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Isolation and Characterization of Polymorphic Microsatellite Loci for *Caridina cantonensis* and Transferability Across Eight Confamilial Species (Atyidae, Decapoda)

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Lai Him Chow, Ka Yan Ma, Jerome H. L. Hui, and Ka Hou Chu (2018) *Caridina cantonensis* is a common freshwater shrimp found in Guangdong Province, China. The species is landlocked, and this life history contributes substantially to its strong population differentiation at the very small geographical scale. Given its widespread distribution, it serves as an excellent model for examining population connectivity and phylogeographical history of freshwater invertebrates in South China. This study isolated ten microsatellite loci from genome sequences of *C. cantonensis* and four from the transcriptome assemblies of *Neocaridina davidi*. The microsatellites were then characterized in 24 *C. cantonensis* individuals from one population. The number of alleles ranged from seven to 18. Observed and expected heterozygosities ranged from 0.050 to 0.958 (averaged 0.649) and 0.754 to 0.919 (averaged 0.833), respectively. We also assessed the cross-species transferability of the markers developed across eight confamilial species. On average, nine markers can be amplified in each species, and five markers can be amplified across all eight species. The markers developed in this study would enable evaluation of genetic diversity and population structure of these species for conservation management planning.

Key words: *Caridina*, *Neocaridina*, SSR markers, Cross-species amplification, Freshwater shrimp.

BACKGROUND

Caridina cantonensis is a common atyid shrimp inhabiting mountain streams and rivulets in Hong Kong, as well as neighbouring areas in Guangdong Province, China (Cai and Ng 1999). It is a typical landlocked shrimp that produces relatively large eggs (0.9-1.2 × 0.6-0.8 mm in diameter, Cai and Ng 1999) and exhibits abbreviated larval development without planktonic stages (Dudgeon 1987). This life history restricts their downstream dispersal and hence prohibits inter-stream dispersal (Dudgeon 2000; Yam and Dudgeon 2005a). Although terrestrial dispersal was found to be feasible in some atyid shrimps - for instance *Paratya australiensis* (Hancock and Hughes 1999) and *C. zebra* (Hurwood and Hughes 2001) - it seems unlikely that *C. cantonensis* travels overland (Tsang et al. 2016). The limited dispersal ability and the highly fragmented nature of freshwater shrimps account for the marked population structure, which was concordantly revealed in two previous studies using nuclear and mitochondrial markers (Tsang et al. 2016; Yam and Dudgeon 2005b). Nonetheless, fine scale population structure and evolutionary history (*e.g.*

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within drainage) were not well resolved because the chosen markers were not variable enough.

In addition to *C. cantonensis*, there are other freshwater obligatory atyid shrimps in Hong Kong (*e.g. C. logemanni*, *C. serrata*, *C. trifasciata*) that share a similar habitat and life history with *C. cantonensis* (Cai and Ng 1999; Yam and Cai 2003; Klotz and von Rintelen 2014). Some of these species are of high conservation value because they are endemic and/or have risks of endangerment in the future owing to rapid urban development in this region (De Grave and Klotz 2013; De Grave et al. 2013). However, to date, no population genetics study has been conducted to elucidate their genetic diversity and population connectivity, severely impeding effective conservation management. To examine the fine scale population connectivity and genetic diversity of the concerned atyid shrimps, we developed microsatellite markers from genome sequences of *C. cantonensis* (albeit with low coverage by Illumina sequencing) and the transcriptome assemblies of *Neocaridina davidi* that can be broadly applied to local *Caridina* species.

MATERIALS AND METHODS

Low-coverage genome sequencing

Genomic DNA was extracted from muscle tissue of a single adult *C. cantonensis* individual with a CTAB protocol (Evans et al. 2013). Approximately 4 μg of genomic DNA (OD260/OD280~1.92) was sent to the BGI, Hong Kong SAR, China for 500-bp DNA library construction (using TruSeq DNA Sample Preparation Kit) and PE250 sequencing on an Illumina Hiseq2500 platform. Raw read (~1Gb) quality was accessed by FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low-quality reads were removed and adapters were trimmed by Trimmomatic (Bolger et al. 2014), while potential bacterial and viral contaminants were further filtered using Kraken (Wood and Salzberg 2014) with default parameters. Paired-end reads were merged to increase the overall read length using PEAR (Zhang et al. 2014).

Transcriptome sequencing of *Neocaridina davidi*

Total RNA was extracted from muscles, hepatopancreas, ovary and gills of adult *N. davidi*, with poly(A) mRNA isolated using Oligo(dT) beads. A 250-bp RNA library was constructed by BGI and subjected to PE151 sequencing on the Illumina HiSeq4000 platform. FastQC was used to access the quality of raw reads, and low-quality reads and adapters were removed by Trimmomatic. Processed raw reads were then *de novo* assembled for each of the organ/tissue using Trinity (Grabherr et al. 2011) with default parameters. Potential bacterial and viral contaminants were filtered using Kraken with default parameters. To reduce redundancy, contigs from the multiple transcriptomes were clustered and further assembled by TGICL (Pertea et al. 2003) with an identity cut-off of 0.94.

Microsatellite markers isolation

Potential microsatellite markers from the *C. cantonensis* genome and the *N. davidi* transcriptome assembled above were detected using MSATCOMMANDER ver. 0.8.2 (Faircloth 2008). In addition, microsatellites with motifs ranging from di-, tri-, and tetra-nucleotides with minimum number of repeat units of eight, six, and six were searched, respectively. Corresponding primer pairs were designed with PRIMER3 (Rozen and Skaletsky 2000) using default parameters.

Characterization of microsatellite markers

Loci with high numbers of motif repeats per locus were selected for assessment of polymorphism to increase the chance of identifying polymorphic loci, and we avoided repeat motifs that may form strong secondary structures (*e.g.* GC, AT) because it would have hindered PCR amplification. We selected 97 loci (13 di-, 38 tri-, and 46 tetra- repeat motifs) to assess polymorphisms. The forward primers were tagged with M13 (-21) (5'-TGTAAAACGACGGCCAGT-3') at their 5' end (Schuelke 2000) to bind with the fluorescent-labelled M13 primer.

Polymorphism assessment was performed on 24 individuals of a *C. cantonensis* population collected from Fung Wong Wat, New Territories, Hong Kong. DNA from these 24 individuals was extracted from pleopods using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. PCRs were performed on two different reaction mix contents, both at final volumes of 8 μl: (A) 0.8 μl template DNA, 1X PCR buffer, 1 mM $MgCl_2$, 0.1 μM forward primer, 0.4 μM reverse primer, 0.4 μM fluorescent-labelled M13 primer, 50 μM each dNTP, 0.6% BSA, and 1.5U *Taq*

polymerase; (B) 0.8 μl template DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM forward primer, 0.6 μM reverse primer, 0.6 μM fluorescent-labelled M13 primer, 50 μM each dNTP, 4% BSA, and 1.5U *Taq* polymerase. Thermocycle conditions were set as follows: an initial denaturation temperature of 95°C for 3 min, then 20 cycles of denaturation at 95°C for 30 s, annealing at 56°C for loci O17, O28, N9, N11 and N19 - 54°C for the rest - for 30 s, extension at 72°C for 30 s, followed by 20 cycles of 95°C (30 s), 53°C (30 s), 72°C (30 s), and a final extension at 72°C for 3 min. Fluorescent-labelled PCR products were pooled and genotyped using ABI3730 Genetic Analyser (Applied Biosystems). R package Fragman (Covarrubias-Pazaran et al. 2016) was used to score alleles. The presence of null alleles was detected using Micro-Checker ver. 2.2 (van Oosterhout et al. 2004). Allelic richness (*Na*) and observed (H_O) and expected (H_E) heterozygosities were calculated using GenAlEx ver. 6.5 (Peakall and Smouse 2012). GenAlEx was used to test if each locus deviated from Hardy-Weinberg equilibrium. Test for deviation from linkage disequilibrium (LD) between all loci was performed using FSTAT ver. 2.9.3 (Goudet 2001).

Cross-species amplification of microsatellite markers

Cross-species PCR amplification was performed on eight or 16 individuals from each of the eight target *Caridina* or *Neocaridina* species: *C. logemanni*, *C. serrata*, *C. trifasciata*, *C. longirostris*, *C. lanceifrons*, *C. typus*, *N. palmata* and *C. aff. apodosis*. The specimens of the last taxon bear resemblance to but show subtle difference from *C. apodosis*, which is endemic to Hong Kong. They are also similar to *C. sphyrapoda* and *C. wumingensis*, which are mainly found in Guangxi Province, China (Sammy De Grave, personal communication). Though the genuine species identity of these specimens was uncertain, they represent an integral part of Hong Kong's atyid community, and hence were included in this study. Sampling details are listed in Appendix 1. PCR reaction mix contents and thermocycling conditions were carried out as described above, except with an annealing temperature of 54°C. Genotyping, allele scoring, and calculation of allelic richness were also conducted as described above. Due to the differences in sample sizes for the nine species including *C. cantonensis*, R package PopGenReport (Adamack and Gruber 2014) was used to rarefy their allelic richness for each locus for comparison of genetic diversity.

Phylogenetic analysis

Phylogenetic relationships between the nine *Caridina* or *Neocaridina* species in Hong Kong (*i.e. C. cantonensis* and the eight target species) were investigated for subsequent correlation between their genetic distance and cross-species amplifiability of microsatellite markers. Partial sequences of mitochondrial cytochrome oxidase I (*COI*) gene of the nine species were generated according to Tsang et al. (2016). Sampling details are listed in Appendix 1. Sequence alignment was performed using MAFFT (Katoh et al. 2002). Aligned sequences were subjected to phylogenetic analysis using maximum likelihood (ML) estimation performed in IQ-TREE ver. 1.6.1 (Nguyen et al. 2015) with *Macrobrachium hainanense* serving as an outgroup. Bootstrapping and SH-like approximate likelihood ratio test (SH-*aLRT*) (Guindon et al. 2010) were performed 1000 times each to assess branch support. The most appropriate model of nucleotide substitution was selected by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE based on the Akaike Information Criterion (AIC).

RESULTS

Characterization of microsatellite markers

By using MSATCOMMANDER, we identified 1839 and 3421 microsatellite loci in the *C. cantonensis* genome and *N. davidi* transcriptome respectively. Among the 97 loci tested, 14 showed high PCR amplification success rate and high sequence polymorphism in *C. cantonensis* (Table 1, GenBank accession number: MH176635-MH176648), out of which ten and four of them were isolated from *C. cantonensis* and *N. davidi* respectively. These loci produced genotypes with sizes ranging from 142 to 470 bp. The number of alleles per locus ranged from seven to 18, averaging at 11.1 (Table 1). The observed and expected heterozygosities ranged from 0.050 to 0.958 (mean = 0.649) and from 0.754 to 0.919 (mean = 0.833) respectively (Table 1). The range of the number of alleles recovered falls within the range observed in other widespread freshwater atyid shrimps, such as *C. gracilipes* (6-17 alleles) (Han et al. 2009) and *Paratya australiensis* (2-14 alleles) (Green et al. 2011). Null alleles were

likely to be present in six of the 14 loci (O17, O28, N15, N19, C20, C34) with frequencies ranging from 0.085 to 0.427. Loci O17, O28, N19 and C34 showed significant deviations from HWE with heterozygote deficits (Table 1). This deviation from HWE might be attributed to the large effective population size but relatively small sample size, in addition to the presence of null alleles, as suggested by Feng and Li (2008). Loci N22 and C8 also deviated from HWE, showing an excess of heterozygosity (Table 1). No evidence of linkage disequilibrium was found between any pair of loci.

Cross-species transferability of microsatellite markers

Among the 14 markers tested, 11 (78.6%) were likely to be useful for population genetics study of *C. serrata*, 10 (71.4%) for *C. trifasciata*, *C. longirostris* and *C. lanceifrons*, nine (64.3%) for *C. logemanni* and *C. typus*, eight (57.1%) for *C. aff. apodosis* and *N. palmata* (Table 2). Markers isolated from *N. davidi* transcriptome, in general, had higher cross-species amplification rates (87.5%) than markers isolated from *C. cantonensis* (58.8%). One possible reason is that as transcriptomes represent expressed genes and are therefore relatively more conserved across species than most of the randomly selected loci from the genome. Markers N11, N15, N20, C1, and C34 were especially useful because they can be amplified and scored with ease across all eight species. Two additional markers (N9, C8) were useful across more than six species.

We found a subtle correlation between genetic distance of the species and cross-species amplifiability of the markers. Regarding the markers isolated from *C. cantonensis*, seven to

Table 1. Characterization of 14 microsatellite loci for *C. cantonensis*

Locus	Primer sequences (5'-3')	Repeat motif	Ta (°C)	PCR reaction mix content[#]	Size range (bp)	*N*	*Na*	H_O	H_E	P_{HW}
O17[†]	F: AGGTAGCTGAGCGATGACC R: CTGCCTTTCGCTGTTCAGT	$(ATC)_8$	56	A	189-288	22	18	0.682	0.919	0.000*
O28[†]	F: AGAGCGACTTGACTGATGC R: CGGGAAGCTAATTTCCTGACT	$(AC)_{16}$	56	A	142-206	20	10	0.050	0.834	0.000*
N9[†]	F: TGTGTTGGCAGATTTCGTCT R: GGCATGCTTAAACACATCCT	$(ATGT)_{17}$	56	A	187-231	23	10	0.870	0.862	0.689
N10[†]	F: TTCACGAAATGCGGCAGAA R: AGTCAGGACAATGGAACACGA	$(GAGT)_{13}$	54	A	155-207	24	10	0.625	0.778	0.502
N11[†]	F: TTCAGTCAGCCAAACGACC R: TGTTGCTAAGTGTGCCTATTCT	$(ACAG)_{16}$	56	A	189-213	22	7	0.818	0.805	0.949
N15[†]	F: ACGCATGATGGAAAGGCAA R: TCACAAAGTCACGACTAAGAT	$(ACAG)_{13}$	54	A	153-241	16	16	0.625	0.916	0.101
N19[†]	F: GTGTGCTTCTTCCTGGCAC R: CTGGAGCTCTCTTCAGCCA	$(ACAT)_{11}$	56	A	238-322	22	13	0.682	0.839	0.005*
N20[†]	F: AGGAAATTACAGCGCATACCAA R: CAGCGAATGACAGCATAACGA	$(CTTT)_{20}$	54	A	406-470	16	11	0.688	0.844	0.116
N22[†]	F: GCGACAACACTCCAGGTTT R: ACCAAACGATAGCAATGTGGAA	$(CTT)_{13}$	54	A	371-410	24	9	0.958	0.813	0.000*
N24[†]	F: GCAATCGGTAAAGGTGGACA R: TCATCTATTCGTCCCGGTTCT	$(AAAG)_{13}$	54	A	379-455	19	11	0.737	0.824	0.407
C1[‡]	F: TCATCCCAGCCGTCCTTTC R: TTGAGCCTACACCATGTCT	$(CCTT)_6$	54	B	176-204	16	10	0.813	0.754	0.142
C8[‡]	F: GGCACAGTAAACAATGCGCT R: TAACAGCCGGTTGAGAGGC	$(AAGG)_6$	54	A	234-278	24	7	0.875	0.787	0.000*
C20[‡]	F: AGAGGCGATGGTTGGCATA R: GGTGCTCGACCGGTAACTA	$(AGG)_8$	54	A	272-353	19	16	0.579	0.904	0.180
C34[‡]	F: AGGTTTGGGCCGGTAAGG R: GTTCGCCACTCTTCTTGGC	$(ACG)_8$	54	B	369-396	24	8	0.083	0.778	0.000*

N: sample size; *Na*: allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; P_{HW}: probability of deviation from HWE (*p*-value). [†]: loci isolated from *C. cantonensis*; [‡]: loci isolated from *N. davidi*; *: loci which deviate from HWE. [#]: A or B type PCR reaction mix (see Materials and Methods).

eight markers could be amplified in species of the clade consisting of the *C. serrata* species group (sensu Dudgeon 1987; including *C. apodosis*, *C. cantonensis*, *C. logemanni*, *C. serrata* and *C. trifasciata*) and *N. palmata* (Table 2, Fig. 1). Eight markers were amplifiable in *C. longirostris*, which is sister to the above clade, and six markers could be amplified in the outlying species *C. lanceifrons* and *C. typus*. For the four markers isolated from *N. davidi*, three to four of them could be amplified in all species (Table 2).

Our preliminary results showed that allelic richness of *C. cantonensis*, the focal species (the species from which the microsatellite loci were isolated; see Jarne and Lagoda 1996), was greater than that of the non-focal species, when comparing their total rarefied allelic richness (Table 3). However, loci in *C. cantonensis* were not always more polymorphic as compared to the other species. It only has the greatest number of alleles after rarefaction in six of the ten loci (O17, O28, N9, N15, N19, N24), and similar result was also observed in two of the four loci isolated from *N. davidi* (C8, C20) (Table 3). The least genetically diverse species was *C. aff. apodosis*, which exhibited the lowest rarefied allelic richness in six of the eight loci (Table 3).

DISCUSSION

Cross-species transferability of microsatellite markers

The success rate for transferring the newly developed microsatellites to the eight confamilial species is quite high compared to that of other studies on caridean shrimp. For example, the success rates of cross-species microsatellite amplification recorded across congeneric *Macrobrachium* species were 0%, 47.5% and 40-60%, while 0-16.7% and 20-50% were reported for species from the family Palaemonidae (based on markers isolated from genomic DNA) (Feng and Li 2008; Song and Kim 2011; Qiao et al. 2011). With an overall high transferability success rate, the markers developed would enable comparison of genetic diversity between a majority of - if not all - freshwater atyid shrimps in Hong Kong, or even South China. However, further studies may be needed to isolate more microsatellite markers for a comprehensive study if considerable null alleles

Table 2. Cross-species transferability of the 14 microsatellite loci in eight *Caridina*/ *Neocaridina* species

Species	Locus	O17†	O28†	N9†	N10†	N11†	N15†	N19†	N20†	N22†	N24†	C1‡	C8‡	C20‡	C34‡
C. logemanni	*N*	/	/	13	/	6	11	1	10	14	/	5	13	/	2
	Na	/	/	7	/	5	12	2	9	5	/	6	4	/	2
C. serrata	*N*	/	/	8	8	8	14	2	15	15	/	4	16	12	7
	Na	/	/	4	6	3	11	1	11	10	/	3	7	7	5
C. trifasciata	*N*	/	6	12	8	8	9	/	11	/	1	6	16	/	7
	Na	/	3	9	5	3	10	/	7	/	1	6	4	/	4
C. longirostris	*N*	/	7	15	/	6	11	5	8	5	/	6	/	12	3
	Na	/	7	6	/	5	11	3	6	4	/	4	/	10	2
C. lanceifrons	*N*	8	/	12	3	8	12	/	4	/	/	5	16	15	2
	Na	3	/	8	3	3	12	/	5	/	/	5	5	5	1
C. typus	*N*	/	/	/	8	6	12	5	16	/	/	5	15	16	4
	Na	/	/	/	5	2	13	2	8	/	/	4	4	5	2
C. aff. apodosis	*N*	8	/	/	/	7	8	/	8	/	/	6	7	8	5
	Na	2	/	/	/	2	5	/	3	/	/	8	3	3	4
N. palmata	*N*	/	/	15	/	4	9	/	7	/	11	8	15	/	7
	Na	/	/	6	/	6	8	/	11	/	3	5	7	/	2
No. individuals tested per species (except *C. aff. apodosis* with a constant sample size of eight individuals)		16	16	16	8	8	16	8	16	16	16	8	16	16	8
PCR reaction mix content#		B	B	B	B	B	B	B	A	A	B	B	B	B	B

†: loci isolated from *C. cantonensis*; ‡: loci isolated from *N. davidi*. *N*: number of individuals with alleles scored; *Na*: allelic richness. /: loci cannot be amplified or with difficulty in allele scoring; #: A or B type PCR reaction mix (see Materials and Methods).

are present in particular species.

Our results agree with the typical finding that transferability success rate progressively declines with increasing genetic distance from the focal specie (*e.g.* Primmer et al. 1996; Hedgecock et al. 2004; Wright et al. 2004). However, the correlation we found in this study was rather subtle, which could be attributed to the limited number of loci tested and the relatively recent divergence of the *Caridina* and *Neocaridina* species examined. In fact, the lack of correlation between genetic distance and transferability success rate is not uncommon. Song and Kim (2011) found no such relationship when they amplified markers across caridean shrimp of different genera and families, and even found an inverse relationship when testing the markers developed by Feng and Li (2008).

Comparison of genetic diversity among the atyid shrimps

It has been pointed out that the focal species usually harbour higher genetic diversity compared with the non-focal species (Goldstein and Pollock 1997). This ascertainment bias arises during the locus selection process. As microsatellite length and mutation rate are positively correlated

Table 3. Rarefied allelic richness of nine *Caridina*/ *Neocaridina* species. Numbers in parentheses following the sum for each species are the values of the corresponding loci for *C. cantonensis*

Locus	*C. cantonensis*	*C. logemanni*	*C. serrata*	*C. trifasciata*	*C. longirostris*
O17†	10.16*	/	/	/	/
O28†	6.28*	/	/	2.91	5.89
N9†	7.44*	5.57	3.51	6.47	4.97
N10†	3.78	/	3.79	3.55	/
N11†	4.43	4.22	2.69	2.86	3.90
N15†	8.45*	7.52	5.70	7.39	7.30
N19†	1.85*	1.67	1.00	/	1.48
N20†	5.11	5.55	5.49	4.69	4.40
N22†	5.11	4.31	6.18	/	3.87
N24†	1.84*	/	/	1.00	/
Sum		28.82 (32.38)	28.37 (36.15)	28.86 (37.31)	31.82 (38.66)
C1‡	4.42	4.73	2.73	4.45	2.96
C8‡	5.16*	3.66	4.87	3.62	/
C20‡	9.50*	/	5.95	/	7.60
C34‡	2.93	1.79	2.96	2.59	1.86

Locus	*C. lanceifrons*	*C. typus*	*C. aff. apodosis*	*N. palmata*
O17†	2.95	/	2.00#	/
O28†	/	/	/	/
N9†	6.72	/	/	5.02
N10†	2.74	3.48	/	/
N11†	2.78	1.98	1.96#	4.99
N15†	7.85	8.04	4.66#	6.21
N19†	/	1.34	/	/
N20†	4.42	5.06	2.41#	6.14
N22†	/	/	/	/
N24†	/	/	/	1.44
Sum	27.47 (39.35)	19.90 (23.61)	11.03 (28.14)	23.80 (27.25)
C1‡	4.07	3.45	4.05	5.20
C8‡	3.96	3.54	2.98#	5.37
C20‡	4.17	4.37	2.90#	/
C34‡	1.00	1.73	2.68	1.48

†: loci isolated from *C. cantonensis*; ‡: loci isolated from *N. davidi*. /: loci cannot be amplified or with difficulty in allele scoring. *: loci for which *C. cantonensis* has the highest allelic richness; #: loci for which *C. aff. apodosis* has the lowest allelic richness.

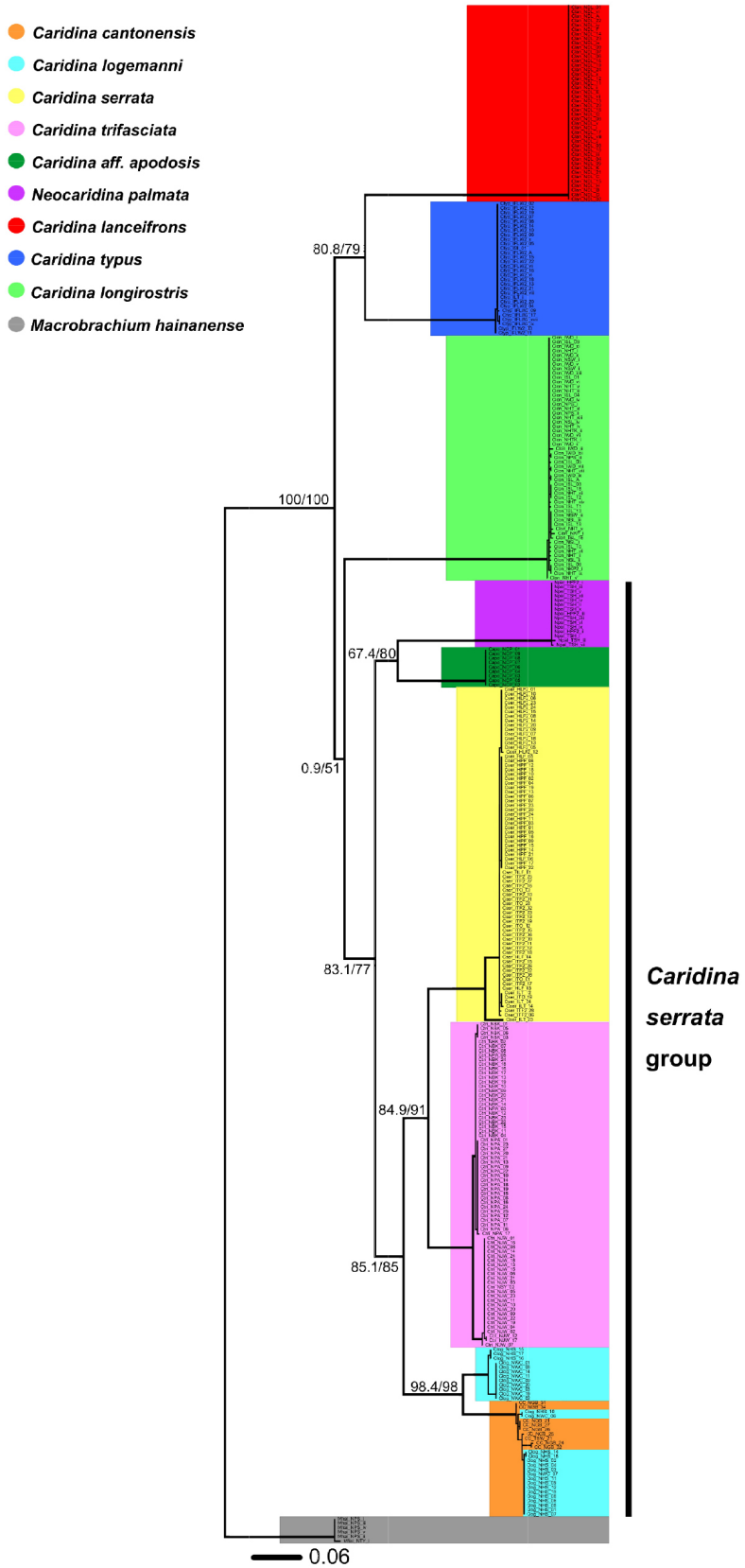

Fig. 1. Maximum likelihood tree generating from a 399-bp long *COI* dataset (GenBank accession no. MH176649-MH176993). SH-alrt/bootstrap support values are indicated at major nodes. Each coloured notation represents one species.

(Jin et al. 1996; Lai and Sun 2003), the chance of identifying polymorphic loci could increase by selecting loci with greater numbers of motif repeats. However, we found loci of *C. cantonensis* are not always more polymorphic as the other species, which may be explained in part by ascertainment bias. It could also be attributed to the large effective population size of the tested *C. cantonensis* population despite the possible presence of null alleles in some loci, which could reduce observed genetic diversity. Nevertheless, interspecific comparison of genetic diversity involving the focal species has to be carefully performed, and ascertainment bias should be taken into account as Goldstein and Pollock note (1997).

Our analyses suggest that *C. aff. apodosis* is generally the least diverse of the species tested. This may be attributed to the fact that these specimens were found in a small population in only one small stream. These specimens bear high resemblance to *C. apodosis*, a critically endangered species that has been recorded in one (different) stream in Hong Kong (De Grave 2013). Confirmation of the identity of *C. aff. apodosis* is impossible due to the lack of *C. apodosis* genetic data in any archives and the inaccessibility of its type specimen. Nevertheless, the clade based on the *C. aff. apodosis COI* sequences suggests that these specimens are distinct from the other species (Fig. 1). Given its low genetic diversity, further investigation on its range and population genetics is warranted, regardless of whether it is truly *C. apodosis* or not.

CONCLUSIONS

We isolated ten microsatellite loci from genome sequences of *C. cantonensis* and four from the transcriptome assemblies of *Neocaridina davidi*, and characterized them for *C. cantonensis*. We then assessed their cross-species transferability across eight confamilial species that occur in Hong Kong. On average, nine markers can be amplified in each species; five markers (N11, N15, N20, C1 and C34) can be amplified across all eight species, making them especially useful. Two additional markers (N9, C8) are useful across more than six species. With an overall high transferability success rate, the markers developed in this study would enable genetic studies of these freshwater atyid shrimps, which would enrich our understanding of their genetic diversity, fine scale population structure, and population connectivity. This is pivotal in conservation management planning for these endemic species and species at risk of endangerment.

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Authors' contributions: KHC devised the study. LHC and KYM conducted the experiments and analyses and prepared the manuscript. JHLH provided the *N. davidi* transcriptome. All authors contributed to revision of the manuscript.

Competing interests: We have no conflicts of interest to disclose.

Availability of data and materials: Microsatellite and COI sequences have been deposited into GenBank.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Appendix 1. Sampling details of the nine *Caridina*/ *Neocaridina* species and *Macrobrachium hainanense*, and the associated analyses done. *N*: sample size. *: analysis performed. If multiple analyses were performed, the portions of individuals included in each analysis were indicated by numbers in parentheses. (download)