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PERMANENT GENETIC RESOURCES NOTE

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Abstract

This article documents the addition of 512 microsatellite marker loci and nine pairs of Single Nucleotide Polymorphism (SNP) sequencing primers to the Molecular Ecology Resources Database. Loci were developed for the following species: *Alcippe morrisonia morrisonia*, *Bashania fangiana*, *Bashania fargesii*, *Chaetodon vagabundus*, *Colletes floralis*, *Coluber constrictor flaviventris*, *Coptotermes gestroi*, *Crotophaga major*, *Cyprinella lutrensis*, *Danaus plexippus*, *Fagus grandifolia*, *Falco tinnunculus*, *Fletcherimyia fletcheri*, *Hydrilla verticillata*, *Laterallus jamaicensis coturniculus*, *Leavenworthia alabamica*, *Marmosops incanus*, *Miichthys miiuy*, *Nasua nasua*, *Noturus exilis*, *Odontesthes bonariensis*, *Quadrula fragosa*, *Pinctada maxima*, *Pseudaletia separata*, *Pseudoperonospora cubensis*, *Podocarpus elatus*, *Portunus trituberculatus*, *Rhagoletis cerasi*, *Rhinella schneideri*, *Sarracenia alata*, *Skeletonema marinoi*, *Sminthurus viridis*, *Syngnathus abaster*, *Uroteuthis* (*Photololigo*) *chinensis*, *Verticillium dahliae*, *Wasmannia auropunctata*, and *Zygochlamys patagonica*. These loci were cross-tested on the following species: *Chaetodon baronessa*, *Falco columbarius*, *Falco eleonorae*, *Falco naumanni*, *Falco peregrinus*, *Falco subbuteo*, *Didelphis aurita*, *Gracilinanus microtarsus*, *Marmosops paulensis*, *Monodelphis Americana*, *Odontesthes hatcheri*, *Podocarpus grayi*, *Podocarpus lawrencei*, *Podocarpus smithii*, *Portunus pelagicus*, *Syngnathus acus*, *Syngnathus typhle*, *Uroteuthis (Photololigo) edulis*, *Uroteuthis (Photololigo) duvauceli* and *Verticillium alboatrum*. This article also documents the addition of nine sequencing primer pairs and sixteen allele specific primers or probes for *Oncorhynchus mykiss* and *Oncorhynchus tshawytscha*; these primers and assays were cross-tested in both species.

This article documents the addition of 512 microsatellite marker loci and nine pairs of Single Nucleotide Polymorphism (SNP) genotyping primers to the Molecular Ecology Resources Database. Table 1 contains information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column. Table 2 presents information on SNP genotyping resources added to the MER database, and presents data on the focal species, the number of sequencing primer pairs, the observed number of SNPs, other species the loci were tested in, and the number of allele specific primers or probes. The MER database and Genbank accession numbers and the authors responsible are also listed. A full description of the development protocol for the loci presented here can be found on the Molecular Ecology Resources Database (http://tomato.biol.trinity.edu/).

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Table 1 Information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and Genbank. The authors responsible for each set of loci are listed in the final column

Species	No. of primers developed	Other species tested	MER database no.	GenBank Accession no.	Authors
Alcippe morrisonia morrisonia	15	n/a	37424–37431 37433–37438	DQ858940–DQ858948, DQ858950–DQ858953, FJ716585,FJ716586	Rong-Chien Lin, Chuan-Chin Huang, Shou-Hsien Li, Cheng-Te Yao
Bashania fangiana	25	n/a	37708–37732	GQ281353–GQ281377	Xiangjiang Zhan, Fuwen Wei, Michael W. Bruford
Bashania fargesii	17	n/a	37691–37707	GQ267715–GQ267731	Xiangjiang Zhan, Lifeng Zhu, Yongqiang Gao, Fuwen Wei, Michael W. Bruford
Chaetodon vagabundus	15	*Chaetodon baronessa*	37840–37854	GQ281437–GQ281451	Michael L. Berumen, Elisabeth Rochel, Glenn R. Almany, Simon R. Thorrold, Geoffrey P. Jones, Morgan S. Pratchett, Craig Syms, Serge Planes
Colletes floralis	9	n/a	37651–37659	FJ041148- FJ041150, EF137744–EF137749	Tomás E. Murray, Emily S. Davis Robert J. Paxton
Coluber constrictor flaviventris	12	n/a	37758–37769	GQ371177–GQ371188	Page E. Klug, Kimberly A. With, Samantha M. Wisely
Coptotermes gestroi	11	n/a	37779–37789	GQ412733–GQ412743	Beng-Keok Yeap, Ahmad Sofiman Othman, Chow-Yang Lee
Crotophaga major	12	n/a	37778, 37790–37800	GQ144418–GQ144429	C. Riehl S. M. Bogdanowicz
Cyprinella lutrensis	29	n/a	37521–37549	GQ169555–GQ169567	PJ Monnahan, Grose, MJ, Landis, JB, Wiley, EO, Hudman, SP
Danaus plexippus	12	n/a	37855–37866	FJ649210, FJ649212–FJ649223	Helen M McCormick, Olivia A Patty, Richard J Wilkins
Fagus grandifolia	10	n/a	37406–37415	GO248754–GO248763	T. Kubisiak, D. Carey, C. Burdine, J. Koch
Falco tinnunculus	10	*F. columbarius*, *F. eleonorae*, *F. naumanni*, *F. peregrinus*, *F. subbuteo*	37310-37319	FJ842386–FJ842395	P.J.G. de Nova, J.A. Dávila, P. Vergara, J.A. Fargallo
Fletcherimyia fletcheri	12	n/a	37679–37690	GQ300842–GQ300853	Gordana Rasic, Nusha Keyghobadi
Fusarium oxysporum f. sp. *Lycopersici F. oxysporum f. sp. radicis-lycopersici*	27	*F. oxysporum f.* sp. *radicis-lycopersici*, *Fusarium oxysporum f.* sp. *Lycopersici*	38143–38117	FJ882019–FJ882025	C. H. Huang, L. E. Datnoff, L. R. Gale P. D. Roberts
Hydrilla verticillata	8	n/a	37770–37777	FJ907306–FJ907313	Amber M. Grajczyk, W.A. Overholt, J.P. Cuda, S.D. Brown, D.A. Williams
Laterallus jamaicensis coturniculus	19	n/a	37660–37678	FJ997575–FJ997593	Philippe Girard, Steven R. Beissinger
Leavenworthia alabamica	12	n/a	37161–37172	FJ860908, FJ860911–FJ860913, FJ860915, FJ860918, FJ860920, FJ860921, FJ860926, FJ860928, FJ860930, FJ860933	Jeremiah W. Busch William J. Werner

Table 1 (Continued)

Species	No. of primers developed	Other species tested	MER database no.	GenBank Accession no.	Authors
Marmosops incanus	15	*Didelphis aurita*, *Gracilinanus microtarsus*, *Marmosops paulensis and Monodelphis americana*	37173–37187	FJ793928–FJ793937, AJ270097, AY386653, AY386655,AY386664, EF486346	Simone Sommer, Anke Schmidt, Fabiano Fernandes, Thomas Püttker, Renata Pardini
Miichthys miiuy	15	n/a	37801–37815	FJ754034–FJ754048	Ru Zhao, W.C.Liu, M. Liu, S. Y. Zhang, R X Wang
Nasua nasua	15	n/a	37498–37512	FJ914573–FJ914587	Mirian Tieko Nunes Tsuchiya-Jerep, Cladinara Roberts Sarturi, Eduardo Eizirik
Noturus exilis	25	n/a	37733–37757	EU760354-EU760378	SP Hudman, MJ Grose, JB Landis, EO Wiley
Odontesthes bonariensis	17	*O. hatcheri*	37559–37575	AB375407-AB375410, AB375412–AB375419, AB375421- AB375423, AB375425,AB375426, AB375428	Eriko Koshimizu, Carlos Augusto Strüssmann, Eugenio Daniel Tejedor, Nobuaki Okamoto, Hideo Fukuda, Takashi Sakamoto
Quadrula fragosa	9	n/a	37301- 37309	FJ785629- FJ785636, FJ785639	Amanda H. Hemmingsen, Kevin J. Roe, Jeanne M. Serb
Pinctada maxima	16	n/a	37867–37882	FJ607747–FJ607749, FJ607751, FJ607753–FJ607756, FJ607759–FJ607760, FJ607762, FJ607764, FJ607767, FJ607768, FJ607770,FJ607774	Yan Wang, Na Liu, Yaohua Shi, Zhifeng Gu, Aimin Wang
Pseudaletia separata	8	n/a	37292–37295, 37297- 37300	FJ896055–FJ896062	Guo-Yan Zhang, Bao-Ping Zhai
Pseudoperonospora cubensis	8	n/a	37513–37520	FJ764997–FJ765004	Loukas Kanetis, Xinwang Wang, Phillip A. Wadl, Katie Neufeld, Gerald Holmes, Peter S. Ojiambo, Marc A. Cubeta, Robert N. Trigiano
Podocarpus elatus	9	*P. grayi*, *P. lawrencei*, *P. smithii.*	37550–37558	FJ935795–FJ935803	Rohan Mellick, Carolyn Porter, Maurizio Rossetto
Portunus trituberculatus	17	*P. pelagicus*	37634–37650	AF410872 FJ660922 FJ660923-FJ660929 GE342670 GE342703 GE342919 GE468057 GE468081 GE468082 GE468121 GE468190	Zhaoxia Cui, Hongxia Wang, Feng Tan, Danhua Wu, Yuan Liu, Weisha Luan Qianqian Li
Rhagoletis cerasi	13	*R. cingulata*, *R. completa*, *R. mendax*, *R. pomonella*	37816–37828	GQ149111–GQ149123	Wolfgang Arthofer, Susanne Krumböck, Hannes Schuler, Bilal Rasool, Markus Riegler, Kirsten Köppler, Christian Stauffer
Rhinella schneideri	11	n/a	37594–37604	FJ847928, FJ847930, FJ847931, FJ847933–FJ847940	Maurício P. de Arruda, Eliana Morielle-Versute, Artur Silva, Maria Paula Cruz Schneider, Evonnildo C. Gonçalves
Sarracenia alata	9	n/a	37585–37593	GQ219717–GQ219725	Margaret M. Koopman, Elizabeth Gallagher, Bryan C. Carstens

Table 1 (Continued)

Species	No. of primers developed	Other species tested	MER database no.	GenBank Accession no.	Authors
Skeletonema marinoi	8	n/a	37621–37626, 37628–37629	EU855763, EU855769–EU855771, EU855775, EU855777, GQ250935, GQ250937	Anna Godhe, Karolina Härnström, V. Saravanan, Christer Halldén, Iddya Karunasagar, Indrani Karunasagar
Sminthurus viridis	14	n/a	37914–37927	FJ971060–FJ971073	John M. K. Roberts, Andrew R. Weeks
Syngnathus abaster	9	*S. acus*, *S. typhle*	37576–37584	GQ168557-GQ168565	Onno E. Diekmann, Licinia Gouveia, Ester T. A. Serrão, Mirjam S. van de Vliet
Uroteuthis (*Photololigo*) *chinensis*	12	*Uroteuthis (Photololigo) edulis* *Uroteuthis (Photololigo) duvauceli*	37954–37965	FJ980010–FJ980021	Y. W. Sin, K. H. Chu, Cynthia Yau
Verticillium dahliae	22	*V. albo-atrum*	37063-37084	FJ851470 FJ851474 FJ851480 FJ851483 GQ160902 FJ851489 FJ851494 FJ851499 FJ851504 FJ851508 FJ851511 FJ851514 FJ851519 FJ851521 GQ160903 FJ851523 FJ851527 FJ851530 FJ851534 FJ851538 FJ851541 FJ851545	Z. K. Atallah, K. K. Maruthachalam, R. M. Davis, S. J. Klosterman, K. V. Subbarao
Wasmannia auropunctata	21	*W. rochai*, *W. sigmoidea*, *A. decemarticulatus*, *L. humile*, *S. saevissima*, *B. spp*	37234–37255	FJ970003–FJ970023	O. Rey, A. Loiseau
Zygochlamys patagonica	11	n/a	38059–38069	FJ937726–FJ937736	Ian G Paterson, María I Trucco, Mario L Lasta, Daniel E Ruzzante

Table 2 Information on the focal species, the sequencing primer pairs developed, the number of single nucleotide polymorphisms observed and any other species the loci were tested in. The next columns contain the number of allele specific primers and probes developed, and the Molecular Ecology Resources Database and Genbank accession numbers, respectively. The authors responsible for each set of loci are listed in the final column

Species	No. primer pairs	No. SNPs in sequence	Others species tested	No. Allele specific primers/ probes	Target gene(s)	MER database numbers	Genbank Accession no.	Authors
Oncorhynchus mykiss/ Oncorhynchus tshawytscha	9/9	87/68	*O. mykiss/ O. tshawytscha*	16	heat shock proteins	37605–37620	FJ772745–FJ772915, FJ772947–FJ773066, FJ773092–FJ772525	Nathan R. Campbell, Shawn R. Narum

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Thirteen new microsatellite loci in *Rhagoletis cerasi* (Diptera: Tephritidae), a model host species for *Wolbachia* symbiosis in field populations

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Running title: *Rhagoletis* microsatellites

Abstract

The European cherry fruit fly *Rhagoletis cerasi*, an important pest in cherry orchards, exhibits *Wolbachia* - induced crossing incompatibilities between two geographic complexes in Europe. Here we present 13 new microsatellite markers in *R. cerasi*. All markers are polymorphic with 4 to 12 alleles per locus and observed heterozygosity values between 0.25 and 0.95. Two markers show significant deviations from Hardy-Weinberg proportions after Bonferroni correction. While the influence of *Wolbachia* on the host's mitochondrial genome is recognised, the markers presented here will provide a useful tool for the analysis of population structure and dynamics of this multiply infected field model species for the first time.

The European cherry fruit fly *Rhagoletis cerasi* infests cherry (*Prunus spp.)* and honeysuckle *(Lonicera spp.)* fruits in continental Europe, the Mediterranean region and temperate regions of Asia (Fimiani 1989, Fischer-Colbrie and Busch-Petersen 1989). It causes substantial losses in cherry orchards (Fimiani 1989) and received attention as one of the target pests for the sterile insect technique (SIT) in the 1970ies. Extensive tests for crossing compatibilities between populations revealed the existence of two geographic complexes with unidirectional incompatibility (Boller and Bush 1974, Boller et al 1976). Riegler and Stauffer (2002) found infections with two distinct *Wolbachia* strains *w*Cer1 and *w*Cer2 and identified *w*Cer2 as the causal agent of incompatibility. Recent screenings revealed superinfections with up to five *Wolbachia* strains in *R. cerasi* (Arthofer et al. 2009). The extensive historic data set on distribution of the geographic complexes (Boller and Bush 1974, Boller et al 1976) coupled with field collections over the entire distribution range of the host species for the last decade (Riegler & Stauffer 2002, Arthofer et al 2009) make *R. cerasi* to one of the most attractive field models of *Wolbachia* dynamics. Here we report the isolation of new polymorphic microsatellite markers of the cherry fruit fly genome that are required for studying the field population dynamics of *Wolbachia* and *R. cerasi*.

Genomic DNA from five individuals of *R. cerasi* was isolated using the GenElute mammalian genomic DNA miniprep kit (Sigma) following the manufacturer's protocol. DNA was quantified photometrically and 250 ng were used in a one-step *MseI* digestion and adaptor ligation reaction according to Zane et al. (2002). A production PCR over 19 cycles using adaptor primers yielded 800 ng

DNA. Microsatellite enrichment was performed by hybridization to biotinylated $(AC)_8$ and $(GA)_8$ oligonucleotides at 50°C and subsequent capture wi th streptavidine MagneSphere paramagnetic particles (PMPs, Promega). PMPs were washed twice with 0.5x SSC and twice with 0.2x SSC. Enriched DNA was thermally eluted to sterile water and a recovery PCR with 32 cycles and adaptor primers was performed. PCR products were purified with the peqGOLD cycle pure PCR purification kit (peqlab), cloned into the pTZ57R/T vector (Fermentas) and used for transformation of JM109 competent *E. coli* cells. 492 white colonies were inoculated onto masterplates and transferred to C/P lift membranes (Biorad). After overnight hybridization at 50°C with digoxigenin (DIG) labelled SSR oligoprobes and subsequent washing steps, screening was performed with the DIG Luminescent Detection Kit (Roche). 44 colonies yielding the darkest signals on x-ray film were transferred to liquid culture and plasmid DNA was extracted by alkaline lysis. In a PCR using $(AC)_8$ and $(GA)_8$ oligonucleotides as microsatellite specific primers in combination with one of the vector primers (M13F or M13R), 28 plasmids yielded products and were selected for sequencing. Only eight unique repeat motifs were found by this approach, and dinucleotide motifs were often accompanied by singe nucleotide repeats. Thus, the whole enrichment was repeated using biotinylated $(C)_{12}$ and $(T)_{12}$ oligoprobes. 144 colonies of this library were pretested by PCR and 72 selected for sequencing. A total of 21 unique mononucleotide repeats and one trinucleotide repeat with flanking regions suitable for primer development were identified.

85 Microsatellite polymorphism was tested on 20 single fly extracts from the
86 eastern Austrian population using a tailed primer technique (Boutin-Ganache et
87 al. 2001). Reactions were carried out on a AB 2720 thermocycler (Applied
88 Biosystems) in 10 μl final volume containing 1x reaction buffer (Fermentas), 1.5
89 mM $MgCl_2$, 100 μM dNTPs, 0.2 μM fluorescent labelled M13 primer, 0.02 μM
90 M13 tailed locus specific forward primer, 0.2 μM untailed specific reverse
91 primer, 0.25 U Taq polymerase (Fermentas) and 0.8 μl template DNA. Cycling
92 conditions were 94℃ for 5 min followed by 35 cycle s at 94℃ for 30 sec, 60℃
93 for 1 min, 72℃ for 45 sec and a final extension at 68℃ for 20 min. Fragment
94 analysis was carried out on an ABI 3100 genetic analyzer and traces were
95 visualized by PeakScanner software and scored manually. 13 primer pairs
96 showed polymorphic patterns. The characteristics of these loci are summarized
97 in table 1. Observed and expected heterozygosities and an exact probability
98 test for deviations from Hardy-Weinberg proportions (HWP) using the Markov
99 chain method with default parameters were calculated in the GENEPOP web
100 interface (http://genepop.curtin.edu.au/; Raymond & Rousset 1995). The
101 number of alleles ranges from 4 to 12 per locus, and H_o values are in the range
102 of 0.25 to 0.96. After sequential Bonferroni correction (Rice 1989) two loci
103 displayed deviations from Hardy-Weinberg proportions ($P<0.01$). No significant
104 departures from gametic disequilibrium at $P<0.01$ were observed in a test
105 involving all pairwise combinations as implemented in FSTAT (Goudet 1995).
106 Applying MICRO-CHECKER software (van Oosterhout et al. 2004), locus 76-3
107 showed a general excess of homozygotes for most allele size classes indicative
108 for null alleles. No other signs for null alleles or large allele dropout were
109 detected.

The primers were also tested on DNA of single individuals of four other species from the genus *Rhagoletis*. Four loci showed limited cross-amplification and results are summarized in table 2.

While the influence of *Wolbachia* on mitochondrial DNA is recognised (Hurst and Jiggins 2005), little is known on the influence of the endosymbiont on nuclear host genome dynamics. The microsatellite markers presented here will provide a useful tool for the study of population structure and dynamics in a *Wolbachia* multiply infected field model species.

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157 **Table 1.** Polymorphic *Rhagoletis cerasi* microsatellite loci listed by locus names, repeat motifs, forward (M13 tailed) and reverse
158 primer sequences in 5' – 3' direction, amplicon size range in bp, number of alleles resolved, observed (H_o) and expected (H_e)
159 heterozygosities, confidence values for the exact probability test for HWP (P) and GenBank accession numbers. Significant
160 deviations from HWP after sequential Bonferroni correction are marked with an asterisk.
161

Locus	Repeat Motif	Primer F	Primer R	Size range	n alleles	Ho	He	P	GenBank
RcMic 76-1	$(TG)_{11}CG(TG)_6$	CACGACGTTGTAAAACGACAACTGTGTCAATTGTGGTGC	GGACGAGATTACCGACTGGA	212-234	8	0.75	0.77	0.138	GQ149111
RcMic 76-3	$(CA)_7$ ... $(T)_7CG(T)_{11}$	CACGACGTTGTAAAACGACTCAGTTAGGCTTCCTTCTACCC	GCAGCTGCTGTTTTCTGTGA	288-305	10	0.55	0.88	0.001	GQ149112
RcMic 76-7	$(T)_9$ ... $(TG)_2TC(TG)_8$	CACGACGTTGTAAAACGACGCCACCGACGTTGACTTACT	AGCGCAGCAAAGGCTTCAGTG	256-261	6	0.55	0.71	0.165	GQ149113
RcMic 79-4	$(GT)_6$ ... $(A)_{13}$	CACGACGTTGTAAAACGACAACAACCTTGACGTAGGGGC	TGCTGTGATGGCTGCACTAG	291-299	5	0.35	0.43	0.225	GQ149114
RcMic 82-10	$(G)_{14}$	CACGACGTTGTAAAACGACGCAACCGCATTTACTCGAAC	CAAAGCTGCTGTAGCTGACG	170-182	10	0.95	0.86	0.000 *	GQ149115
RcMic 82-28	$(G)_{14}A(G)_4$	CACGACGTTGTAAAACGACGGGGTCGAGAGATGTTCAC	TAGTTTTAGCGTTCTTCTTG	178-189	9	0.85	0.78	0.443	GQ149116
RcMic 82-46	$(G)_{16}$	CACGACGTTGTAAAACGACCAGGAGACCAGAAGCGAATC	AGATGGCCTACCGAACCTTT	196-196	6	0.55	0.56	0.465	GQ149117
RcMic 82-47	$(G)_{17}$	CACGACGTTGTAAAACGACTAATCGCGTCGCAGATGTAG	GATGCCACTTGTCCAGATCA	205-217	12	0.95	0.79	0.999	GQ149118
RcMic 83-16	$(A)_6T(A)_6C(A)_5T(A)_{11}$	CACGACGTTGTAAAACGACCGCTGCAGAAGTGAGAATGC	ATCCACTCCCACATTGAAG	149-153	5	0.65	0.71	0.656	GQ149119
RcMic 83-26	$(G)_{13}$	CACGACGTTGTAAAACGACCACAAGGGCCTAGGTTGGTA	CCTGCAGTGATGTCGGAGTA	221-232	10	0.75	0.78	0.339	GQ149120
RcMic 83-44	$(GGT)_6$	CACGACGTTGTAAAACGACACCTGTCACCTATCTGAGCG	CAATAGCTCCACAGCCGATT	214-225	4	0.25	0.31	0.097	GQ149121
RcMic 84-35	$(C)_{12}A(C)_6$	CACGACGTTGTAAAACGACATGTGCATGTTTTAGCGTTC	CCCTTCCGCCTATAACAAC	236-242	7	0.80	0.83	0.002	GQ149122
RcMic 84-42	$(G)_{12}$	CACGACGTTGTAAAACGACTCAGCGCATTGAGTATTTGG	GTCTCGGGTTTGTCTGCAAT	170-184	7	0.50	0.62	0.000 *	GQ149123

162

163 **Table 2.** Cross-amplification of four *R. cerasi* microsatellite loci with other species of the genus *Rhagoletis*. The remaining 9 loci did

164 not show any bands.

Species	RcMic 76-3	RcMic 76-7	RcMic 82-47	RcMic 83-26
R. cingulata		✓	✓	✓
R. completa	✓	✓		✓
R. mendax		✓		
R. pomonella				✓

165