conducted in Sequencher, ver. 4.9 (Gene Codes Corporation, Ann Arbor). Potentially heterozygous bases in the ATPSα intron were scored by close inspection of the chromatograms and manual editing as required. A heterozygous position was proposed if the peak height of the potential alternative base in the chromatogram was at least 50% of the height of the most abundant base. Phase (Stephens et al 2001) as implemented in DnaSP, ver. 5.1, (Librado and Rozas 2009) was used to predict haplotypes for the ATPSα intron using default parameters except that the number of iterations was increased to 200. Bioedit (Hall 1999) was used for deducing COI amino acid sequences with an invertebrate coding table.

Maximum parsimony analysis was conducted by PAUP 4.0a (Swofford 2002) using the bandb search which is guaranteed to

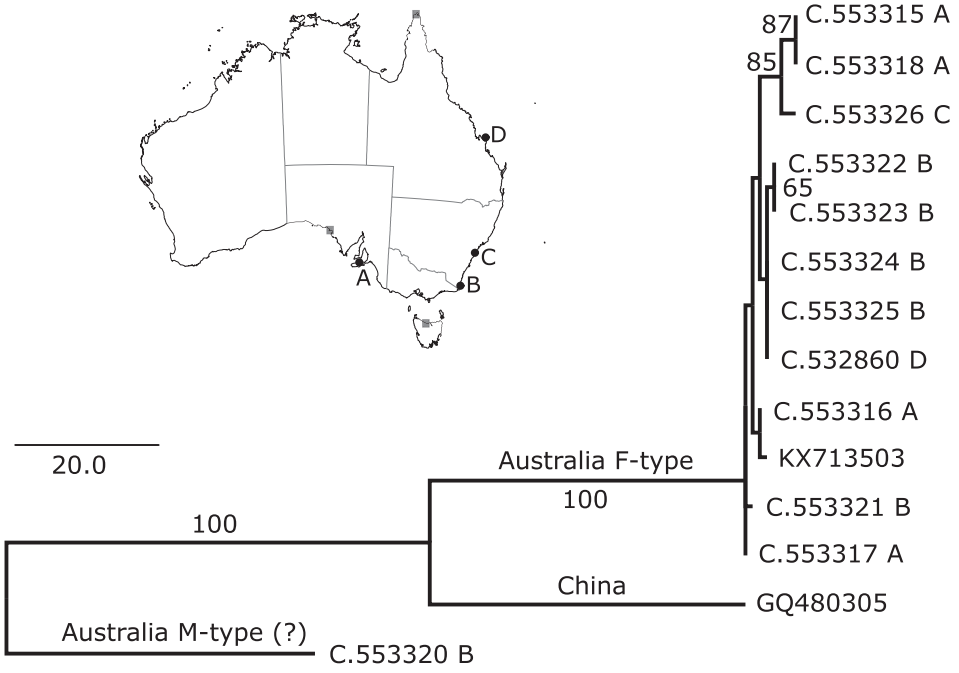

Figure 1. Phylogenetic relationships between cytochrome *c* oxidase subunit I sequences in *Trichomya hirsuta* based on maximum parsimony analysis. The scale bar represents 20 substitution steps. Australian Museum specimens are identified by their registration number (prefix C.) and a letter indicating specimen location that refers to the inset map. Other specimens are identified by their GenBank Accession number. Bootstrap values over 50 are written near nodes. Only branches with such values were observed in all most parsimonious trees. The squares on the inset map show the location of the most northern, western and southern Australian specimens recorded in Museum collections.