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Neither variation loss, nor change in selfing rate is associated with the worldwide invasion of *Physa acuta* from its native North America

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Abstract Whether bioinvasions are associated with a loss of genetic diversity and a change in mating system is instrumental for understanding the evolutionary fate of invasive species. Little loss is expected under strong propagule pressure which might be a general situation in widespread, efficient invader. In hermaphroditic species, we have few examples of a transition between outcrossing and selfing as a consequence of invasion, though this is classically predicted (as a corollary to Baker's law). We estimated micro-satellite variation in 44 populations of the widespread freshwater snail *Physa acuta* sampled at worldwide scale (including several populations from its native North America). Neither loss of variation (or bottleneck), nor increase in selfing rate was detected in invaded areas. Moreover there was no isolation by distance at large geographic scale, and limited

geographic coherence in genetic patterns was detected using STRUCTURE software—the West Mediterranean area being an exception. Such patterns might be explained by (1) multiple introductions in the invaded areas, presumably fostered by aquarium trade, followed by regional spread in some cases—in which case mating partners might be numerous enough to prevent transition towards higher selfing rates, and (2) invasions from the whole native area. This suggests that *P. acuta* is an exceptionally efficient invader (which is not true of related species), but the reasons of its success remain elusive.

Keywords Bioinvasions · Genetic diversity · Mating systems · Phylogeography · *Physa acuta* · Snail

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Introduction

Bioinvasions offer excellent opportunities for studying the evolutionary process, as their spatio-temporal dynamics depends not only on ecological, but also on evolutionary forces (Lee 2002; Sax et al. 2005; Facon et al. 2006; Davis 2008). Evolutionary biologists have been particularly interested by several questions with regard to bioinvasions, including (1) their origin and history (see Dlugosh and Parker 2008; Estoup and Guillemaud 2010; Lawson Handley et al. 2011), (2) the evolutionary forces at work (Lee 2002; Sax et al. 2005; Facon et al. 2006), and (3) their consequences

on the distribution of genetic variation, and therefore on adaptation and mating systems (Kolbe et al. 2004; Barrett et al. 2007; Facon et al. 2008). We are interested here in the first aspect, and especially in the last one. With regard to the origin and history of invaders, freshwater organisms deserve special attention (see Roman and Darling 2007; Beisel and Levêque 2010). As other organisms, they usually rely on migration to reach new environments, especially on passive migration, for example through birds, over long distances. However the trade of aquarium plants (e.g., in invertebrates) and intentional introductions (e.g., in fish) now are much more efficient vectors of passive migration (Padilla and Williams 2004; Hayes et al. 2008; Duggan 2010). Migration, whether active or passive, can strongly affect the subsequent build-up and distribution of variation in the invaded areas (Sax et al. 2005; Facon et al. 2008).

The consequences of bioinvasions on variation have essentially been evaluated on neutral molecular variation (Roman and Darling 2007; Dlugosh and Parker 2008; Lawson Handley et al. 2011). Bioinvasions have for some time been thought to lead to depressed variation as they were assumed to coincide with bottlenecks and founder effects. Recent reviews indicate that the loss of variation is on average limited (Dlugosh and Parker 2008), and enhanced variation might also be observed as the result of both repeated introductions and local hybridization (Wares et al. 2005; Roman and Darling 2007; Dlugosh and Parker 2008). For the same reason, invasions might not lead to increased differentiation in the invaded areas compared to the native areas following founder effect, but this has been less considered (Novak and Mack 2005; Barrett et al. 2007). The mating system might also interfere with variation loss following invasion. For example, selfing plants seem to lose variation whereas this does not occur in outcrossers, perhaps because variation segregates more among than within populations in selfers, limiting the build-up of variation through recurrent migration (Novak and Mack 2005). In self-compatible hermaphrodites, strong genetic bottlenecks, low density of mating partners or absence of pollinators might also lead to increased selfing rates (Kirkpatrick and Jarne 2000; Cheptou 2012), but very few examples have been documented (see Barrett et al. 2007 in plants). Hermaphroditic species of freshwater snails exhibit variation in the selfing rate, though intermediate

selfing rates (i.e. around 0.5) are rare (Jarne and Auld 2006; Escobar et al. 2011). In this group, both predominantly outcrossing and predominantly selfing species have become biological invaders in various places (e.g., Pointier et al. 2005), but whether invasion can induce a transition in mating system remains to be documented.

We focus on the freshwater snail *Physa acuta*, an hermaphroditic species belonging to the Physidae (freshwater Pulmonates). The systematics of this family has been recently clarified by the phylogenetic work of Wethington and Lydeard (2007) suggesting that several named species indeed belong to a complex of closely allied species. On the whole there might be no more than 20 species of Physidae (see also Jarne et al. 2010). *P. acuta* itself includes several deep mtDNA clades, i.e. exhibiting rather large molecular divergence (of the order of 6 % at 16S rDNA and COI genes; Wethington and Lydeard 2007). This species which has been known under a plethora of species names (see Dillon et al. 2002) is the Physidae (and perhaps the mollusc) species exhibiting the largest distribution area as a result of recent expansion. Knowledge about its native area and invasion history is sketchy (Dillon et al. 2002). *P. acuta* originates from East of the Rocky Mountains in North America, but the available information prevents to be more specific in geographic terms. The first argument about this origin relies on the fact that most physid species originates from this very same area (New World), especially the species most closely allied to *P. acuta* (Wethington and Lydeard 2007). A stronger argument derives from the timing of species description in the physids. *P. acuta* was first described in Europe in 1805, and in North America in 1817 only (Dillon et al. 2002). However several common species, such as *Radix peregra* or the congeneric *Physa fontinalis*, were described as early as 1760. Given that *P. acuta* is easily distinguished from all other European freshwater snails and is also a very common species, its late description is suggestive of a first European invasion at the beginning of the nineteenth century. That it was described later in North America than in Europe is explained by the fact that the first descriptions of North American freshwater gastropods were performed by Say in 1817. Other continents might have been invaded later than Europe, but this is poorly documented (see e.g. Brown 1994; Albrecht et al. 2009). *P. acuta* is today ubiquitous on Earth, occupying

freshwater environments from the Equator to the Polar circles, including remote islands. Note that no other physid species is invasive to the exception of the neotropical *Aplexa marmorata* (Bony et al. 2007) which invasion remains though limited to a few African countries. *P. acuta* has been studied with regard to various issues in evolutionary biology, especially species limit (Dillon et al. 2002, 2011), life-history traits, mating systems, sexual selection and senescence (e.g. Jarne et al. 2000; Escobar et al. 2008, 2009; Péliissié et al. 2012). Its population structure has been analyzed using genetic markers at local scale (Bousset et al. 2004; Dillon and Wethington 2006; Escobar et al. 2008; Van Leeuwen et al. 2013), but never at a geographic scale that can provide insights into its recent expansion.

Given the scale at which invasion took place in *P. acuta*, this species can be considered as an excellent biological model for analyzing bioinvasions at a very large geographic scale. We studied the distribution of microsatellite variation (preferred here to DNA sequences given the pretty recent history of invasion) in 44 populations sampled from both the native and invaded (worldwide scale) areas. Our objectives were (1) to improve our knowledge about the invasion history of *P. acuta*, and (2) to evaluate whether invasion has been associated with depletion of genetic variation and change in selfing rate.

Materials and methods

Sampling and characterization of microsatellite variation

Physa acuta is a cosmopolitan species occupying a wide range of habitats. Populations fluctuate widely, sometimes rising to extremely high densities (e.g., thousands of individuals/m²; adults are about 10–15 mm in shell length), in connection with variation in water availability. Four hundred and fifty-five individuals were sampled in 44 sites representing a variety of habitats at worldwide scale, essentially between 1999 and 2002 (Table 1). They were euthanized in hot water for 30–60 s before being stored in 90° ethanol, and sent to our laboratory in Montpellier. Species status was checked upon arrival based on outside individual phenotypes (see also below for molecular identification). Populations Mos1, FL, FRJ,

Co and UCh have already been used in a population genetic analysis at much smaller geographic scale (Bousset et al. 2004). We also sampled individuals (N = 66) in one to three sites of the allied species *Physa gyrina*, *Physa fontinalis* and *Aplexa marmorata* (SI Table 1).

Twelve individuals were generally analyzed per site, sometimes less (Table 2). Population genetic estimates should therefore be regarded with caution. DNA extraction (QiAmp Tissue kit, Qiagen), microsatellite characterisation and locus scoring on an ABI 310 sequencer were performed according to Sourrouille et al. (2003) and Bousset et al. (2004). Nine loci were analyzed (Monsutti and Perrin 1999; Sourrouille et al. 2003), four of which are dinucleotide repeats (Pac1, AF108758, AF108762, AF108764) and the other five are tetranucleotide repeats (Pac2, Pac4, Pac5, Pac7, Pac14). PCR amplification was performed either on single loci (Pac 14, AF108758), or on locus groups (Pac1, Pac2 and Pac5; Pac4 and Pac7; AF108762 and AF108764). Electrophoretic runs were conducted separately on two locus groups (Pac1, Pac2, Pac4, Pac5, Pac7, AF108762 and AF108764; Pac14 and AF108758). The subsequent analyses were limited to six loci (Pac1, Pac2, Pac5, AF108758, AF108762 and AF108764), because of the high frequency of null alleles at the three remaining loci. Null alleles were for example fixed in 6, 2 and 2 samples (181, 105 and 18 individuals respectively out of 455) at loci Pac4, Pac7 and Pac14 respectively.

Within-population genetic diversity and demography

The genetic variability was described in each population using the number of alleles (N_{all}) rarefied to six individuals (see Petit et al. 1998), the observed heterozygosity (H_o) and the gene diversity (H_e) adjusted for small samples, averaged over all loci (Nei 1987). Deviations from the genotypic proportions expected at Hardy–Weinberg equilibrium (HWE) at each locus and from genotypic equilibrium between locus pairs were tested in each population using exact tests (Rousset and Raymond 1995). Departure from HWE over all loci was evaluated using Fisher's method for combining probabilities. The unbiased estimator \hat{f} of Wright's F_{is} was calculated according to Weir and Cockerham (1984). The calculations and

Table 1 Location of the 44 *P. acutia* populations studied

| Country | #. Acro | Site | Latitude | Longitude | Habitat | Sampling date | Sampler |
|--------------|---------|--------------------------------|----------|-----------|-----------|---------------|------------------|
| France | 1. Mos1 | Clairac, Agen | N 44°30' | E 00°35' | River | Sep 2001 | Authors |
| France | 2. FL | La Roche Jagu, Païmpol | N 48°70' | W 03°00' | Pond | Jul 2002 | Authors |
| France | 3. FRJ | Mosson, Montpellier | N 43°40' | E 03°46' | River | Sep 2000 | Authors |
| Ukraine | 4. UKD | Dnestr, Novodnestrovsk | N 50°00' | E 32°50' | River | Sep 1991 | A. V. Komushin |
| Denmark | 5. DaB | Bornholm, Aakirkeby | N 55°30' | E 14°70' | Ditch | Aug 2002 | R. Bromley |
| Switzerland | 6. SwV | Vursys, Neuchatel | N 46°75' | E 06°75' | Pond | Mar 2002 | S. Trouvé |
| Ireland | 7. IrG | Glastry Clay Pits, County Down | N 54°50' | W 06°00' | Clay Pits | Aug 2000 | R. Anderson |
| Portugal | 8. PoB | Fonte da Benémula, Algarve | N 37°20' | W 08°80' | River | Jun 2002 | M. Malaquias |
| Spain | 9. SpM | Moli La Font, Benicassim | N 40°30' | E 00°50' | – | Nov 1999 | Authors |
| Italy | 10. ItP | Lake Trasimeno, Perugia | N 43°10' | E 12°00' | Lake | Sep 1999 | Authors |
| Malta | 11. MIF | Floriana, Lavallette | N 35°90' | E 14°50' | – | May 2000 | Authors |
| Turkey | 12. TKI | Isparta Egirdir | N 37°76' | E 30°56' | – | May 2002 | M. Zeki Yildirim |
| UEA | 13. DuD | Dubai | N 25°13' | E 55°17' | Fish farm | Jan 2002 | G. Feulner |
| Egypt | 14. EgQ | Lake Qarun, Fayoum | N 29°50' | E 30°50' | Fish farm | Mar 2002 | P.A. Crochet |
| Tunisia | 15. TuB | Oued Beta | N 34°25' | E 09°00' | Wadi | Jan 1990 | Authors |
| Morocco | 16. MoE | Erfoud | N 31°30' | W 04°10' | Ditch | Apr 2000 | Authors |
| Cameroon | 17. CaO | Obili, Yaoundé | N 04°00' | E 12°00' | Pond | May 2002 | F. Nijokou |
| Cameroon | 18. CaN | Ngda-Ekelle, Yaoundé | N 04°00' | E 12°00' | Lake | May 2002 | F. Nijokou |
| South Africa | 19. SaD | Phoenix, Durban | S 30°00' | E 31°00' | Pond | Apr 2002 | C. Appleton |
| Madagascar | 20. MaF | Facacativa | S 17°50' | E 47°50' | River | Aug 2000 | Authors |
| Mauritius | 21. MrB | Bel Air, St Felix | S 20°50' | E 57°50' | – | Sept 1987 | Authors |
| Australia | 22. AuD | Dunlop creek, Brisbane | S 27°50' | E 153°00' | River | Feb 2000 | A. Estoup |
| New Zealand | 23. NzA | Lake Albert | S 44°00' | E 169°00' | Lake | Oct 2000 | M.J. Wallace |
| New Zealand | 24. NzK | Kekerengu river, Kaikoura | S 42°00' | E 173°00' | River | Jan 2002 | M.J. Wallace |
| Malaysia | 25. MyK | Kuala Lumpur | N 02°50' | E 102°00' | Fish farm | Feb 2002 | H.A. Jambari |
| Vietnam | 26. ViD | Dam Sen Park, Saigon | N 10°50' | E 106°50' | Pond | Apr 2001 | Authors |
| Japan | 27. JaK | Kabukuri marsh, Sendai | N 38°37' | E 141°06' | Marsh | Apr 2002 | M. Kawata |
| Tonga | 28. ToT | Tongatapu island | S 18°00' | W 174°00' | Pond | Aug 2002 | Authors |
| Chile | 29. ChM | Rio Mataquito, Curico | S 35°00' | W 72°00' | River | Feb 2002 | A.M. Ramos |
| Chile | 30. ChE | Rio Elqui, La Serena | S 29°50' | W 71°50' | River | Feb 2002 | A.M. Ramos |
| Colombia | 31. CoB | Funza, Bogota | N 05°00' | W 74°00' | – | Jul 2000 | Authors |
| Venezuela | 32. VeC | Canal Calicanto, Maracay | N 10°17' | W 67°35' | Canal | Nov 1998 | Authors |
| Martinique | 33. MiP | Pagerie | N 14°90' | W 61°50' | River | Apr 1999 | Authors |

Table 1 continued

| Country | #. Acro | Site | Latitude | Longitude | Habitat | Sampling date | Sampler |
|-------------|---------|--|----------|-----------|-----------|---------------|-----------------|
| Guadeloupe | 34. GuP | Petit Pérou | N 16°50' | W 62°00' | River | Dec 2000 | Authors |
| Puerto Rico | 35. PrS | Salinas | N 18°00' | W 67°00' | Pond | Oct 1998 | Authors |
| Cuba | 36. CuH | La Havane | N 22°50' | W 82°50' | - | Mar 2002 | Authors |
| Cuba | 37. CuJ | Isla Juventud, La Fe | N 21°50' | W 82°50' | - | May 2002 | Authors |
| Mexico | 38. MxV | Xalapa, Xallitic, Veracruz | N 20°00' | W 97°00' | Laundries | Feb 2002 | R. Rojas Garcia |
| Mexico | 39. MxT | Tamaulipas | N 22°00' | W 98°00' | - | Mar 2002 | R. Rojas Garcia |
| USA | 40. UsB | Barton Spring, Austin, Texas | N 30°50' | W 97°60' | River | Feb 2001 | A. Tsitrona |
| USA | 41. UsC | Charles Town Landing, Charleston, South Carolina | N 34°00' | W 80°00' | Pond | Dec 2001 | R. Dillon |
| USA | 42. UsF | Fairmount park, Pennsylvania | N 40°00' | W 75°00' | River | Jul 2000 | A. Wethington |
| USA | 43. UsN | New Harmony, Indiana | N 38°00' | W 87°50' | Pond | Oct 2000 | A. Wethington |
| USA | 44. UsD | Douglas Lake, Michigan | N 45°50' | W 84°50' | Lake | Jul 2000 | R. Dillon |

#.Acro site number and acronym (acronym: three-letters code giving country and site, except for the French populations for homogeneity with Bousset et al. 2004). The habitat type is indicated when known

tests above were performed using GENEPOP 3.1 (Raymond and Rousset 1995). We estimated the selfing rate using the relationship $\hat{s} = 2\hat{f}/(1 + \hat{f})$ (Pollak 1987). \hat{f} is upwardly biased by null alleles and other technical artifacts transforming heterozygotes into homozygotes (see Jarne and David 2008). The selfing rate was therefore also estimated using the multilocus method implemented in RMES software (David et al. 2007) which has been designed to be insensitive to such artifacts.

The demographic history of populations was evaluated using the method of Cornuet and Luikart (1996). These methods are based on the excess of heterozygosity expected in a population which size has suddenly decreased, and then remained constant. Tests were conducted for the three types of mutation models proposed (Infinite Allele Model, IAM; Stepwise Mutation Model, SMM; Two-Phased Model, TPM). This last model is arguably the most realistic given our knowledge of mutation models at microsatellites (Jarne and Lagoda 1996; Ellegren 2004). The heterozygosity excess was evaluated over loci in the populations with more than 10 individuals using Wilcoxon signed-ranks tests implemented with BOTTLENECK software (Cornuet and Luikart 1996).

The allelic richness and expected heterozygosities were compared between populations exhibiting signals of bottleneck versus other populations, as well as between populations from the native area and other populations. Given the uncertainty about the exact limits of the native area, tests were conducted considered as native, either USA populations, or USA and Mexican populations (the later ones are located on the Caribbean coast, not far south from the USA boarder). This was done using an analysis of variance (using an ArcSin (square root) transformation for expected heterozygosity data), allowing to test whether invasion was associated with variability loss.

Differentiation among populations and phylogeography

Genetic differentiation was analysed per locus for all population pairs using a homogeneity test (Goudet et al. 1996) computed as an exact test. The estimator $\hat{\theta}$ of F_{st} (Weir and Cockerham 1984) was calculated over all loci, both over all populations and all population pairs. All tests and calculations were performed with

Table 2 Variation at six microsatellite loci in the 44 populations sampled

| Population | N | N _p | R _a | H _e | H _o | \hat{f} | P | S |
|------------|----|----------------|----------------|----------------|----------------|-----------|-------|--------------|
| 1. Mos1 | 12 | 5 | 3.438 (1.337) | 0.619 (0.153) | 0.694 (0.172) | -0.128 | 0.034 | 0.004 |
| 2. FL | 12 | 5 | 2.371 (0.895) | 0.387 (0.293) | 0.264 (0.295) | 0.327 | 0.003 | 0.019 |
| 3. FRJ | 21 | 5 | 2.973 (0.674) | 0.579 (0.155) | 0.548 (0.150) | 0.056 | 0.234 | 0.010 |
| 4. UkD | 12 | 4 | 2.463 (1.058) | 0.363 (0.242) | 0.306 (0.228) | 0.164 | 0.070 | 0.254 |
| 5. DaB | 12 | 6 | 3.692 (1.636) | 0.557 (0.204) | 0.489 (0.216) | 0.128 | 0.073 | 0.064 |
| 6. SwV | 12 | 5 | 3.045 (0.800) | 0.533 (0.190) | 0.375 (0.302) | 0.306 | 0.000 | 0.016 |
| 7. IrG | 12 | 4 | 2.904 (1.196) | 0.516 (0.235) | 0.294 (0.255) | 0.440 | 0.000 | 0.044 |
| 8. PoB | 12 | 6 | 2.476 (0.597) | 0.433 (0.195) | 0.389 (0.195) | 0.106 | 0.185 | 0.230 |
| 9. SpM | 12 | 5 | 3.300 (1.201) | 0.528 (0.240) | 0.486 (0.200) | 0.082 | 0.186 | 0.008 |
| 10. ItP | 12 | 6 | 2.960 (0.634) | 0.579 (0.093) | 0.361 (0.164) | 0.386 | 0.000 | 0.031 |
| 11. MIF | 12 | 4 | 2.384 (0.902) | 0.444 (0.266) | 0.431 (0.266) | 0.032 | 0.402 | 0.023 |
| 12. TkI | 3 | 1 | 1.700 (0.837) | 0.211 (0.333) | 0.222 (0.344) | -0.066 | 0.332 | - |
| 13. DuD | 12 | 5 | 2.557 (1.086) | 0.440 (0.230) | 0.438 (0.274) | 0.005 | 0.517 | 0.000 |
| 14. EgQ | 6 | 4 | 2.167 (0.753) | 0.389 (0.230) | 0.361 (0.195) | 0.078 | 0.394 | - |
| 15. TuB | 10 | 5 | 2.714 (0.607) | 0.568 (0.064) | 0.367 (0.103) | 0.366 | 0.000 | 0.021 |
| 16. MoE | 12 | 5 | 2.564 (1.094) | 0.441 (0.282) | 0.361 (0.256) | 0.188 | 0.044 | 0.194 |
| 17. CaO | 12 | 5 | 2.623 (1.025) | 0.486 (0.257) | 0.319 (0.331) | 0.352 | 0.000 | 0.005 |
| 18. CaN | 12 | 3 | 1.940 (1.063) | 0.279 (0.286) | 0.278 (0.287) | 0.005 | 0.554 | 0.071 |
| 19. SaD | 9 | 5 | 2.315 (0.492) | 0.487 (0.127) | 0.468 (0.391) | 0.043 | 0.376 | 0.158 |
| 20. MaF | 12 | 5 | 3.071 (1.575) | 0.463 (0.301) | 0.333 (0.167) | 0.289 | 0.000 | 0.016 |
| 21. MrB | 12 | 6 | 1.890 (0.597) | 0.338 (0.240) | 0.259 (0.208) | 0.242 | 0.038 | 0.088 |
| 22. AuD | 9 | 5 | 2.483 (0.888) | 0.480 (0.251) | 0.389 (0.208) | 0.200 | 0.088 | - |
| 23. NzA | 12 | 5 | 2.821 (1.163) | 0.471 (0.307) | 0.444 (0.297) | 0.059 | 0.305 | 0.013 |
| 24. NzK | 6 | 5 | 2.167 (0.753) | 0.303 (0.211) | 0.333 (0.236) | -0.111 | 0.153 | - |
| 25. MyK | 12 | 6 | 2.720 (1.658) | 0.407 (0.263) | 0.389 (0.287) | 0.046 | 0.358 | 0.010 |
| 26. ViD | 12 | 3 | 2.349 (0.941) | 0.311 (0.258) | 0.194 (0.146) | 0.385 | 0.000 | 0.698 |
| 27. JaK | 12 | 6 | 2.927 (0.865) | 0.501 (0.175) | 0.319 (0.178) | 0.373 | 0.000 | 0.093 |
| 28. ToT | 12 | 5 | 2.871 (1.139) | 0.509 (0.219) | 0.264 (0.123) | 0.492 | 0.000 | 0.213 |
| 29. ChM | 6 | 4 | 2.667 (1.366) | 0.470 (0.366) | 0.250 (0.329) | 0.491 | 0.000 | - |
| 30. ChE | 12 | 5 | 3.148 (1.540) | 0.530 (0.308) | 0.375 (0.267) | 0.301 | 0.001 | 0.295 |
| 31. CoB | 12 | 3 | 1.484 (0.531) | 0.178 (0.208) | 0.208 (0.246) | -0.179 | 0.050 | 0.012 |
| 32. VeC | 12 | 6 | 2.638 (0.934) | 0.569 (0.123) | 0.458 (0.156) | 0.201 | 0.036 | 0.115 |
| 33. MtP | 12 | 4 | 1.828 (0.566) | 0.193 (0.137) | 0.184 (0.142) | 0.047 | 0.345 | 0.685 |
| 34. GuP | 12 | 5 | 3.378 (1.380) | 0.553 (0.194) | 0.403 (0.162) | 0.300 | 0.001 | 0.015 |
| 35. PrS | 6 | 4 | 2.833 (1.169) | 0.578 (0.303) | 0.417 (0.274) | 0.299 | 0.016 | - |
| 36. CuH | 6 | 5 | 2.569 (1.002) | 0.450 (0.178) | 0.306 (0.324) | 0.341 | 0.012 | - |
| 37. CuJ | 12 | 6 | 2.720 (0.823) | 0.523 (0.295) | 0.312 (0.211) | 0.414 | 0.000 | 0.399 |
| 38. MxV | 12 | 3 | 1.777 (0.731) | 0.248 (0.250) | 0.222 (0.328) | 0.109 | 0.251 | 0.295 |
| 39. MxT | 12 | 5 | 2.943 (1.737) | 0.431 (0.285) | 0.264 (0.193) | 0.398 | 0.000 | 0.367 |
| 40. UsB | 12 | 5 | 4.786 (2.291) | 0.684 (0.294) | 0.375 (0.137) | 0.463 | 0.000 | 0.011 |
| 41. UsC | 10 | 6 | 3.603 (1.004) | 0.602 (0.163) | 0.583 (0.232) | 0.032 | 0.386 | 0.009 |
| 42. UsF | 6 | 4 | 2.333 (0.816) | 0.407 (0.287) | 0.417 (0.361) | -0.027 | 0.333 | - |
| 43. UsN | 6 | 4 | 3.167 (1.722) | 0.528 (0.347) | 0.583 (0.391) | -0.117 | 0.078 | - |

Table 2 continued

| Population | N | N _p | R _a | H _e | H _o | \hat{f} | <i>P</i> | <i>S</i> |
|------------|----|----------------|----------------|----------------|----------------|-----------|----------|----------|
| 44. UsD | 12 | 3 | 1.619 (0.573) | 0.127 (0.134) | 0.139 (0.155) | −0.094 | 0.000 | 0.110 |

N is the sample size and *N_p* the number of polymorphic loci. *R_a*, *H_e* and *H_o* are the allelic richness (rarefied to *N* = 6 individuals), Nei's unbiased gene diversity and the observed heterozygosity over all loci respectively. Their standard deviations are indicated into parentheses. \hat{f} is the estimate of Wright's *F_{is}* and *P* is the probability associated with the corresponding exact test (deviation) of Hardy–Weinberg proportions. *S* is the estimate of the selfing rate using the maximum likelihood method of David et al. (2007) (estimated when *N* > 10). The only significant value (*P* < 0.05) is indicated in bold characters

GENEPOP 3.1. We also estimated *F_{st}* using the method of Chapuis and Estoup (2007) in order to evaluate the influence of null alleles on population differentiation. This was done using FreeNA software (Chapuis and Estoup 2007). Independence between geographic and genetic distances of population pairs (isolation by distance) was tested using the Mantel-like procedure implemented in GENETIX 4.05 (Belkhir et al. 2004). The geographic distance was the shortest distance between two sampling points, estimated according to the formula provided in Viard et al. (1997) that accounts for earth curvature. The genetic distance was the logarithm of *F_{st}*/(1 − *F_{st}*) as advocated by Rousset (1997).

Population clustering was inferred using STRUCTURE v2.3 software (Falush et al. 2003) under the admixture model using all populations. Runs were conducted for a number of clusters (*K*) ranging from 1 to 15, and 20 runs were performed for each *K* value. The ranked likelihood (*K* from 1 to 15) saturated at *K* = 8–10, and we followed Evanno et al. (2005) for inferring the most likely cluster number. Populations were attributed to a cluster when their probability was larger than 0.8—the population probability is returned as the average unweighted probability over all individuals (we also discuss below the case of populations with probability larger than 0.6).

Results

Within-population genetic diversity and demography

None of the nine loci used provided any amplification products in *P. gyrina*, *P. fontinalis* and *A. marmorata*, whatever the geographical origin of the samples (Table S1). This was expected given that the phylogenetic distances between *P. acuta* and these species

are of the order of several millions of years, whatever the molecular clock assumed (Wethington and Lydeard 2007). These microsatellite loci can therefore be used to exclude individuals of these species falsely identified as *P. acuta* individuals, as can also be mitochondrial DNA sequences (Wethington and Lydeard 2007).

The six loci used in *P. acuta* exhibited from six (1B) to 30 (58B) alleles (overall mean 14.66, s.d. 9.39) at study scale. One to six loci were polymorphic per population (Table 2). The allelic richness averaged over loci (rarefied to six individuals) ranged from 1.48 to 4.91, and gene diversity (*H_e*) from 0.127 to 0.684 (Table 2). The observed *H_o* was generally lower than gene diversity, often resulting in heterozygote deficiencies. Of the 235 possible tests of deviation from HW proportions at locus-population combinations, 110 were significant at the 5 % level (46 when applying a brute-force Bonferroni test). Significant deviations were evenly distributed across loci although more significant values were detected at locus Pac5. The multilocus tests per population were significant in 28 populations (Table 2). The estimates of *F_{is}* over all loci ranged from −0.179 to 0.492 (Table 2). However the RMES multilocus population estimates of the selfing rate were not significantly different from 0, to the exception of that from population ViD (Vietnam). The estimate over all populations was 3.7×10^{-4} (95 %CI 0–0.084). None of the tests of genotypic disequilibrium between loci pairs over all populations were significant and only 2.5 % of the tests performed within population for 1,210 locus pairs were significant, which is lower than the number of significant tests expected by chance.

The tests for bottleneck detection gave different results depending on the mutation model (Table 3). The null hypothesis of mutation—drift equilibrium was not rejected in many populations, whatever the mutational model. A signal of bottleneck (heterozygosity excess)

Table 3 Results of tests on mutation-drift equilibrium

| Pop | IAM | | TPM | | SMM | |
|---------|-------|------|-------|------|-------|------|
| 1. Mos1 | 0.002 | Exc. | 0.01 | Exc. | 0.13 | |
| 2. FL | 0.03 | Exc. | 0.19 | | 0.47 | |
| 3. FRJ | 0.04 | Exc. | 0.19 | | 0.27 | |
| 4. UkD | 0.23 | | 0.34 | | 0.71 | |
| 5. DaB | 0.50 | | 0.88 | | 0.94 | |
| 6. SwV | 0.03 | Exc. | 0.37 | | 0.77 | |
| 7. IrG | 0.19 | | 0.37 | | 0.68 | |
| 8. PoB | 0.13 | | 0.63 | | 0.02 | Def. |
| 9. SpM | 0.21 | | 0.59 | | 0.79 | |
| 10. ItP | 0.005 | Exc. | 0.10 | | 0.5 | |
| 11. MIF | 0.004 | Exc. | 0.02 | Exc. | 0.03 | Exc. |
| 13. DuD | 0.10 | | 0.37 | | 0.68 | |
| 15. TuB | 0.002 | Exc. | 0.002 | Exc. | 0.002 | Exc. |
| 16. MoE | 0.13 | | 0.13 | | 0.16 | |
| 17. CaO | 0.01 | Exc. | 0.02 | Exc. | 0.23 | |
| 18. CaN | 0.04 | Exc. | 0.08 | | 0.42 | |
| 20. MaF | 0.18 | | 0.50 | | 0.88 | |
| 21. MrB | 0.10 | | 0.41 | | 0.5 | |
| 23. NzA | 0.16 | | 0.32 | | 0.47 | |
| 25. MyK | 0.02 | Exc. | 0.18 | | 0.5 | |
| 26. ViD | 0.88 | | 0.02 | Def. | 0.005 | Def. |
| 27. JaK | 0.10 | | 0.41 | | 0.79 | |
| 28. ToT | 0.03 | Exc. | 0.13 | | 0.27 | |
| 30. ChE | 0.04 | Exc. | 0.13 | | 0.37 | |
| 31. CoB | 0.01 | Exc. | 0.02 | Exc. | 0.04 | Exc. |
| 32. VeC | 0.001 | Exc. | 0.001 | Exc. | 0.003 | Exc. |
| 33. MtP | 0.71 | | 0.95 | | 0.98 | |
| 34. GuP | 0.41 | | 0.59 | | 0.63 | |
| 37. CuJ | 0.001 | Exc. | 0.01 | Exc. | 0.15 | |
| 38. MxV | 0.04 | Exc. | 0.05 | | 0.28 | |
| 39. MxT | 0.41 | | 0.71 | | 0.88 | |
| 40. UsB | 0.10 | | 0.19 | | 0.53 | |
| 41. UsC | 0.01 | Exc. | 0.10 | | 0.5 | |
| 44. UsD | 1.00 | | 0.004 | Def. | 0.004 | Def. |

BOTTLENECK software was run under the IAM, the TPM and SMM in populations with more than 10 individuals (see Table 2). The table gives the probability associated with Wilcoxon signed-ranks test of the null hypothesis of equilibrium. 'Exc.' and 'Def.' hold respectively for excess and deficit (when $P \leq 0.05$) indicating respectively a bottleneck and an expansion

occurred under the IAM in half of the 34 populations tested, but this model is arguably the least likely of the three models used. More conservatively considering only those cases in which the conclusions were similar

under the three mutation models, no heterozygosity deficit (genetic signal of population expansion) was detected. Two cases were detected if we consider the TPM and SMM only (ViD and UsD). A genetic signal of bottleneck was detected in four populations (MIF, TuB, CoB and VeC) for the three mutation models (Table 3). Considering the (most likely) TPM only the number of bottleneck signals rises to seven, all in populations outside USA/Mexico.

No significant difference in allelic richness and gene diversity was detected between populations showing or not a signal of bottleneck whether considering the four or seven populations just mentioned (F test, $P > 0.160$, $N = 34$ populations). This was also true when considering allelic richness in USA and other populations, or USA/Mexico *versus* other populations ($P = 0.905$ and 0.095 respectively; $N =$ all 44 populations), although there was slightly less variation in invaded areas. For example, the allelic richness for the USA/Mexico populations was 3.10 (s.d. 1.126) vs. 2.68 (s.d. 0.602) for other populations. We note that the USA population UsD exhibits extremely low variability. The same comparisons for gene diversity were not significant either ($P = 0.771$ and 0.678 respectively).

Differentiation among populations and phylogeography

Differentiation tests between population pairs over all loci were all significant ($P < 10^{-4}$), a single one (between the two populations from Switzerland) approaching the 5 % significance level ($P = 0.043$) and 18 tests only exhibiting a probability larger than 0.01 (Table S2). The overall F_{st} estimate was 0.352 (CI 0.326–0.389; based on 1,000 bootstraps), and the pairwise estimates over all loci ranged from 0.035 to 0.821 (mean = 0.359; Table S2). The extremely high values are partially explained by small sample sizes. We also estimated F_{st} among population pairs using FreeNA software in order to account for null alleles. The mean value over all population pairs was higher by 2 % only than the one estimated without taking into account null alleles (0.359 vs. 0.366). No isolation by distance was detected at overall scale (Mantel-like test, $P = 0.363$).

STRUCTURE analysis indicated that the most likely number of clusters is six based on the approach of Evanno et al. (2005) (Table 4; Figure

Table 4 Population clusters returned by STRUCTURE analysis

The six clusters are numbered from 1 to 6. (A) Twenty-eight populations were affected to a cluster with $P > 0.8$ (italic characters) and nine with $P > 0.6$. (B) For the remaining seven populations, we provide the two clusters with the highest probability. North-American populations are indicated in bold characters. See Table 1 for acronyms and Fig. 1 for spatial distribution

| A. | | | | | |
|----------------|----------------|----------------|----------------|----------------|----------------|
| 1 | 2 | 3 | 4 | 5 | 6 |
| <i>6. SwV</i> | <i>1. FL</i> | <i>32. VeC</i> | <i>13. DuD</i> | <i>2. FRJ</i> | 38. MxV |
| <i>31. CoB</i> | <i>3. MosI</i> | <i>33. MtP</i> | <i>25. MyK</i> | <i>4. UkD</i> | 39. MxT |
| <i>36. CuH</i> | <i>8. PoB</i> | 41. UsC | <i>26. ViD</i> | <i>16. MoE</i> | 44. UsD |
| | <i>9. SpM</i> | | <i>27. JaK</i> | <i>18. CaN</i> | |
| | <i>11. MIF</i> | | <i>34. GuP</i> | <i>21. MrB</i> | |
| | <i>14. EgQ</i> | | | <i>28. ToT</i> | |
| | <i>19. SaD</i> | | | | |
| | <i>23. NzK</i> | | | | |
| 42. UsF | <i>10. ItP</i> | <i>29. ChM</i> | <i>7. IrG</i> | <i>5. DaB</i> | <i>35. PrS</i> |
| | <i>15. TuB</i> | | | <i>17. CaO</i> | |
| | <i>24. NzA</i> | | | | |

| B. | | | | | |
|----------------|-----------|-------|-----------|-------|--|
| Population | Cluster 1 | P | Cluster 2 | P | |
| 12. TkI | 2 | 0.458 | 5 | 0.389 | |
| 20. MaF | 2 | 0.247 | 4 | 0.405 | |
| 22. AuD | 2 | 0.484 | 4 | 0.207 | |
| 30. ChE | 3 | 0.323 | 6 | 0.359 | |
| 37. CuJ | 3 | 0.335 | 4 | 0.310 | |
| 40. UsB | 4 | 0.158 | 6 | 0.472 | |
| 43. UsN | 1 | 0.375 | 4 | 0.264 | |

S1). Twenty-eight populations were attributed to a cluster with a probability higher than 0.8, and a further group of nine populations had probability larger than 0.6. The seven remaining populations were attributed to at least two clusters (both probabilities higher than 0.2 in all cases but one). The USA populations were attributed to different clusters, and two of them to two clusters. Populations around the Mediterranean sea (South Africa and New Zealand as well) belong to a single cluster, but were not connected to USA populations. Asian populations also belong to a single group. There was otherwise little geographic cohesion. Interestingly the two Mexican populations clustered with the USA population UsD (Michigan). We further analyzed the data by considering pairwise estimates of F_{st} on a regional basis under the idea that single introductions followed by local spread should result in limited differentiation among populations compared to that among North American populations (0.351). The only remarkable result is the low mean pairwise estimates of F_{st} (0.120) exhibited by the Mos1, FL, SpM, PoF and ItP group (i.e. populations from Italy,

Southern France, Spain and Portugal) which belong to STRUCTURE cluster 2 (Fig. 1).

Discussion

Consequences of null alleles on population structure

We detected null alleles at very high frequencies at three out of the nine loci initially considered. This result is not surprising given that *P. acuta* exhibits rather long phylogenetic branches (Wethington and Lydeard 2007) and these loci were initially cloned using individuals sampled in Southern France and Switzerland. Individuals belonging to a different branch are indeed expected to have accumulated mutations, including in the flanking regions of microsatellite loci serving as primers, leading to amplification failure (see (Dakin and Avise 2004; Selkoe and Toonen 2006). Null alleles are also likely to occur at the other six loci. RMES analysis indeed showed that



Fig. 1 Position of sampling sites (see Table 1 for geographical coordinates). The (approximative) native area of *P. acuta* (Eastern North America) is circled. Shape codes refer to STRUCTION clusters (for populations affected to a cluster with $P > 0.6$; cluster 1 = empty circles; 2 = stars; 3 = empty

squares; 4 = triangles; 5 = arrows pointing to the left; 6 = arrows pointing to the right). Populations affected to two clusters are represented as plain circles. See Table 4 for more details

the populations studied are essentially outbred while the mean F_{is} was 0.38. In such a situation the frequency of null alleles is about $F_{is}/2$ i.e. 0.18 (Jarne and David 2008). It is therefore not surprising that few null homozygotes were detected since their expected frequency is $0.0324 (=0.18^2)$ and sample size was generally 12 individuals.

Intuitively the occurrence of null alleles should have a depressing effect on the variability within populations since a null allele might hide several alleles not occurring in the population (Estoup et al. 2002; but see Lemer et al. 2011). We therefore probably underestimated the within-population variation to a small extent. However populations from the native areas did not show a higher frequency of null alleles (mean F_{is} was 0.41). Moreover a closer look at the distribution of null homozygotes at the three loci initially excluded from the analysis of population structure and showing a high frequency of such genotypes points to the same direction: null homozygotes had a slightly higher frequency in native populations at two loci. If any, the variability in populations from the native area might have been slightly underestimated, and our conclusions on

variation loss (see below) are not affected. A similar conclusion can be made with regard to genetic differentiation. Estimates of genetic divergence are hardly affected when null alleles are accounted for (FreeNA analysis), as expected given the low frequency of null alleles at the six loci studied (Chapuis and Estoup 2007).

Within-population genetic diversity and demography

With up to 30 alleles per locus and 1.5–5 alleles per locus and population, the amount of variability was large enough to conduct meaningful tests. Most populations showed medium to high variation with at least four polymorphic loci (out of six) in 38 populations (out of 44), and mean gene diversity over 0.3 in 39 populations. Both allelic richness and gene diversity were not significantly lower in invaded than in native areas, whether Mexican populations were counted as native or not. Four to seven bottlenecks (out of 34 populations tested) were detected depending on the mutation model assumed, all in populations outside the native area of *P. acuta* (note though that

sample size was limited requiring caution when interpreting this result). These populations were no less variable than other populations. Even if the native area was represented by five (or seven) populations only, calling for more extensive sampling in future studies, it seems that the invasion of *P. acuta* has not been associated with variation loss within populations. Moreover wide variation was detected among populations. This is consistent with recent reviews which detected no (Wares et al. 2005; Roman and Darling 2007) or limited (Dlugosh and Parker 2008) loss of variation across a wide range of organisms. In freshwater gastropods, contrasted results have been reported. Marked loss of variation was associated with invasion of New Zealand by *Lymnaea stagnalis* (Kopp et al. 2012), of the Bolivian Altiplano by *Lymnaea truncatula* (Meunier et al. 2001) or of North America by *Potamopyrgus antipodarum* (Dybdahl and Drown 2011 and references therein). On the other hand, no loss occurred in *Biomphalaria pfeifferi* in Madagascar (Charbonnel et al. 2002) or in *Melanooides tuberculata* in the French Antilles (Facon et al. 2008). Invasion dates of the sites considered in the present work are not known with precision, preventing an analysis of time since invasion versus diversity. However we know that *P. acuta* has been in Europe for two centuries and in Africa for at least half a century (Brown 1994) providing ample time for multiple introductions from various sources, and the build-up of genetic variability. Such a result has been reported in other freshwater gastropods, including *M. tuberculata* (Facon et al. 2008) and *Pomacea* species (Hayes et al. 2008; Matsukara et al. 2013).

Heterozygote deficiencies were detected in a large number of populations, but they are essentially due to null alleles (see discussion above). After correction using David et al.'s method, some selfing was detected in a single population. This is consistent with what we know of the essentially outcrossing mating system of *P. acuta*, most populations being outcrossing and a few exhibiting selfing rates of up to ca. 30 % (Jarne et al. 2000; Henry et al. 2005; Escobar et al. 2008). The population showing here some selfing is located in the invaded area. However invasion is not associated with a change in mating system towards more selfing. Such a change might be expected if inbreeding depression is purged requiring that populations go through marked bottlenecks of a few individuals (Kirkpatrick and Jarne 2000). This might also occur for purely

demographic or environmental reasons: invaders might be faced with difficulties for finding reproductive partners (or pollinators in plants) (Barrett et al. 2007; Barrett 2011; Cheptou 2012). As mentioned above, this is unlikely to have been the case in *P. acuta* because of marked propagule pressure. Biological invasions are rarely associated with a change in selfing rate, as shown in plants (Barrett et al. 2007; Barrett 2011). This seems to be true in *P. acuta* as well, and no other case has been reported in hermaphroditic freshwater snails. It should though be noted that invading species of hermaphroditic freshwater snails are more often selfers than outcrossers (see Pointier et al. 2005, 2011), and that populations of these species in invaded areas are generally deprived in variation, showing a few genotypes only, compared to populations from native areas, in line with a suggestion by Roman and Darling (2007) and reviews in plants (Barrett et al. 2007, Barrett 2011).

Among-population differentiation and invasion phylogeography

Almost all tests revealed significant genetic differentiation among population pairs with an average F_{st} value of 0.35. This is not utterly surprising given the geographic scale of our study, and the intraspecific phylogenetic scale as well (we covered a good part of the species diversity). Wethington and Lydeard (2007) have shown that *P. acuta* exhibits pretty long phylogenetic branches based on mtDNA analysis, and Dillon and co-workers that it is a good species based on intraspecific crossings (Dillon et al. 2002, 2007, 2011). A wide range of genetic distances among populations was therefore expected (see also the discussion on null alleles above), as already observed with the same type of markers at species scale in other hermaphroditic freshwater snails (Viard et al. 1997; Mavárez et al. 2002).

The five US populations showed a similar range of F_{st} values (0.212–0.708; similar conclusion when adding the two Mexican populations) suggesting that the genetic differentiation captured at worldwide scale is representative of that in the native area. We might highlight that the overall R_{st} (0.337) falls within the confidence interval of the overall F_{st} (0.326–0.389) suggesting that mutation contributes little to genetic differentiation, and the observed pattern can be interpreted as a migration-drift process (Hardy et al.

2003). The spatial analysis did not reveal any strong geographic pattern or isolation by distance at study scale, even if STRUCTURE analysis detected some local genetic cohesion; for example, Western Mediterranean samples fell within cluster 2. Neither did our analysis reveal clustering of North American populations to the exception of one population from Michigan, which together with the two Mexican populations (and the one from Puerto Rico), formed cluster 6. Seven populations, including two American ones, could not be attributed to any STRUCTURE cluster. Moreover North American populations occurred in three (to four) STRUCTURE clusters (Fig. 1).

From these results, we can draw several conclusions: (1) although our North-American sampling is somewhat weak, the variation detected in this (native) area is also found in invaded areas. Given the geographic distribution of the USA populations, this suggests that invasive populations of *P. acuta* are distributed throughout the species range. However the origin of some STRUCTURE clusters, whether geographically homogeneous (cluster 2) or heterogeneous (cluster 4), remains undetermined. (2) The geographic heterogeneity of some clusters suggests that invasion vectors may transfer *P. acuta* from local North-American areas to largely separated areas. This presumably results from human activities since it is difficult to see which biological or ecological processes would lead to such a pattern. (3) The genetic heterogeneity of some populations might be indicative of multiple invasions, or invasions from populations that are already heterogeneous (as true for at least two US populations). (4) Geographic cohesion of e.g. cluster 2 (at rather large scale) suggests invasion followed by spread, or introduction from the same source at wide geographic scale. Both multiple introductions and subsequent spread are likely given that *P. acuta* has been reported in Southern Europe for almost two centuries (see Introduction). Limited sample in North America presumably limited our inference capacity. We were also not able to use methods based on ABC (approximate Bayesian computation) inference which have proven to be efficient for reconstituting invasion routes in simpler situations (Estoup and Guillemaud 2010; Lawson Handley et al. 2011). Here the introduction histories are complex and information on introduction dates is not available preventing to implement efficiently these methods.

Limited data are available in other outcrossing hermaphroditic species—a reason is that few such species are invasive. In selfing species, strong genetic patterns have been observed, i.e. the occurrence of a few markedly different lines per site, for example in *Lymnaea truncatula* (Meunier et al. 2001) and *B. pfeifferi* (Charbonnel et al. 2002). Similar patterns have been detected in parthenogenetic freshwater gastropods, such as *Melanoides tuberculata* (Facon et al. 2003) and *Potamopyrgus antipodarum* (Städler et al. 2005), but also in bisexual gastropods such as *Pomacea* species (Hayes et al. 2008, Matsukara et al. 2013). Some lines (clones) have gained wide distribution at a fast pace, and it has sometimes be possible to trace back invasion routes (e.g., Meunier et al. 2001; Facon et al. 2003). In all groups, recurrent introduction and recombination may contribute to the subsequent build-up of variation blurring the genetic structure associated with the initial introduction and potentially contributing to fuel the evolutionary potential of invaders (Kolbe et al. 2004; Facon et al. 2008).

Physa acuta: an exceptionally efficient invader

Predicting what makes a good invader is a major challenge in the field of bioinvasions, perhaps an issue without general answer. Judging from its native and current distributions, *P. acuta* is an extremely efficient invader, still in the process of invading new habitats and areas (e.g., Albrecht et al. 2009). More generally, very few species have gained a cosmopolitan distribution from a rather regional one. We can offer several explanations. The first is that the migration barrier has been trespassed (Facon et al. 2006; Davis 2008) because of extensive aquarium plant trade (Padilla and Williams 2004; Duggan 2010) and transport by waterbirds (Figuerola and Green 2002; Kappes and Haase 2012; Van Leeuwen et al. 2013). Strong propagule pressure perhaps resulted in multiple introductions. *P. acuta* is also an efficient invader at local scale: as an example, the pond network of Grande-Terre, Guadeloupe (French West Indies, ca. 800 km² and 2,000 ponds) has been invaded in the course of a few years (PD, PJ and JPP, unpublished data). However we note that other freshwater gastropods should also have benefitted from these dispersal facilities. The second explanation has more to do with the evolutionary potential: *P. acuta* exhibits a wide range of variation including substantial molecular

(this study; Bousset et al. 2004) or quantitative (e.g., Escobar et al. 2008) variation and deep evolutionary branches (Wethington and Lydeard 2007), but this is found in other poorly invasive species, such as *Lymnaea peregra* or *Biomphalaria glabrata* (Mavárez et al. 2002; Cordellier and Pfenninger 2009). It is also possible that *P. acuta* is extremely plastic with regard to both aquarium trade and natural conditions. An indirect argument is that it fares extremely well under laboratory conditions. It is also found in a wide range of environmental conditions, including strong water currents or freshwaters heavily loaded with organic matter (authors' observations). Such a niche is occupied by few species of freshwater snails, and *P. acuta* might have been able to draw the best on this kind of new ('dirty') niches opened by human activities. An interesting parallel can be drawn with the clonal species *M. tuberculata* and *Tarebia granifera*, other efficient invaders occupying heavily loaded waters. Testing the idea of wide plasticity might be performed under laboratory or mesocosms conditions.

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