

Microsatellite evidence of invasion and rapid spread of divergent New Zealand mudsnail (*Potamopyrgus antipodarum*) clones in the Snake River basin, Idaho, USA

Robert Hershler · Hsiu-Ping Liu ·
William H. Clark

Received: 2 March 2009 / Accepted: 10 August 2009 / Published online: 4 September 2009
© U.S. Government 2010

Abstract We used microsatellites to assess genetic diversity and spatial structuring of the invasive apomictic New Zealand mudsnail (*Potamopyrgus antipodarum*) in the initial focal area of its recent North American invasion, a portion of the upper Snake River basin (Idaho) that is segmented by a series of hydropower dams. Thirty-four samples (812 total snails) from a 368 km reach of this drainage were genotyped for six loci. Sixty-five distinct clones were detected and grouped into four divergent clusters based on chord distances. Genetic structuring of populations was generally low. Our results indicate that the founding population(s) of this invasion was composed of a small number of putative clonal lineages which spread rapidly within this fragmented watershed owing to the enhanced dispersal ability of these parthenogens. The substantial genetic variation

documented in this study suggests that caution should be used in the application of biological control measures for this pest species.

Keywords Invasive species · New Zealand mudsnail · *Potamopyrgus* · Microsatellites · Genetic diversity · Population structuring

Introduction

The New Zealand mudsnail, *Potamopyrgus antipodarum* (Gray 1843), is a small aquatic gastropod native to New Zealand (Winterbourn 1970) that has been widely introduced in Australia (Ponder 1988), Europe (Ponder 1988), North America (Zaranko et al. 1997) and parts of Asia (Kornijów et al. 2001; Urabe 2007; Kalyoncu et al. 2008) over the past 200 years. *Potamopyrgus antipodarum* was first discovered in North America in 1987 in the Snake River and tributary springs near Hagerman, south-central Idaho (Bowler 1991); the source and mode of this introduction are unknown. This invasive snail subsequently spread within the Snake River basin and thence colonized other western drainages (MSU 2009) and crossed the continental divide into the headwaters of the Missouri River basin (Bowler and Frest 1996), establishing huge populations (e.g.,

R. Hershler (✉)
Department of Invertebrate Zoology, Smithsonian
Institution, P.O. Box 37012, NHB W-305, MRC 163,
Washington, DC 20013-7012, USA
e-mail: hershler@si.edu

H.-P. Liu
Department of Biology, Metropolitan State College of
Denver, Denver, CO 80217, USA

W. H. Clark
Orma J. Smith Museum of Natural History, Albertson
College of Idaho, Caldwell, ID 83605-4432, USA

500,000/m², Banbury Springs, Idaho; Richards et al. 2001) that are altering ecosystem food web functioning (Hall et al. 2006; Riley et al. 2008) and negatively impacting colonization (Kerans et al. 2005) and growth (Lysne and Koetsier 2008) of native invertebrates. This species has also independently colonized the Great Lakes region in eastern North America (Zaranko et al. 1997). *Potamopyrgus antipodarum* is currently recognized as a major aquatic pest in North America (ANSTF 2009) and is expected to spread over much of continent unless prevention measures are undertaken (Loo et al. 2007).

The rapid spread and tremendous densities which characterize *P. antipodarum* as a successful invader in North America (and other parts of its introduced range) may be attributed in large part to its parthenogenetic reproductive mode (Ponder 1988; Alonso and Castro-Díez 2008), which is shared by only a few mollusks (Suomalainen et al. 1987). Native populations of this species are composed of sexually reproducing diploids; and apomictic triploid females (Wallace 1992), which are thought to arise through the occasional fertilization of unreduced eggs (Hughes 1996). Invasive populations are predominantly or entirely female (e.g., Wallace 1985; Zaranko et al. 1997; Schreiber et al. 1998) and believed to be exclusively clonal (e.g., Hughes 1996; Anderson 2006; Alonso and Castro-Díez 2008).

Previous investigations of the genetic diversity of *P. antipodarum* outside its native range have consisted of broad-scale surveys in western Europe, where this species was first discovered in the late 1800's (Ponder 1988), which showed that regional populations are predominantly composed of a small number of widely distributed clones (Hauser et al. 1992; Jacobsen et al. 1996; Weetman et al. 2002; Städler et al. 2005). Here we provide an additional genetic perspective on this nuisance species by examining microsatellite variation on smaller spatial scales within the initial focal area of its recent North American invasion, a portion of the upper Snake River basin that is segmented by a series of hydro-power dams on the master stream. Our specific goals are to examine the extent and spatial structuring of genotypic and clonal diversity among mainstem Snake River and tributary habitats along a 368 km reach of this fragmented drainage; and to use these results to make inferences about regional invasion dynamics.

Materials and Methods

Sampling

Thirty-four (34) sites were sampled during 2006–2007 for this study; 23–24 specimens were collected from each site (Fig. 1; Table 1). Collections were made from the Snake River (19 samples) and seven tributary systems (15 samples) between Niagara Springs and the vicinity of Ontario, spanning six river reaches separated by hydropower dams (Fig. 1). The collection localities included multiple sites in the Thousand

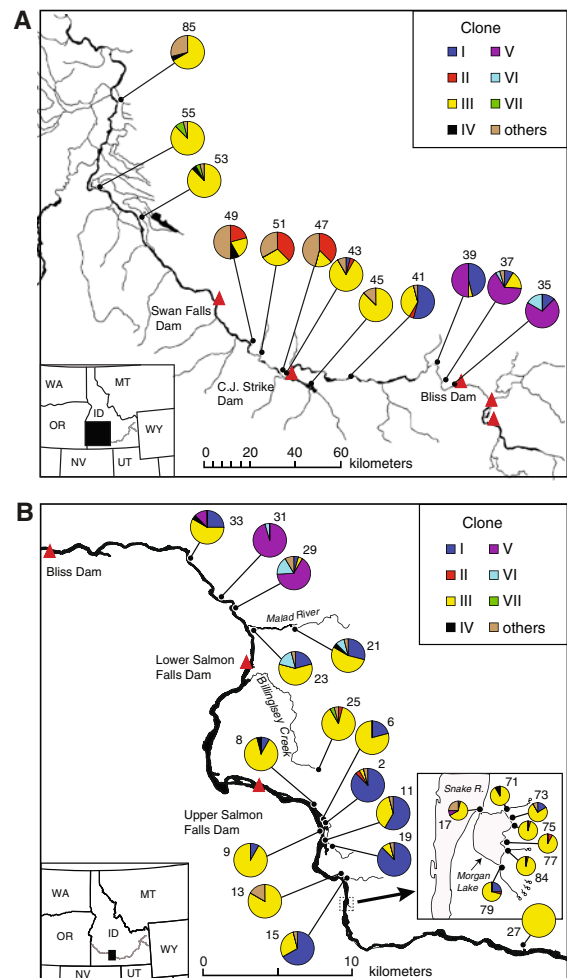


Fig. 1 Maps showing location of sampling sites within the upper Snake River basin, south-central Idaho. **a** Localities below Bliss Dam. **b** Localities above Bliss Dam. Numbers refer to samples listed in Table 1. The proportional representation of the seven most common clones (with the remaining clones lumped into “others”) in each sample is also depicted

Table 1 Sample locality information and summary statistics for microsatellite data

Sample code	Collection locality	Easting ^a	Northing ^a	N ^b	G ^b	G/N ^b	Eff ^b	Eve ^b	Shw ^b
<i>Above upper Salmon Falls Dam</i>									
27	Niagara Springs	690558	4726111	24	1	0.042	1	1	0
79	Banbury Springs (F)	678476	4728681	24	3	0.125	1.767	0.589	0.314
84	Banbury Springs (G)	678444	4728606	24	2	0.083	1.087	0.544	0.075
77	Banbury Springs (E)	678469	4728752	24	2	0.083	1.18	0.59	0.125
75	Banbury Springs (C)	678501	4728816	23	2	0.087	1.091	0.546	0.078
73	Banbury Springs (B)	678500	4728860	24	4	0.167	1.684	0.421	0.338
71	Banbury Springs (A)	678484	4728888	24	2	0.083	1.18	0.59	0.125
17 ^c	Snake River at outflow of Morgan Lake	678343	4728909	24	9	0.375	2.556	0.284	0.638
15 ^c	Snake River at mouth of Box Canyon	678181	4730523	24	3	0.125	1.947	0.649	0.338
13 ^c	Snake River at Blue Springs	677748	4730990	24	5	0.208	1.426	0.285	0.296
19	Sand Springs, middle reach	677238	4732800	24	3	0.125	1.291	0.43	0.198
11	Sand Springs, lower reach	676615	4733083	24	3	0.125	2.072	0.691	0.354
09 ^c	Spring outflow south of powerhouse	676555	4733491	24	2	0.083	1.18	0.59	0.125
02	Thousand Springs, south inlet (Lemon Falls)	676793	4734195	24	4	0.167	1.297	0.324	0.223
06	Thousand Springs, south of Minnie Miller	676594	4734783	24	2	0.083	1.492	0.746	0.222
08 ^c	Snake River at outflow of spring south of Bickel Spring	675670	4735798	24	3	0.125	1.291	0.43	0.198
<i>Between upper and Lower Salmon Falls Dams</i>									
25	Spring along Billingsley Creek	676028	4738221	24	4	0.167	1.297	0.324	0.223
<i>Between lower Salmon Falls and Bliss Dams</i>									
21	Malad River, above diversion	674134	4748020	24	5	0.208	2.692	0.538	0.515
23	Malad River, near Snake River confluence	671367	4747703	24	4	0.167	2.42	0.605	0.466
29 ^c	Snake River, Sidewinder site	670017	4749202	23	5	0.208	2.142	0.428	0.464
31 ^c	Snake River, unnamed point	669108	4749775	23	2	0.087	1.091	0.546	0.078
33 ^c	Snake River, at outflow of large spring	667304	4752162	24	4	0.167	2.38	0.595	0.457
<i>Between Bliss and C.J. Strike Dams</i>									
35 ^c	Snake River at Pilgrim Springs	652668	4751411	24	3	0.125	1.834	0.611	0.349
37 ^c	Snake River at outflow of Bancroft Springs	650470	4755099	23	5	0.208	2.142	0.428	0.464
39 ^c	Snake River at mouth of Clover Creek	648244	4762313	24	3	0.125	2.165	0.722	0.363
41 ^c	Snake River, C.J. Strike Reservoir, at Hooley Pump	607518	4754959	24	4	0.167	2.286	0.572	0.419
45	C.J. Strike Reservoir, Bruneau Arm	590076	4751926	24	3	0.125	1.291	0.43	0.198
43 ^c	Snake River, C.J. Strike Reservoir, just above dam	583693	4755811	24	5	0.208	1.426	0.285	0.296
<i>Between C.J. Strike and Swan Falls Dams</i>									
47 ^c	Snake River, below C.J. Strike Dam	579664	4756656	24	13	0.542	5.333	0.41	0.922
51 ^c	Snake River, west of Chattin Flats	569303	4763269	24	9	0.375	4.114	0.457	0.751
49 ^c	Snake River, south of Black Butte	564876	4766767	24	15	0.625	8.727	0.582	1.064
<i>Below Swan Falls Dam</i>									
53 ^c	Snake River, Marsing	515913	4822243	24	4	0.167	1.297	0.324	0.223
55 ^c	Snake River, below Homedale	498379	4836195	24	3	0.125	1.291	0.43	0.198
85 ^c	Snake River, above Ontario Island	504028	4876272	24	7	0.292	2.149	0.307	0.527

^a Universal Transverse Mercator NAD 83, Zone 11

^b N = sample size, G = number of genotypes, G/N = proportion of distinct genotypes, Eff = effective number of genotypes, Eve = evenness of Eff, Shw = uncorrected Shannon-Wiener index

^c Sample from mainstem Snake River

Springs complex and the mainstem Snake River near Hagerman (above Lower Salmon Falls Dam) where *P. antipodarum* was first discovered in North America (Bowler 1991). Specimens were collected by hand in small (ca. 30 m²) shallow water areas and preserved in 90% ethanol in the field. Collection locality coordinates were determined using a global positioning system in the field. Additional locality and collection data, and museum voucher numbers for our samples are available from the first author upon request.

Microsatellites

Genomic DNA was isolated from individual snails using a CTAB protocol (Bucklin 1992). Extractions were performed for 24 specimens from each sample (812 total). Individual snails were screened with a panel of six microsatellite markers (Pa217, Pa254, Pa56, Pa143, Pa121, Pa112) that were developed for *P. antipodarum* by Weetman et al. (2001). (A seventh locus, Pa132, could not be scored unambiguously in some individuals even after multiple attempts to redesign primers and optimize PCR conditions and thus was excluded from this study.) A forward primer dye-labeled with Beckman Coulter dye D2 was used in polymerase chain reactions for locus Pa56. Each 12.5 µl reaction contained 1X Promega buffer, 3.5 mM of MgCl₂, 2.5 mM of dNTPs, 0.5 µM dye-labeled forward primer, 0.5 µM of reverse primer, 0.31 U of *Taq* polymerase and 50–100 ng of template DNA. For the other five loci, PCR was performed using an M13-tailed forward primer as described by Boutin-Ganache et al. (2001). Each 12.5 µl reaction contained 1X Promega buffer; 3.5 mM of MgCl₂; 2.5 mM of dNTPs; 0.034 µM of M13-tailed forward primer; 0.5 µM of reverse primer; 0.5 µM of M13 primer dye-labeled with Beckman Coulter dyes D2, D3 or D4 (Prologo); 0.31U of *Taq* polymerase and 50–100 ng of template DNA. The thermal profile for both the forward dye-labeled and the M13 dye-labeled reactions was as follows: the PCR reaction consisted of an initial 2 min denature step at 94°C, followed by 35 cycles of denature 1 min at 94°C, optimal anneal temperature for 1 min (Pa217, Pa254, Pa56, Pa121 and Pa112: 59°C; Pa143: 63°C), extend 1 min at 72°C and a final extension step at 72°C for 5 min. The PCR products were run on the CEQ8000 XL

DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analyzed using the Frag3 default method.

Data analysis

Clonal diversity was assessed using GENOTYPE/GENODIVE v2.0b13 (Meirmans and Van Tienderen 2004). As the first step in this process, the clonal membership of each multilocus genotype was assessed using GENOTYPE, which reduces the upward bias in the estimation of clonal diversity attributable to variation within clones that is due to somatic mutations and genotyping errors. GENOTYPE performs this task by generating a frequency distribution histogram of genetic distances between individuals (which is presumed to be multimodal in asexual taxa) and establishing a threshold value for intracolonial distance based on the valley between the first and second peaks (Meirmans and Van Tienderen 2004). The infinite allele model (with missing data not counted) option was used for this analysis. After multilocus genotypes were assigned to clones using the selected threshold value, the number of genotypes (clones) (G), effective number of genotypes (Eff), evenness of Eff (Eve), and uncorrected Shannon-Wiener index (Shw) were generated for each sample using GENODIVE. The proportion of distinct genotypes (G/N, N = sample size) was calculated by hand.

Isolation by distance was assessed with a Mantel test (based on 10,000 randomizations) implemented in the program IBD (Isolation By Distance; Bohonak 2002), which correlates chord distance (calculated using GENODIVE) with geographic distance. We used the chord metric because it has a low sampling error and makes no assumptions about population size and loci mutation rates (Takezaki and Nei 1996). Pairwise geographic distances between sampling localities were measured as stream distances, which were modeled using ESRI ArcGIS software with the Network Analyst extension (<http://www.esri.com/software/arcgis/extensions/networkanalyst/index.html>). Stream network data were obtained from the National Hydro Dataset (<http://nhd.usgs.gov>). Partitioning of genetic variation was assessed by analysis of molecular variance (AMOVA; Excoffier et al. 1992) performed in GENODIVE using an infinite allele

model (Fst-analogue). We tested for genetic divisions between the mainstem Snake River and its tributaries, and between tributaries. We also evaluated partitions between drainage segments separated by dams, with separate analyses conducted for all samples and mainstem Snake River samples only.

As a means of examining relationships among clones we calculated chord distance (D_{cc} , Cavalla-Sforza and Edwards 1967) values with GENODIVE and used the resulting distance matrix to construct an unrooted neighbor-joining network with NEIGHBOR (PHYLIP, version 3.57c; Felsenstein 1995). Topological confidence was evaluated with 1000 bootstrap replicates using SEQBOOT and CONSENSE in the PHYLIP package.

Results

Four of the 816 specimens could not be amplified or confidently scored and thus were excluded from the dataset. Seventy-five (75) multilocus genotypes were detected among the 812 snails (see Appendix Table 4). The frequency distribution of pairwise genetic distances between individual snails contained two main peaks, the first of which was close to zero (Fig. 2). We chose a threshold distance of “one” (recommended by GENOTYPE for our dataset), below which individuals were considered clone mates (Fig. 2). At this selected threshold, the 75 multilocus

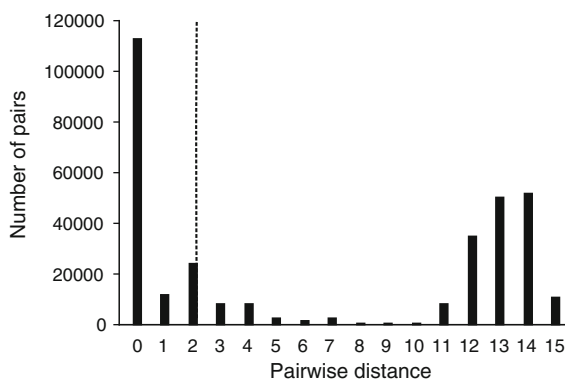


Fig. 2 Frequency distribution of pairwise genetic distances among 812 specimens of *P. antipodarum* from the upper Snake River basin. The dashed line represents the threshold of intraclonal distance

genotypes were assigned to 65 clones, only one of which (clone I) was composed of multiple genotypes (see Appendix Table 4). Clonal diversity is summarized in Table 1. The proportion of distinguishable genotypes (G/N) was < 0.3 in 30 samples and 0.375–0.625 in the other four samples. The effective number of genotypes, evenness, and Shannon-Weiner index ranged from 1–8.73, 0.284–1 and 0–1.064, respectively. The highest clonal diversity was observed at mainstem Snake River localities between the C.J. Strike and Swan Falls Dams (samples 47, 49; Table 1).

The number of clones (G) detected within samples ranged from one to 15 (Table 1) and averaged 4.36. Only a single sample (sample 27, Niagara Springs) was monoclonal. The frequency distributions of the seven most common clones are detailed in Table 2 and Fig. 1. The two most common clones (III, I) were observed in 32/34 (94%) and 19/34 (56%) samples and accounted for 56 and 17% of the analyzed snails, respectively; the seven most common clones comprised 92% of the total sample (744/812). The remaining rare clones were scattered within the study area (Table 2) and consisted of 49 singletons and nine clones that were composed of two–three specimens from one–two sites (Table 2). Six of the seven most common clones were observed in both tributary and mainstem Snake River samples while clone V was only detected in mainstem samples (Fig. 1; Table 2). All seven of these clones ranged across at least one of the Snake River dams and the most widely distributed clone (III) was detected in all six drainage reaches (Fig. 1; Table 2).

The correlation between genetic and stream distances among samples was non-significant (Mantel test, $P = 0.9040$) (Fig. 3). In each AMOVA most of the variance was distributed within samples (89.5–92.1%) (Table 3). Variation between mainstem river and tributary habitats and among tributary systems was small (0.45, 4.86%, respectively) and non-significant. Variation among drainage reaches separated by dams, based on all samples or only those from mainstem Snake River localities, was significant, but also very small (2.11, 4.54%, respectively).

The relationships between clones based on chord distances are shown in Fig. 4. Sixty-three of the 65 clones clustered into four divergent groups which we interpret as putative clonal lineages. The remaining

Table 2 Frequency distribution of *P. antipodarum* clones

Sample code	Clone							
	I	II	III	IV	V	VI	VII	Others
27 ^a	–	–	24	–	–	–	–	–
79 ^a	6	1	17	–	–	–	–	–
84 ^a	–	1	23	–	–	–	–	–
77 ^a	–	2	22	–	–	–	–	–
75 ^a	–	1	22	–	–	–	–	–
73 ^a	4	–	18	–	–	–	–	2
71 ^a	–	–	22	2	–	–	–	–
17	1	–	15	–	2	–	–	6
15	16	–	7	–	–	–	–	1
13	–	–	20	–	–	–	–	4
19 ^a	21	–	2	–	–	–	–	1
11 ^a	14	–	9	–	–	–	–	1
9	2	–	22	–	–	–	–	–
2 ^a	21	1	1	–	–	–	–	1
6 ^a	5	–	19	–	–	–	–	–
8	2	–	21	1	–	–	–	–
25 ^a	–	1	21	–	–	–	1	1
21 ^a	7	–	13	1	–	2	–	1
23 ^a	5	–	14	–	–	4	–	1
29	1	–	1	–	15	4	–	2
31	–	–	–	–	22	1	–	–
33	6	–	14	1	3	–	–	–
35	3	–	–	–	17	4	–	–
37	2	–	4	–	15	1	–	1
39	11	–	1	–	12	–	–	–
41	13	1	9	–	–	–	–	1
45 ^a	–	–	21	–	–	–	–	3
43	1	1	20	–	–	–	–	2
47	–	9	4	–	–	–	–	11
51	–	9	7	–	–	–	–	8
49	–	5	5	2	–	–	–	12
53	–	–	21	1	–	–	1	1
55	–	–	21	–	–	–	2	1
85	–	–	16	1	–	–	–	7
Total	141	32	456	9	86	16	4	68

Values in italics distinguishes samples from (six) drainage reaches separated by Snake River dams

^a Sample from tributary habitat

two clones were singletons detected in tributary samples. Fifty-seven (57) clones were clustered in one of the putative lineages whereas each of the three

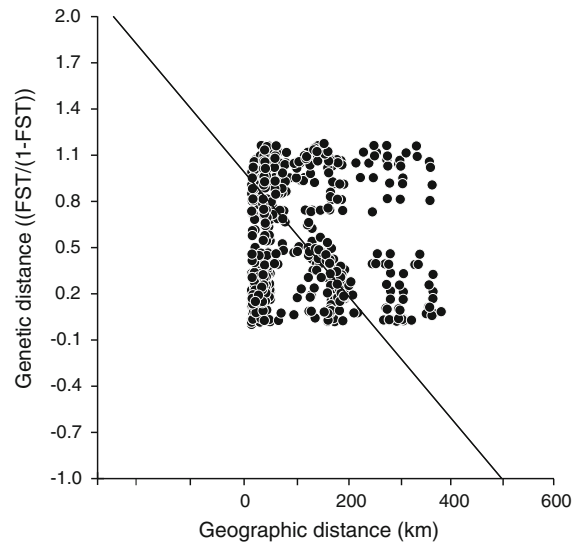


Fig. 3 Reduced major axis regression of genetic and geographic distances based on all pairwise combinations of *P. antipodarum* samples

other lineages was composed of two clones. Note that bootstrap support for this topology was low.

Discussion

Introduced populations of *P. antipodarum* in North America have been assumed to be entirely clonal based on sex ratios (e.g., Zaranko et al. 1997). Our microsatellite data from the upper Snake River basin are consistent with this claim. All 812 specimens that we analyzed had three alleles for each locus, based on detection of three peaks or two peaks with unequal height biased towards the longer fragment (short-allele dominance, Armour et al. 1996; Wattier et al. 1998). These results imply triploidy, which is associated with clonal reproduction in *P. antipodarum* and almost every other such species owing to the problem of equally distributing three sets of chromosomes during meiosis (Suomalainen 1950; see Stock et al. 2002 for a rare exception). The general patterns of clonal diversity that we have documented for invasive *P. antipodarum* are closely similar to those observed in other apomictic species (e.g., see compilations in Parker 1979; Ellstrand and Roose 1987) in that most samples (33/34) are multiclonal, most

Table 3 Analysis of molecular variance (AMOVA) for four population structures of *P. antipodarum*

Source of variation	df	Variance components	% Variation	P
By tributary versus mainstem Snake River habitat ^a				
Among groups	1	0.015	0.45	0.158
Among samples within groups	32	0.285	8.64	0.001
Within samples	1,588	2.999	90.91	1.000
By tributary system ^b				
Among groups	6	0.152	4.86	0.059
Among samples within groups	8	0.096	3.07	0.001
Within samples	699	2.877	92.06	1.000
By drainage reach (all samples) ^c				
Among groups	5	0.070	2.11	0.025
Among samples within groups	28	0.242	7.30	0.001
Within samples	1,588	2.999	90.58	1.000
By drainage reach (mainstem Snake River samples only) ^d				
Among groups	4	0.157	4.54	0.017
Among samples within groups	14	0.205	5.93	0.001
Within samples	887	3.097	89.53	1.000

^a Two groups: tributary (2, 6, 11, 19, 21, 23, 25, 27, 45, 71, 73, 75, 77, 79, 84), mainstem Snake River (8, 9, 13, 15, 17, 29, 31, 33, 35, 37, 39, 41, 43, 47, 49, 51, 53, 55, 85)

^b Seven groups: Niagara Springs (27), Banbury Springs (71, 73, 75, 77, 79, 84), Sand Springs (11, 19), Thousand Springs (2, 6), Billingsley Creek (25), Malad River (21, 23), Bruneau River (45)

^c Six groups: above Upper Salmon Falls Dam (2, 6, 8, 9, 11, 13, 15, 17, 19, 27), between Upper and Lower Salmon Falls Dams (25), between Lower Salmon Falls and Bliss Dams (21, 23, 29, 31, 33), between Bliss and C.J. Strike Dams (35, 37, 39, 41, 43, 45), between C.J. Strike and Swan Falls Dams (47, 49, 51), below Swan Falls Dam (53, 55, 85)

^d Five groups: above Upper Salmon Falls Dam (8, 9, 13, 15, 17), between lower Salmon Falls and Bliss Dams (29, 31, 33), between Bliss and C.J. Strike Dams (35, 37, 39, 41, 43), between C.J. Strike and Swan Falls Dams (47, 49, 51), below Swan Falls Dam (53, 55, 85)

clones (52/65) are restricted to single samples, and few clones (1/65) occur in >75% of the samples.

Clonal diversity in this invasive assemblage of *P. antipodarum* (overall G/N, 65/812, 0.08) is low relative to that observed in the native range of this species, where the frequent and iterative transition to asexuality generates remarkable levels of local variation—e. g., 165 clones were detected among 605 clonal specimens (G/N, 0.27) in a small (570 km²) lake using allozyme markers (Fox et al. 1996; for other examples see Dybdahl and Lively 1995; Jokela et al. 1999). The large genetic distances (>1.0 chord distance) between the four putative clonal lineages of invasive *P. antipodarum* suggests that they diverged prior to the recent introduction of this species to the upper Snake River basin. Variation within each of the lineages is minor; and most of the clones (57/65) are

unique to one or two specimens, suggesting that they originated recently via mutation. The scattered spatial distribution of these rare clones (see Table 2, “others” column) also suggests that they evolved subsequent to the presumed point introduction of *P. antipodarum* to this region. These patterns of microsatellite variation collectively suggest that only a small number of founder clones of *P. antipodarum* were introduced to the upper Snake River basin. A similar finding based on microsatellite data was reported for introduced colonies of *P. antipodarum* in Great Britain (Weetman et al. 2002).

Although it seems likely that a single colonization event was involved based on the history of regional spread (Bowler 1991; Bowler and Frest 1996) and the overlapping geographic distributions of clonal lineages, the possibility of multiple introductions of

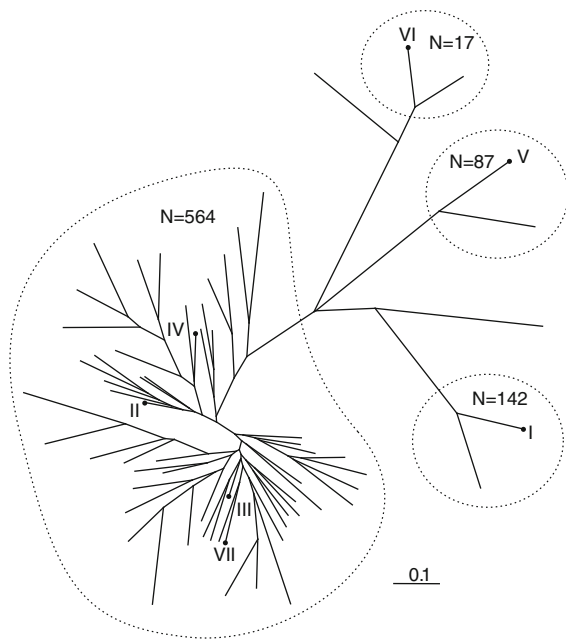


Fig. 4 Neighbor-joining network based on chord distances for 65 clones of *P. antipodarum* from the upper Snake River basin. The four putative clonal lineages are delineated by stippled lines and the total number of specimens in each (N) is given. Labels refer to the seven most common clones (I–VII, see Appendix Table 4)

P. antipodarum to the upper Snake River basin of Idaho cannot be excluded. Our data suggest that the initial point of introduction may not have been in the vicinity of Hagerman, where this species was first discovered in North America (Bowler 1991), as the highest clonal diversities were observed well downflow from this area (Table 1). Note, however, that identification of the point of introduction may be clouded by subsequent dispersal of clones within the upper Snake River basin. We cannot assess the origin of this invasion because the only other microsatellite data available for *P. antipodarum* are from (introduced) British populations (Weetman et al. 2002). Nonetheless, we note that the allelic differences across multiple loci mitigate against a close relationship between these two clonal assemblages (compare Appendix Table 4 with Weetman et al. 2002, Table 1).

The microsatellite variation that we have documented implies little genetic structuring among

populations and is consistent with the rapid (less than a decade; Bowler 1996) spread of *P. antipodarum* within the upper Snake River basin; and with previously published evidence of the great dispersal ability of this invasive snail (e.g., Ponder 1988), which is presumably enhanced by its parthenogenetic reproductive mode (e.g., avoidance of cost of sex, potential for single individuals to found new colonies; White 1973). Our finding that populations are not differentiated according to an isolation by distance model provides additional evidence of occasional dispersal of this species over long distances (see Alonso and Castro-Díez 2008). The occurrence of multiple clones across segmented reaches of the Snake River (Fig. 4) suggests an ability to traverse dams, either by anthropogenic overland transport or passage through these structures. The distributions of clones and results of the AMOVA also imply an absence of genotypic specialization for mainstem Snake River and tributary habitats.

Potamopyrgus antipodarum is currently a candidate for biological control in the United States (Proctor et al. 2007). Our finding of genetically divergent lineages in the Snake River basin suggests that caution should be used in applying control interventions pending study of clonal diversity and distribution on larger spatial scales and assessment of possible inter-clonal variation in resistance. Our results may also prove useful to the ongoing development of other management strategies for *P. antipodarum* (Proctor et al. 2007) and can serve as baseline data for future studies of the spread and evolution this species in North America.

Acknowledgments Barry Bean and Mike Stephenson (IPC) provided a large amount of field assistance. Mike Radko (IPC) calculated the stream distances between sampling sites and Molly Ryan (Smithsonian Institution) assisted with preparation of the figures. Tom Quinn (University of Denver) and Sara Oyler-McCance (United States Geological Survey) generously shared bench space and equipment in the Rocky Mountain Center for Conservation Genetics and Systematics. This project was supported (in part) by a contract (to RH) from the Idaho Power Company (Award #1600).

Appendix

See Appendix Table 4.

Table 4 Allelic combinations of the 65 distinct multilocus genotypes (clones) of *P. antipodarum* detected in the upper Snake River basin

Clone	Pa254	Pa112	Pa121	Pa143	Pa217	Pa56
I	147/147/147	231/237/237	99/99/99	165/171/174	356/356/356	318/318/322
I	147/147/147	231/237/237	99/99/99	165/171/174	356/356/356	159/318/322
I	147/147/147	231/237/237	99/99/99	165/171/174	356/356/356	318/322/322
I	147/147/147	231/237/237	99/99/99	165/171/174	354/354/354	318/318/320
I	147/147/147	231/237/237	99/99/99	165/171/174	354/354/354	318/318/318
I	147/147/147	231/237/237	99/99/99	165/171/174	195/195/356	318/318/322
I	147/147/147	231/237/237	99/99/99	165/171/174	195/195/195	106/106/108
I	147/147/147	231/237/237	99/99/99	165/171/174	193/193/356	318/318/318
I	147/147/147	231/237/237	99/99/99	165/171/174	195/354/354	318/318/320
I	147/147/147	231/237/237	99/99/99	165/171/174	354/354/354	318/320/324
I	147/147/147	231/237/237	99/99/99	165/171/174	354/354/356	318/318/320
I	147/147/159	231/237/237	99/99/99	165/171/174	354/354/354	318/318/320
II	157/157/157	237/237/243	99/118/164	168/171/171	195/195/343	159/159/308
III	157/157/157	237/237/243	99/118/164	168/171/171	195/343/354	159/308/320
	157/157/157	237/237/243	99/118/164	168/171/171	195/343/354	159/159/320
	157/157/157	237/237/243	99/118/164	168/171/171	343/354/354	308/320/324
	157/157/157	237/237/243	99/118/164	168/171/171	195/343/354	159/159/159
IV	157/157/157	237/237/243	99/118/164	168/171/171	195/195/354	159/159/320
	157/157/157	237/237/243	99/118/164	168/171/171	189/343/354	159/308/320
	157/157/157	237/237/243	99/118/164	168/171/171	195/195/195	159/159/308
	157/157/157	237/237/243	99/118/164	168/171/171	195/343/354	159/159/308
	157/157/157	237/237/243	99/118/164	168/171/171	195/195/343	159/308/354
	157/157/157	237/237/243	99/118/164	168/171/171	343/343/354	308/308/320
	157/157/157	237/237/243	99/118/164	168/171/171	195/195/343	159/159/159
	157/157/157	237/237/243	99/118/167	168/171/171	195/343/354	159/308/320
	157/157/157	237/237/243	99/111/118	168/171/171	195/343/354	159/159/159
	157/157/157	237/237/243	99/111/118	168/171/171	195/343/354	159/308/320
	157/157/157	237/237/237	99/99/99	168/171/171	195/195/195	159/308/320
V	143/143/187	237/237/240	99/133/136	159/171/174	328/338/354	292/302/318
	143/143/187	237/237/240	99/133/136	159/171/174	328/328/354	292/292/318
	147/147/157	231/237/243	99/118/164	168/168/171	195/354/356	159/308/320
	147/157/157	237/237/243	99/118/164	168/171/171	195/343/354	159/308/320
	147/157/157	237/237/243	99/118/164	168/171/171	195/343/356	159/308/320
	147/157/157	234/237/243	99/118/164	168/171/171	195/343/356	159/308/320
	147/157/157	231/237/243	99/118/164	168/171/171	195/356/356	159/308/320
VI	155/155/155	231/237/240	99/99/136	168/168/174	338/338/340	302/304/314
	155/155/155	231/237/240	99/99/136	168/168/174	338/338/340	314/314/314
	155/155/155	231/237/240	99/99/136	168/168/174	338/340/356	302/304/314
	157/157/157	237/237/243	99/108/118	168/171/171	195/195/354	159/308/320
VII	157/157/157	237/237/243	99/118/164	168/168/171	195/343/354	159/308/320
	157/157/157	237/237/243	99/118/164	168/168/171	195/343/354	159/159/159
	147/157/157	237/237/237	99/99/99	168/171/171	195/195/195	159/159/308
	157/157/157	237/237/243	99/99/118	168/171/171	195/343/354	159/308/320
	157/157/157	237/237/243	99/99/118	168/171/171	195/195/195	159/159/159

Table 4 continued

Clone	Pa254	Pa112	Pa121	Pa143	Pa217	Pa56
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/118</i>	<i>168/171/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/118</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/159/308</i>
	<i>157/157/157</i>	<i>231/237/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/195/343</i>	<i>159/308/320</i>
	<i>157/157/157</i>	<i>231/237/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/157</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>159/171/174</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/157</i>	<i>237/240/243</i>	<i>99/99/118</i>	<i>159/171/174</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>343/343/354</i>	<i>308/308/320</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/99/118</i>	<i>159/168/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/187</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>159/171/174</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/187</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/187</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/187</i>	<i>237/240/243</i>	<i>99/99/118</i>	<i>159/168/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/187</i>	<i>237/237/243</i>	<i>99/99/99</i>	<i>159/168/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>157/157/157</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/187</i>	<i>237/237/237</i>	<i>99/99/99</i>	<i>159/171/174</i>	<i>195/195/354</i>	<i>159/159/320</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/157</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/195/343</i>	<i>159/159/308</i>
	<i>143/157/157</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/157</i>	<i>237/237/243</i>	<i>99/99/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>143/157/157</i>	<i>237/240/243</i>	<i>99/118/164</i>	<i>159/168/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/164</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/164</i>	<i>168/171/171</i>	<i>195/195/354</i>	<i>159/159/320</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/164</i>	<i>168/171/171</i>	<i>165/165/343</i>	<i>159/159/308</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/99/99</i>	<i>168/171/171</i>	<i>195/195/354</i>	<i>159/159/320</i>
	<i>157/157/157</i>	<i>237/237/243</i>	<i>99/164/167</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/308/320</i>
	<i>157/157/157</i>	<i>237/237/237</i>	<i>99/118/164</i>	<i>168/171/171</i>	<i>195/195/195</i>	<i>159/159/159</i>
	<i>157/157/157</i>	<i>237/237/237</i>	<i>99/99/118</i>	<i>168/171/171</i>	<i>195/343/354</i>	<i>159/159/159</i>

The seven most common clones (I–VII) are given in italics

References

- Alonso A, Castro-Díez P (2008) What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae: Mollusca)? *Hydrobiol* 614:107–117
- Anderson TR (2006) New Zealand Mudsnail *Potamopyrgus* [sic] *antipodarum*. In: Boersma PD, Reichard SH, Van Buren AN (eds) *Invasive species in the Pacific Northwest*. University of Washington Press, Seattle, pp 102–103
- Aquatic Nuisance Species Task Force (ANSTF) (2009) New Zealand mudsnail (*Potamopyrgus antipodarum*) <http://www.anstaskforce.gov/spoc/nzms.php> (accessed 23/II/2009)
- Armour JA, Crosier LM, Jeffreys AJ (1996) Distribution of tandem repeat polymorphisms within minisatellite MS621 (D5S110). *Ann Hum Genet* 7:11–20
- Bohonak AJ (2002) IBD (Isolation by distance): a program for analyses of isolation by distance. *J Hered* 93:153–154
- Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotech* 2001:24–26, 28
- Bowler PA (1991) The rapid spread of the freshwater hydrobiid snail *Potamopyrgus antipodarum* Gray in the middle Snake River, southern Idaho. *Proc Desert Fish Counc* 21:173–179

- Bowler PA, Frest TJ (1996) The advancing distribution of the New Zealand mud snail, *Potamopyrgus antipodarum* (Gray), in North America. Am Malacol Union 62nd Annual Meeting Program and Abstracts:31
- Bucklin A (1992) Use of formalin-preserved samples for molecular analysis. Newsl Crustac Mol Tech 2:3
- Cavalla-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Evol 21:550–570
- Dybdahl MF, Lively CM (1995) Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). J Evol Biol 8:385–398
- Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. Am J Bot 74:123–131
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances along DNA haplotypes: application to human mitochondrial DNA restriction data. Genet 131:479–491
- Felsenstein J (1995) PHYLIP (Phylogeny Inference Package) Version 3.57c. Distributed by the author. University of Washington, Seattle
- Fox JA, Dybdahl MF, Jokela J, Lively CM (1996) Genetic structure of coexisting and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evol 50:1541–1548
- Hall RO Jr, Dybdahl MF, VanderLoop MC (2006) Extremely high secondary production of introduced snails in rivers. Ecol Appl 16:1121–1131
- Hauser L, Carvalho GR, Hughes RN, Carter RE (1992) Clonal structure of the introduced freshwater snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae), as revealed by DNA fingerprinting. Proc R Soc Lond B249:19–25
- Hughes RN (1996) Evolutionary ecology of parthenogenetic strains of the prosobranch snail, *Potamopyrgus antipodarum* (Gray) (= *P. jenkinsi* (Smith)). Malacol Rev Suppl 6:101–113
- Jacobsen R, Forbes VE, Skovgaard O (1996) Genetic population structure of the prosobranch snail *Potamopyrgus antipodarum* (Gray) in Denmark using PCR-RAPD fingerprints. Proc R Soc Lond B263:1065–1070
- Jokela J, Dybdahl MF, Lively CM (1999) Habitat-specific variation in life-history traits, clonal population structure and parasitism in a freshwater snail (*Potamopyrgus antipodarum*). J Evol Biol 12:350–360
- Kalyoncu H, Barlas M, Yildirim MZ, Yorulmaz B (2008) Gastropods of two important streams of Gökova Bay (Muğla, Turkey) and their relationships with water quality. Int J Sci Technol 3:27–36
- Kerans BL, Dybdahl MF, Gangloff MM, Jannot JE (2005) *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone Ecosystem. J N Am Benthol Soc 24:123–138
- Kornijów R, Szerbowski JA, Krywosz BartelR (2001) The macrozoobenthos of the Iraqi Lakes Tharthar, Habbaniya and Razzazah. Archiv Pol Fish 9(suppl. 1):127–145
- Loo SE, MacNally R, Lake PS (2007) Forecasting New Zealand mudsnail invasion range: model comparisons using native and invaded ranges. Ecol Appl 17:181–189
- Lysne S, Koetsier P (2008) Comparison of Desert Valvata snail growth at three densities of the invasive New Zealand mudsnail. West N Am Nat 68:103–106
- Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794
- Montana State University (MSU) (2009) New Zealand mudsnails in the western USA. <http://www.esg.montana.edu/aim/mollusca/nzms/status.html> (Accessed 23/II/2009)
- Parker ED Jr (1979) Ecological implications of clonal diversity in parthenogenetic morphospecies. Am Zool 19:753–762
- Ponder WF (1988) *Potamopyrgus antipodarum*—a molluscan colonizer of Europe and Australia. J Moll Stud 54:271–285
- Proctor T, 18 co-authors (2007) National management and control plan for the New Zealand mudsnail (*Potamopyrgus antipodarum*). Prepared for the Aquatic Nuisance Species Task Force by the New Zealand Mudsnail Management and Control Plan Working Group. Available from http://www.anstaskforce.gov/Documents/NZMS_MgmtControl_Final.pdf (accessed 23/II/2009)
- Richards DC, Cazier LD, Lester GT (2001) Spatial distribution of three snail species, including the invader *Potamopyrgus antipodarum*, in a freshwater spring. West N Am Nat 61:375–380
- Riley LA, Dybdahl MF, Hall RO Jr (2008) Invasive species impact: asymmetric interactions between invasive and endemic freshwater snails. J N Am Benthol Soc 27:509–520
- Schreiber ESG, Glaister A, Quinn GP, Lake PS (1998) Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in Lake Purrembete, Victoria, Australia. Mar Freshw Res 49:73–78
- Städler T, Frye M, Neiman M, Lively CM (2005) Mitochondrial haplotypes and the New Zealand origin of clonal European *Potamopyrgus*, an invasive aquatic snail. Mol Ecol 14:2465–2473
- Stock M, Lamatsch DK, Steinlen C et al (2002) A bisexually reproducing all-triploid vertebrate. Nat Genet 30:325–328
- Suomalainen E (1950) Parthenogenesis in animals. Adv Genet 3:193–253
- Suomalainen E, Saura A, Lokki J (1987) Cytology and evolution in parthenogenesis. CRC Press, Boca Raton, Florida
- Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genet 144:389–399
- Urabe M (2007) The present distribution and issues regarding the control of the exotic snail *Potamopyrgus antipodarum* in Japan. Jpn J Limnol 68:491–496 (Japanese, with English abstract)
- Wallace C (1985) On the distribution of the sexes of *Potamopyrgus jenkinsi* (Smith). J Moll Stud 51:290–296
- Wallace C (1992) Parthenogenesis, sex and chromosomes in *Potamopyrgus*. J Moll Stud 58:93–107
- Wattier R, Engel CR, Saumitou-Laprade P, Valero M (1998) Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). Mol Ecol 7:1569–1573
- Weetman D, Hauser L, Carvalho GR (2001) Isolation and characterisation of di- and trinucleotide microsatellites in the freshwater snail *Potamopyrgus antipodarum*. Mol Ecol Notes 1:185–187

- Weetman D, Hauser L, Carvalho GR (2002) Reconstruction of microsatellite mutation history reveals a strong and consistent deletion bias in invasive clonal snails, *Potamopyrgus antipodarum*. *Genet* 162:813–822
- White MJD (1973) *Animal cytology and evolution*, 3rd edn. Cambridge, London
- Winterbourn M (1970) The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacol* 10:283–321
- Zaranko DT, Farara DG, Thompson FG (1997) Another exotic mollusc in the Laurentian Great Lakes: the New Zealand native *Potamopyrgus antipodarum* (Gray 1843) (Gastropoda, Hydrobiidae). *Can J Fish Aquat Sci* 54:809–814