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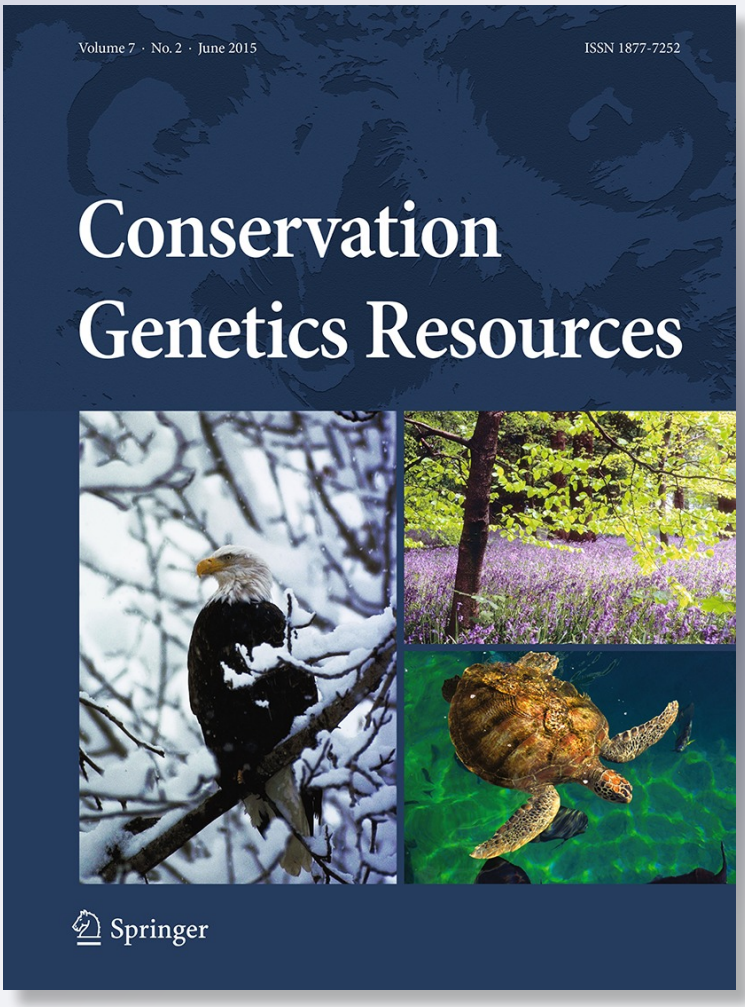
Conservation Genetics Resources

ISSN 1877-7252
Volume 7
Number 2

Conservation Genet Resour (2015)
7:569-572
DOI 10.1007/s12686-015-0426-6



Springer

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MICROSATELLITE LETTERS

Highly polymorphic microsatellite markers in two species, the invasive shore crabs *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* (Decapoda, Varunidae)

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Received: 6 January 2015 / Accepted: 7 January 2015 / Published online: 18 January 2015

Abstract Varunid crabs *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* are species native from intertidal coastal areas in North-western Pacific and have become invasive along the Atlantic European coasts. To gain insights into population genetic features, we developed and characterized 16 (*H. sanguineus*) and 11 (*H. takanoi*) novel polymorphic microsatellite loci from next-generation sequencing. The number of alleles ranged from 2 to 20 for *H. sanguineus*, and from 8 to 21 for *H. takanoi*. Expected heterozygosity ranged from 0.470 to 0.947 and from 0.313 to 0.781, with mean multilocus F_{IS} estimates suggesting rapid turnover of populations. Overall, these microsatellite markers showed very high levels of polymorphism that will facilitate population genetic studies devoted to track down the most likely sources of introduction.

Keywords *Hemigrapsus* · Biological invasion · Intertidal coastal areas · Microsatellites

Electronic supplementary material The online version of this article (doi:10.1007/s12686-015-0426-6) contains supplementary material, which is available to authorized users.

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Biological invasions are often closely linked to anthropogenic activities. Maritime trade promotes recurrent human-induced migration from native ranges, disrupting the dynamic of natural coastal ecosystems. Among maritime intertidal alien species identified in Europe, two Asian shore crab species *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* (Asakura and Watanabe, 2005) have been reported along the Atlantic coast, from southern France to North Sea in Germany. Both species are native from the North-western Pacific. To track down invasive spread and the most likely sources of introduction, we isolated set of microsatellites markers for both species.

Total genomic DNA from *H. sanguineus* and *H. takanoi* was extracted using the NucleoSpin® Tissue kit (Macherey–Nagel, Duren, Germany) following protocols outlined in the manufacturer's handbook and sent to GenoScreen, Lille, France (www.genoscreen.fr). DNA libraries were constructed by coupling multiplex microsatellite enrichment and next generation sequencing as described in Malausa et al. (2011). 1 µg of DNA was used to develop microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Of 144,462 (*H. sanguineus*) and 26,232 (*H. takanoi*) randomly fragmented sequences, 33,741 (*H. sanguineus*) and 8,428 (*H. takanoi*) non-compound sequences containing microsatellite motifs were retained. Finally, 1,363 (*H. sanguineus*) and 433 (*H. takanoi*) sequences likely to contain suitable loci including microsatellite motif longer than five repeats were then returned, of which 47 loci with the longest repeat sequences (at least eight repeat motif) were initially tested for reliable amplification on eight individuals for both species.

As in Favre-Bac et al. (2014), forward primers of successfully amplified loci were labelled with 6-FAM, NED, PET or VIC fluorescent dye (Applied Biosystem). PCR

Table 1 Name, species, primer sequence (5′–3′), repeat motif from the original sequence, allelic size range (bp), multiplex number, Dye used, the total number of allele (A_n), the mean observed (H_o) and expected (H_e) heterozygosity, the mean intra-population fixation index (F_{IS}) estimated over one populations for both species ($N = 32$ individuals for each species) and the accession numbers for 16 and 11 polymorphic microsatellite loci isolated in *Hemigrapsus sanguineus* (Hs) and *Hemigrapsus takanoi* (Ht), respectively

Locus name	Species	Primer sequences (5′-3′)	Repeat motif	Allelic size range	Multiplex number	Dye	A_n	H_o	H_e	F_{IS}	Accession no.
Hs-01	Hs	F: AGGAACAGCAACAATAGCGG R: TCGTGTTTTCTCCGTTTCTTT	$(AAC)_{28}$	113–165	1	6-FAM	19	0.839	0.932	0.102	KP281465
Hs-03	Hs	F: TCAAAACAACAAACAAAACAATCA R: TCGTCTGTAAGGGTATGGTAAGAA	$(ACA)_{23}$	106–155	1	VIC	18	0.742	0.918	0.194**	KP281458
Hs-04	Hs	F: AAACTGTTCAGTTGTGCAAAGG R: TGCTATTCCTCTTCTACTACTTCTGC	$(GTA)_{22}$	100–146	1	NED	14	0.643	0.892	0.283**	KP281463
Hs-08	Hs	F: GTCAACCGGAGGCACATAAA R: AAAGACAAACATATTTAGACCTGACA	$(AGT)_{20}$	90–156	2	6-FAM	18	0.935	0.944	0.009	KP281462
Hs-13	Hs	F: TACTATCCTCCCCATTGCCA R: TAACCCACTATCCCACACCC	$(GTA)_{20}$	158–188	2	VIC	11	0.806	0.856	0.058	KP281460
Hs-17	Hs	F: AGGAATATAACCAGAATAGAGCAGAG R: CGTTTACTAAGAAAGGGGTTCG	$(TAG)_{18}$	174–233	2	NED	18	0.839	0.926	0.096	KP281468
Hs-18	Hs	F: GGAAGAGGTGGATGCATAGG R: CGGATAGCCAACACAGACAT	$(GAG)_{18}$	93–118	2	PET	6	0.645	0.714	0.098	KP281466
Hs-23	Hs	F: TGAAGAAGTGGTGACCCAGC R: TCTTGCCAGACCAGGATCTC	$(TTG)_{18}$	215–295	1	NED	2	0.448	0.470	0.047	KP281461
Hs-31	Hs	F: GTGGTGATGGTGATGTGATG R: AGCCACTCCTTGACCAGTCG	$(GTT)_{17}$	235–295	1	6-FAM	18	0.806	0.933	0.137*	KP281473
Hs-36	Hs	F: AGGAGAGTGTTTACAGCATCCA R: CTTCGGAGGTTACGCAAGAG	$(AGG)_{16}$	172–190	3	6-FAM	7	0.258	0.732	0.651**	KP281470
Hs-37	Hs	F: TTGCGAGTCTGTGTTTGCTT R: TGAGGGGAACTGACAACCTT	$(TTG)_{16}$	208–261	1	VIC	17	0.710	0.919	0.230**	KP281469
Hs-40	Hs	F: ATGATTAGGGATTACCGCGA R: TGCACAGTTCTCAGCCTCAT	$(AGG)_{15}$	89–113	3	6-FAM	9	0.323	0.818	0.609**	KP281464
Hs-41	Hs	F: CTCTTGCATGGCCTGATTTC R: GAATGCAAAGAATAGAAACTGCAT	$(GTT)_{15}$	95–129	3	VIC	12	0.903	0.890	−0.015	KP281467
Hs-42	Hs	F: TCCCTCTGCCTTTTACTGTTC R: TGAAGAAGGATAAGATGATGCAG	$(CTA)_{15}$	133–219	3	NED	20	0.536	0.947	0.439**	KP281459
Hs-44	Hs	F: CAGCAATTCAGAAGAACGCA R: TACGCGATTCTTAAGGCCAC	$(ACT)_{15}$	171–218	3	PET	15	0.935	0.924	−0.013	KP281471
Hs-46	Hs	F: AATCTCGCTCGTTATCACACC R: GGGAAGAATGAACGGAAGAG	$(GGA)_{14}$	192–206	2	6-FAM	6	0.160	0.795	0.802**	KP281472

Table 1 continued

Locus name	Species	Primer sequences (5′-3′)	Repeat motif	Allelic size range	Multiplex number	Dye	A_n	H_o	H_e	F_{IS}	Accession no.
Ht-02	Ht	F: GAATAGCAGGCGACACATTG R: CGGACACATTTAGTCCACGTT	$(AGT)_{22}$	151–222	1	VIC	21	0.419	0.942	0.559**	KP281479
Ht-08	Ht	F: AAAGAAAATCAGCAACAAGTACG R: GGCTGAATATGAGAAGGGTGA	$(ACA)_{20}$	99–159	1	NED	13	0.406	0.902	0.553**	KP281482
Ht-12	Ht	F: TGGATAGTCTTCGGCCACTC R: ATCCTTTCATCCCACGCTC	$(AGT)_{17}$	267–360	1	6-FAM	20	0.548	0.910	0.401**	KP281484
Ht-14	Ht	F: GTTTGCAGTAGCGTGTAGTGG R: CATGAACCAACACGACATGA	$(GAT)_{17}$	132–191	1	PET	16	0.742	0.925	0.201**	KP281478
Ht-20	Ht	F:ACGGATAAAGAGAAATAGAGGAAAGA R: CGAACATGTGGATTGCTGAA	$(TGT)_{15}$	192–257	2	VIC	20	0.700	0.944	0.262**	KP281480
Ht-22	Ht	F: CGCCCACGAAATTCTGTACT R: AAATAATCACCACCATCACCA	$(GTA)_{15}$	126–200	2	NED	19	0.700	0.932	0.252**	KP281474
Ht-28	Ht	F: AACAAACATCAATGCAACACG R: ACAGGGAAATAATGCAGCGT	$(GAG)_{14}$	123–167	2	6-FAM	13	0.313	0.835	0.630**	KP281483
Ht-29	Ht	F: GAGAAAAGAAGGAAAAGAAAGGC R: TCCCTACTGTTGCTACCGCT	$(ACA)_{14}$	123–144	2	PET	8	0.781	0.772	−0.012	KP281475
Ht-34	Ht	F: GCGACGTGGAGGTAAATGAT R: TCTTTCGTCTGGATAGCTGC	$(CAA)_{13}$	135–220	3	VIC	22	0.677	0.926	0.272**	KP281476
Ht-39	Ht	F: ACACATGCGTCAACAACACA R: TGGTGATGCTGATGGTGATT	$(ATC)_{12}$	126–164	3	6-FAM	13	0.613	0.889	0.314**	KP281477
Ht-47	Ht	F: GCCCAAAAGCGGAAATAGAG R: ACGTAAAGCCAACGACCAAC	$(AGG)_{11}$	96–135	3	PET	13	0.387	0.915	0.581**	KP281481

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

reactions were performed in 10 µl volume containing 20 ng of genomic DNA, 1X multiplex PCR master mix (QIAGEN Hilden, Germany), 0.1 µM of forward and reverse primer. The PCR cycling program had an initial denaturation of 95 °C for 15 min; 30 cycles of 94 °C for 30 s, annealing temperature of 57 °C for 1 min 30 s, and 72 °C for 1 min; and a final extension at 60 °C for 30 min.1 µl of PCR product were pooled in 9.75 µl of deionized formamide (Applied Biosystems) and 0.25 µl of GeneScan 500 LIZ size standard (Applied Biosystems). PCR amplicons were subsequently electrophoresed and sized using a 3130 X DNA Sequencer (Applied Biosystems) and the software GeneMapper version 4.0, respectively.

Polymorphism of isolated suitable microsatellite loci was tested on individuals collected from 3-years successive sampling in Dunkerque (50°03′018″N, 2°22′106″E), Northern France (N = 32 individuals per species). 16 microsatellites (*H. sanguineus*) and 11 microsatellites (*H. takanoi*) had easily readable chromatograms and were polymorphic. Primer pairs were successfully combined into three multiplex per species (Table 1). No linkage disequilibrium was observed for any pairs of loci for both species. Measures of genetic diversity were estimated using FSTAT, version 2.9.3 (Goudet 1995). For *H. sanguineus*, the number of alleles ranged from 2 to 20 among loci (mean = 13.125) for a total of 210 alleles observed. For *H. takanoi*, 8–21 alleles were observed among loci (mean = 16.182) for a total of 178 alleles. Mean observed heterozygosity (H_O) values ranged from 0.160 to 0.935 (*H. sanguineus*) and from 0.313 to 0.781 (*H. takanoi*). F_{IS} estimates ranged from −0.015 to 0.802 (*H. sanguineus*) and from −0.012 to 0.630 (*H. takanoi*) for a mean multilocus value of 0.229 and 0.368, respectively. A large part of loci suggested a genotypic structure that departed from panmixia expectations (Table 1). For both species, individuals were collected in three different years (springs 2012–2014). Along with the very high number of observed alleles, this suggested a temporal Wahlund effect due to a mixture of different cohorts (Table 1). Concordantly, Gothland et al. (2014) showed that up to four different cohorts of *H. takanoi* can be collected within a small geographical range.

Overall, these microsatellite loci suggest complex spatial and temporal dynamics and will be appropriate for fine and large-scaled population genetic studies devoted to unravel genetic signatures of introduction and expansion of these two invasive Asian shore crabs.

Acknowledgments This work received financial support from the French program "EC2CO".

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