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Genetic uniformity and long-distance clonal dispersal in the invasive androgenetic *Corbicula* clams

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Abstract

The clam genus *Corbicula* is an interesting model system to study the evolution of reproductive modes as it includes both sexual and asexual (androgenetic) lineages. While the sexual populations are restricted to the native Asian areas, the androgenetic lineages are widely distributed being also found in America and Europe where they form a major aquatic invasive pest. We investigated the genetic diversity of native and invasive *Corbicula* populations through a worldwide sampling. The use of mitochondrial and nuclear (microsatellite) markers revealed an extremely low diversity in the invasive populations with only four, undiversified, genetic lineages distributed across Europe and America. On the contrary, in the native populations, both sexual and androgenetic lineages exhibited much higher genetic diversity. Remarkably, the most abundant and widely distributed invasive forms, the so-called form A and form R found in America and Europe respectively, are fixed for the same single *COI* (cytochrome c oxydase subunit I) haplotype and same multilocus genotype. This suggests that form R, observed in Europe since the 1980s, derived directly from form A found in America since the 1920s. In addition, this form shares alleles with some Japanese populations, indicating a Japanese origin for this invasive lineage. Finally, our study suggests that few androgenetic *Corbicula* individuals successfully invaded the non-native range and then dispersed clonally. This is one striking case of genetic paradox raising the issue of invasive and evolutionary success of genetically undiversified populations.

Keywords: androgenesis, invasive species, phylogenetics, phylogeography, reproductive strategies

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Introduction

The impact that the mode of reproduction can have upon genetic divergence and adaptation of lineages is a widely debated topic (Barraclough *et al.* 2003; Simon *et al.* 2003; Schön *et al.* 2009). Asexuality can provide short-term evolutionary advantages (Lehtonen *et al.* 2012), such as through the removal of costs for producing males, for conducting meiosis or for finding a mate. Furthermore, asexuality provides a colonization advantage as only one new founding individual is required. However, the absence of mechanisms for rapid genetic change has earned asexual organisms the label of evolutionary dead ends (Maynard Smith 1978). Mixed reproductive systems, in which both sexual and asexual lineages occur within a species, can be a beneficial combination of evolutionary strategies with outcrossing sexual stages co-occurring with asexual ones.

The clam genus *Corbicula* is an excellent model of mixed reproductive system, enabling the examination of the relative impact of the reproductive mode on genetic divergence and adaptation. It includes asexual

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and sexual lineages that present a differential distribution linked to anthropogenic introduction (Pigneur *et al.* 2012): the sexuals are restricted to native Asian regions while asexuals are both in the native regions (Asia – Komaru *et al.* 2013; Australia – Byrne *et al.* 2000) and in the invaded range (America and Europe, Hedtke *et al.* 2008; Pigneur *et al.* 2011a). The sexual lineages of *Corbicula* are dioecious while the asexuals are hermaphrodites and reproduce through androgenesis, also known as 'male-parthenogenesis' (reviewed in Pigneur *et al.* 2012). Androgenetic lineages of *Corbicula* produce biflagellate unreduced sperm while sexuals have reduced monoflagellate sperm (Konishi *et al.* 1998; Glaubrecht *et al.* 2003). When the unreduced sperm fertilizes the egg, the maternal nuclear DNA of the egg is extruded as two polar bodies. As a consequence, only the paternal pronucleus is kept in the zygote of androgenetic *Corbicula,* while the mitochondria of the egg are retained (Komaru *et al.* 1998; Ishibashi *et al.* 2003). As androgenetic *Corbicula* lineages are hermaphrodites and capable of both cross- and self-fertilization, a single individual may found a population if the conditions are suitable. The two different reproductive strategies within *Corbicula* also differ by their ploidy status. Sexuals are strictly diploid (Okamoto & Arimoto 1986) whereas diploid, triploid and tetraploid individuals have been found in asexuals (Qiu *et al.* 2001; Lee *et al.* 2005; Hedtke *et al.* 2008; Skuza *et al.* 2009; Houki *et al.* 2011, E. Etoundi, personal observation).

Among androgenetic *Corbicula* clams, the unreduced spermatozoon from one genetic lineage can also fertilize the egg of another lineage. This results in the combination of the nuclear genome of the first lineage with the mitochondrial genome of the second, a phenomenon known as egg parasitism or mitochondrial capture, and this results in cytonuclear mismatches (Park *et al.* 2002; Lee *et al.* 2005; Hedtke *et al.* 2008; Pigneur *et al.* 2011a, 2012). In addition, egg parasitism enables a mixing of different nuclear genomes when the maternal nuclear genome is incompletely extruded (Komaru *et al.* 2006; Hedtke *et al.* 2008, 2011). As a consequence, in androgenetic *Corbicula*, outcrossing and recombination may occur. Indeed, these clams cannot be considered paternal versions of true parthenogens as they maintained the ability to incorporate maternal genetic material (Pigneur *et al.* 2012).

Nowadays, *Corbicula* clams are considered one of the major exotic invasive pests in American and European freshwater ecosystems. Their extant native range includes Asia, the Middle East, Australia and Africa (Araujo *et al.* 1993). Their first record outside the native range was in British Columbia (USA) in the 1920s (McMahon 1982). The clams then rapidly spread throughout North America and reached both Central and South America (Counts 1986; Ituarte 1994). In Europe, invasive *Corbicula* clams were recorded only in the 1980s (Mouthon 1981). They are now well established in watersheds of most European countries, as far West as Ireland or Portugal, to Eastern Europe, as far as Ukraine (DAISIE 2014).

The successful invasion of *Corbicula* clams has been mainly attributed to their rapid maturation, high fecundity and high dispersal (Sousa *et al.* 2008). However, the reproductive mode may also play an important role in facilitating the establishment of introduced species (Roman & Darling 2007). Recent genetic studies particularly underlined the importance of androgenetic reproduction in the invasive success of *Corbicula* clams (Pigneur *et al.* 2011a, 2012).

Within the genus *Corbicula*, phylogenetic studies revealed an 'estuarine clade' (mainly the sexual *C. japonica*) along with a 'freshwater clade', the latter including both sexual dioecious (e.g. *C. sandai*) and hermaphroditic androgenetic lineages. Currently, the origin and taxonomic status of the invasive *Corbicula* lineages in America and Europe remains unclear, despite several morphological and genetic studies (e.g. Renard *et al.* 2000; Siripattrawan *et al.* 2000; Pfenninger *et al.* 2002; Lee *et al.* 2005; Hedtke *et al.* 2008; Pigneur *et al.* 2011a). Kinzelbach (1991) hypothesized that the individuals of *Corbicula* that invaded Europe were introduced by ballast waters from America and that there was no direct geographic link between the European and the native Asian populations. Among the three European morphotypes found (R, S and Rlc; Marescaux *et al.* 2010), the form R seems fixed for the same, single *COI* haplotype as the American form A (Siripattrawan *et al.* 2000; Hedtke *et al.* 2008; Pigneur *et al.* 2011a). The American form B and the European form Rlc are each fixed for a single, closely related, *COI* haplotype (Hedtke *et al.* 2008; Pigneur *et al.* 2011a). The South American form C and the European form S share the same *COI* haplotype despite divergent morphologies (Pigneur *et al.* 2011a). The mitochondrial (mt) haplotypes found in the invasive forms appear closely related to Asian lineages, except the haplotype found in forms C and S which has, to date, never been recorded in Asian populations (Park & Kim 2003). Nevertheless, inferring relationships based on mitochondrial data are particularly tricky in androgenetic *Corbicula* clams due to egg parasitism (see Pigneur *et al.* 2011a for a discussion on the 'pitfall of mt phylogenies in androgenetic *Corbicula* populations'). Preliminary microsatellite data have revealed that, in Western Europe, each of the three European morphotypes (R, S and Rlc) is fixed for a single multilocus genotype (Pigneur *et al.* 2011a). In America, forms A and B appear also to be fixed at nuclear markers as demonstrated by Hillis & Patton (1982) and Hedtke

et al. (2008). Although many invasive species exhibit an important genetic diversity due to high propagule pressure with a combination of high inoculum size, multiple introduction events and multiple origins (reviewed in Roman & Darling 2007), there are also some cases of 'genetic paradoxes' with successful invasive species exhibiting a very low genetic polymorphism resulting from asexuality, genetic bottlenecks or founder effect (e.g. Tsutsui *et al.* 2000; Golani *et al.* 2007; Dlugosch & Parker 2008; Puillandre *et al.* 2008; Zhang *et al.* 2010). In *Corbicula* clams, the ability of selfing (Kraemer *et al.* 1986) combined with the high fecundity and early maturity (Sousa *et al.* 2008) of these hermaphroditic androgenetic clams may quickly enable re-establishment of populations after massive bottlenecks.

The present study examines the genetic diversity of asexual and sexual lineages in the genus *Corbicula* and the invasion pattern of the androgenetic lineages. We surveyed both sexual and asexual lineages of the native area (Asia, Africa) and asexual lineages of the invasive regions (America and Europe) to unravel their relationships and the invasion pattern of the androgenetic lineages. This is the first study including samples from their worldwide distribution. We used the *COI* mt marker to pre-identify the lineages and to detect egg parasitism. Ten microsatellite markers (Pigneur *et al.* 2011b) were used to assess the genetic diversity and population structure of both native and invasive *Corbicula* populations. By studying both sexuals and asexuals, we can further evaluate the evolutionary history of this genus and describe biogeographical patterns.

Materials and methods

Specimen collection and DNA extraction

A large-scale collecting campaign was conducted to obtain *Corbicula* specimens from 20 European localities, from 6 sites in North and South America and from 16 sites in the native regions Africa and Asia (Fig. 1, Table 1). The individuals were directly sampled by the authors or obtained thanks to collaborators. Individuals were preserved in 96% ethanol. The general shell morphology was described, and the individuals from Europe were classified into the three previously described morphotypes R, S and Rlc (Pfenninger *et al.* 2002;

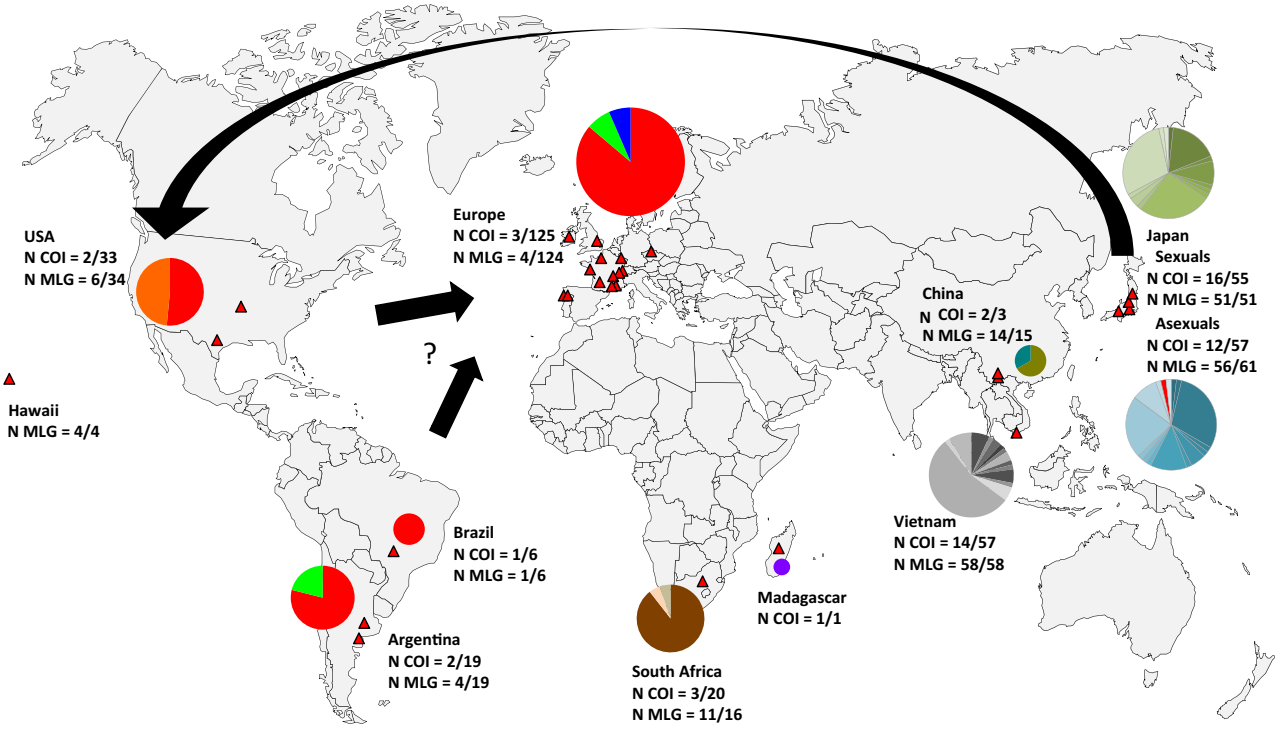

Fig. 1 Map of *Corbicula* sampling presenting the *COI* haplotype distribution and diversity. N = number of individuals analysed, N COI = number of *COI* haplotypes found, N microsat = number of genotypes found. For the invasive lineages, haplotype FW1 is in orange, FW4 in blue, FW5 in red, and FW17 in green. Arrows indicate the putative invasion pathways from Asia towards America and then Europe. Question mark indicates that the origin of European populations can be in either North or South America. For Japan, the haplotype diversity of the sexual and asexual lineages is presented separately; the sexuals are framed. No MLG data available for the single individual from Madagascar. No *COI* data available for individuals from Hawaii.

Marescaux *et al.* 2010) while the American samples were classified into the three morphotypes A, B and C following Lee *et al.* (2005). The 'mixed' (found in Ohio River, USA) or 'intermediate' (population I from River Seine, France) forms were considered separately.

Total genomic DNA was extracted from the adductor muscles, the foot or the mantle from each *Corbicula* specimen using the DNeasy blood & tissue kit (Qiagen). The number of analysed individuals per population is indicated in Table 1; in total, 403 *Corbicula* individuals were included in the present study.

Mitochondrial COI gene study

A fragment of 710 bp of the *COI* gene was amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Amplification was performed following the protocol described in Pigneur *et al.* (2011a). PCR products were purified and sequenced with each universal primer on an automated ABI 3730XL Genetic Analyzer (Macrogen Inc., Genoscreen). The *COI* mtDNA fragment was successfully amplified and sequenced in 380 *Corbicula* samples (Table 1). Sequences were visualized and aligned using BIOEDIT 7.0.5.3 (Hall 1999). Of the 545 nucleotides scored, 98 variable sites were detected, defining 50 haplotypes (Table 1, Appendix S1, Supporting information). The newly discovered sequences were deposited into GenBank (Accession nos: KC211240-89).

The number of haplotypes found in the different areas is indicated in the general map (Fig. 1), which has been designed under QGIS version 1.8.0 Lisboa (Quantum GIS Development Team 2013. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). Regarding the invasive lineages, we have considered the distinct haplotypes as distinct 'populations' for the analyses described below. The 'mixed' and 'intermediate' forms were also analysed separately.

Diversity indices (H, haplotype diversity, and π, nucleotide diversity) were calculated using DNASP v5 (Librado & Rozas 2009). The genetic diversity of *Corbicula* clams was calculated for each population corresponding to a sampling location for native individuals and to a lineage/morphotype for invasive individuals (Tables 1 and 2).

A maximum-likelihood tree and a Bayesian phylogeny were constructed with PHYML 2.4.5 (Guindon & Gascuel 2003) and MRBAYES 3.2 (Huelsenbeck & Ronquist 2001) respectively, with the model GTR+I+G selected using JMODELTEST 0.1 software (Posada 2008). The best-fitting models were chosen with the Akaike Information Criterion. For ML phylogeny, bootstrap values were obtained for 1000 replicates. BI trees were constructed based on a cold chain and five heated chains, running for 1,500,000 generations with a sample frequency of 500. The first 25% of the trees were discarded, and the remaining trees were used to build a consensus tree and estimate Bayesian posterior probabilities (PP). We considered that convergence occurred at a stable standard deviation of split frequencies $\leq 0,01$. To confirm the Bayesian analysis reached the convergent state, two independent runs were executed. We used *Neocorbicula limosaCOI* sequence as outgroup, and trees were visualized under MEGA4 (Tamura *et al.* 2007).

Microsatellite study

We used ten microsatellite loci developed by Pigneur *et al.* (2011b) as nuclear markers: ClA01, ClA02, ClA03, ClB03, ClB11, ClC01, ClC12, ClD06, ClE01 and ClD12. For each locus, the amplification was performed following the protocol of Pigneur *et al.* (2011a). The fragments were analysed on an ABI 3130XL Genetic Analyzer with GeneScan-500 (LIZ) size standard (Applied Biosystems). Results were visualized using GENEMAPPER (Applied Biosystems). Electropherograms did not show triple peaks although the ploidy status of most studied populations is ignored and may include triploids. The data were treated here as diploids.

For each individual, the multilocus genotype (here, the unique combination of alleles for the 10 microsatellite loci) was defined (Appendix S2, Supporting information). The multilocus genotype (MLG) assignment was performed using GENCLONE (Arnaud-Haond & Belkhir 2006).

MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to estimate stutter errors, and the proportion of null alleles (NA) at each locus, calculated for both the overall sampling and the 15 predefined populations. The genotypes were then corrected accordingly. The 15 populations were defined based on the sampling country, the reproductive mode and *COI* data.

With GENETIX software (Belkhir *et al.* 2001), we calculated several nuclear genetic diversity statistics: number of alleles per locus (A), observed (Ho) and expected (He) heterozygosity, and genotypic richness (R).

The Bayesian clustering method implemented in the commonly used STRUCTURE software (Pritchard *et al.* 2000) assumes minimum linkage disequilibrium and populations in Hardy–Weinberg equilibrium (HWE). Working on asexual *Corbicula* lineages violates these assumptions and can therefore lead to spurious clustering through this Bayesian method. We therefore used a discriminant analysis of principal components (DAPC) (Jombart *et al.* 2010) that does not rely on population genetics models and is thus robust to deviations from HWE and Linkage equilibrium. DAPC is a multivariate

Table 1 Origin and specification of the studied populations of *Corbicula*

Taxon	Population	Country	River (or lake)	Location	Geographic coordinates		N COI	COI haplotypes	N microsat	Voucher number
C. sp. form A	AA	USA	San Gabriel River	Georgetown, Texas	30°37′59.5″N	97°41′04.4″W	14	FW5	14	AA1-14
C. sp. form B	AB	USA	San Gabriel River	Georgetown, Texas	30°37′59.5″N	97°41′04.4″W	16	FW1	16	AB1-16
C. sp. form A	Oh	USA	Ohio River	Mouth of Hess Bayou/ Chestnut Hills Nature Preserve (Pulaski County, Illinois)	37°09′92″N	89°14′08″W	3	FW5	4	Oh2-5 (INHS33606, 31670,32736)
C. sp. form A	Arg	Argentina	–	–	NA	NA	19	FW5	19	Arg1-15
C. sp. form A	BRA	Brazil	Parana River & Baia River	–	NA	NA	6	FW5	6	Bra1-6
C. sp. form C	C	Argentina	Arroyo El Pescado	South of La Plata	34°57′37″S	57°46′37″W	4	FW17	4	C1-4
C. sp.	Hw	USA (Hawaii)	–	Maui	NA	NA	0	?	4	Hw2-8, 10
C. sp. form R	R	Ireland	–	–	NA	NA	8	FW5	8	Ir1-8
C. sp. form R	R	Switzerland	Neufchâtel Lake	–	NA	NA	6	FW5	6	Su1-6
C. sp. form R	R	Spain	Centeans Ponds	Centeans	42°07′N	8°37′W	6	FW5	6	Es1-6
C. sp. form R	R	UK	New Bedford	Sutton Gault	52°23.7′N	0°05.9′E	6	FW5	6	UK1-6
C. sp. form R	R	Czech Republic	Elbe	Křivenice	50°24′32.04″N	14°23′45.42″E	6	FW5	6	CZ1-6
C. sp. form R	R	Portugal	Minho estuary		42°04′46.02″N	8°30′52.02″W	8	FW5	6	Po13-18
C. sp. form R	R	Portugal	Lima estuary		41°43′32.06″N	8°4113.73″W	6	FW5	12	PLi4-5, Po5-10
C. sp. form R	R	Switzerland	Rhine River	Augst	47°32′20″N	7°42′52″E	2	FW5	2	5.1, 5.2
C. sp. form S	S	Switzerland	Rhine River	Birsfelden	47°33′39″N	7°37′56″E	1	FW17	1	12.2
C. sp. form R	R, Rlc	France	Doubs	Saunières	NA	NA	5	FW5, FW4	5	Db1, 2, 4-6
C. sp. form Rlc	Rlc	France	Doubs	Saunières	NA	NA	6	FW4	7	Db3, 21-26
C. sp. I (intermediate)	I	France	Seine	Poses	NA	NA	2	FW17	2	S1, S3
C. sp. form R	R	France	Saône	Ile Barbe, Lyon	NA	NA	6	FW5	6	Sa1-6
C. sp. form S	S	France	Saône	Ile Barbe, Lyon	NA	NA	6	FW17	6	Sa21-26
C. sp. form R	R	France	Vidourle		43°41′53.2″N	4°09′83.0″E	6	FW5	6	Vd1-6
C. sp. form R	R	France	Somme Canal	Cappy	49°55′24.3″N	2°44′49.9″E	6	FW5	6	C.som1-6
C. sp. form R	R	France	Nantes-Brest Canal		47°26′72.3″N	01°33′95.7″W	6	FW5	3	CNB2, 5, 6
C. sp. form R	R	France	Loire		46°07′35″N	4°05′86″E	6	FW5	3	Loi1-3
C. sp. form R	R	France	Charente		45°4′3.65″N	0°35′11.2″W	6	FW5	3	Cha1, 3, 5
C. sp. form R	R	France	Tarn	Moissac	44°05′99″N	1°04′66″W	6	FW5	3	Tar2-4
C. sp. form R	R	France	Hérault	Saint-Guilhem-le-Désert	43°42′34.5″N	3°33′48.3″E	3	FW5	3	Her1-3
C. sp. form Rlc	Rlc, R	France	Gard		43°57′24.7″N	4°16′19.7″E	4	FW4, FW5	7	Gar1-3, Gard2-3, 5-6
C. sp. form R	R	France	Gard		43°51′26.6″N	4°36′48.4″E	2	FW5	2	Gard1, 4
C. sp. form R	R	France	Moselle		48°49′50.6″N	6°06′50″E	6	FW5	6	Mos1-6
C. largillierti ?	BA	China	Tributaries of Lake Dianchi	Baxin shiping (Yunnan)	NA	NA	2	BA1, BA6	5	BA1-6

Table 1 *Continued*

Taxon	Population	Country	River (or lake)	Location	Geographic coordinates		N COI	COI haplotypes	N microsat	Voucher number
C. largillierti ?	Muj	China	Mujiang River	Yunnan	NA	NA	0	?	4	Muj1-5,8
C. sp.	FU	China	Fuxian Hu Lake	Yunnan	NA	NA	1	BA1	6	Fu1-6
*C. japonica**	Jp	Japan		Local market, Tokyo	NA	NA	10	Jp1,2,3,10	10	Jp1-11
*C. japonica**	KA	Japan	Kano River	Kyoto	NA	NA	5	Ka1,Ka2, Ka5	6	Ka1-6
*C. japonica**	CJ	Japan	Sinji Lake	Shimane (collected by fisherman, precise location not known)	35°28′21.84″N	133° 1′18.63″E	20	CJ1,7,12, 19, Jp3	16	CJ1-7,9-15, 17-22
*C . sandai**	CS	Japan	Lake Biwa	Hikone, Shiga prefecture (collected by fisherman, precise location not known)	35° 9′24.27″N	135°56′36.93″E	20	SandaiA, sandaiB, CS10,23,27	19	CS4,6,9-15, 18-19,21-27, 31-32
C. fluminea/leana morph	EHM	Japan	Shigenobu river ?	Shigenobu Takubo, Ehime	33°47′3.97″N	132°51′40.72″E	18	EHM102, 107,111, 113,119, FW1	16	EHM102-113, 119-124
C. fluminea/leana morph	KMT	Japan	Canal close to Mifune town hall	Kumamoto	32°42′53.44″N	130°48′9.35″E	10	FW5	14	KMT211-224
C. fluminea/leana morph	KMT	Japan	Canal close to Mifune town hall	Kumamoto Mifune Machiyakuba	32°42′53.44″N	130°48′9.35″E	10	KMT201,208, FW1,FW5	10	KMT201-210
C. fluminea/leana morph	Cl	Japan	Miyazaki Gongenbaru Canal		32° 24′53.39′′N	131°36′54.97″E	19	Cl201,221, 223, FW1	21	Cl201-208, 210-216,218, 220-224
C. sp.	Vt	Vietnam		Thoi An Dong, Binh Thuy district, Can Tho	NA	NA	18	Vt1,10,13, 14,FW14, FW15	20	Vt1-20
C. fluminea ?	CR	Vietnam	–	Can Tho	NA	NA	20	CR2,4,5,20, 23,26, Vt13,14	18	CR1-20
C. fluminea ?	CR′	Vietnam	–	Can Tho	NA	NA	19	CR′3,9, CR2,20, Vt14	20	CR′1-21
C. fluminalis africana	ZA	South Africa	Mooi River	Potchefstroom	20°41′18.2″S	27°05′55.2″E	20	ZAH1-3	16	ZA1-20
C. madagascariensis	Mad	Madagascar	Menentanana River		NA	NA	1	Mada	0	Mad1
							Total = 380	Total = 50	Total = 389	Total =403

Taxon = species designation, Population = population code used in the text and figures, Country = country of the sampled locality, River (or lake) = river drainage or lake when available, N COI= number of individuals sequenced for *COI*, COI haplotypes = names of specific haplotypes, N microsat = number of individuals studied for the microsatellite study, Voucher number = university or museum catalogue number of stored specimens. Asterisks indicate sexual *Corbicula* lineages.

Table 2 Reproductive mode and genetic diversity in the *Corbicula* populations

Form or population code	*Corbicula* taxon	Reproductive mode	H	π	Mean A	Mean He	Mean Ho	R
	Invasive							
	America							
A/R (3 sites)	C. sp. form A	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.6	0.3^	0.6^	0
A/R (1 site)	C. sp. form A (Oh)	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	2,00	0.39	0.67	0.5
B (1 site)	C. sp. form B	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.9	0.31^	0.56^	0
C/S (1 site)	C. sp. form C	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	2.5	0.39^	0.58^	0
	Europe							
A/R (20 sites)	C. sp. form R	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.5	0.25^	0.5^	0
Rlc (2 sites)	C. sp. form Rlc	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.38	0.19^	0.38^	0
C/S (2 sites)	C. sp form S	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.75	0.38^	0.75^	0
I (1 site)	C. sp. (intermediate)	Androgenetic	0.0000 ± 0.0000	0.0000 ± 0.0000	1.5	0.25^	0.5^	0
	Native							
	Asia							
Fu	C. sp. (China)	*?*	–	–	2	0.32	0.47	0.83
BA	C. sp. (China)	*?*	1.0000 ± 0.2500	0.00001 ± 0.00320	2.6	0.35	0.36	1
Muj	C. sp. (China)	*?*	–	–	2.4	0.47	0.46	1
Vt	C. sp. (Vietnam)	Androgenetic	0.648 ± 0.007	0.00185 ± 0.00044	5	0.59	0.46	1
CR	C. sp. (Vietnam)	Androgenetic	0.747 ± 0.098	0.00250 ± 0.00070	4.4	0.57	0.39	1
CR′	C. sp. (Vietnam)	Androgenetic	0.596 ± 0.122	0.00125 ± 0.00036	3.6	0.58	0.54	1
KMT	C. sp. (Japan)	Androgenetic	0.442 ± 0.133	0.00346 ± 0.00212	2.9	0.45	0.48	0.91
EHM	C. sp. (Japan)	Androgenetic	0.667 ± 0.106	0.00232 ± 0.00072	1.7	0.27	0.35	0.93
Cl	C. sp. (Japan)	Androgenetic	0.298 ± 0.133	0.00119 ± 0.00056	2.6	0.42	0.52	0.9
Jp	*C. japonica*	Sexual	0.533 ± 0.180	0.00090 ± 0.00040	2.8	0.44	0.41	1
Ka	*C. japonica*	Sexual	0.700 ± 0.048	0.00250 ± 0.00080	2.6	0.43	0.47	1
CJ	*C. japonica*	Sexual	0.774 ± 0.065	0.00310 ± 0.00036	3.6	0.62	0.3	1
CS	*C. sandai*	Sexual	0.621 ± 0.109	0.00175 ± 0.00050	2.5	0.31	0.24	1
	Africa							
ZA	*C. fluminalis africana*	?	0.284 ± 0.128	0.00112 ± 0.00062	2.5	0.39	0.58	0.67
	Unknown status							
	America							
Hw	C. sp. (Hawaii)	?	–	–	3	0.52	0.5	1

H = haplotype diversity, π = nucleotide diversity, mean A= mean number of alleles per locus, mean He = mean expected heterozygosity, mean Ho = mean observed heterozygosity, R = genotypic richness. ^ = All individuals having the same MLG within each of these groups, the mean He and Ho values are biased and only reflect that all individuals are heterozygotes for some loci.

analysis that identifies genetic clusters and their relationships. It involves transformation of the data through principal component analysis (PCA) before subjection to discriminant analysis (DA). Indeed, DA is the most suitable multivariate analysis to achieve the best between-population differentiation but necessitates variables being uncorrelated and less numerous than the number of observations (Jombart *et al.* 2010). We performed DAPC using the package ADEGENET (Jombart 2008) implemented in R version 2.15.2 (R Development Core Team 2008). The number of putative populations was first determined using the k-means clustering algorithm (Legendre & Legendre 1998) for $K = 1$ to $K = 20$, via the function find.clusters. The appropriate number of clusters is defined using the Bayesian information criterion (BIC) through the distribution of BIC corresponding to all possible clustering and with the lowest value generally indicative of the best clustering. The distribution obtained in the present study shows no particular elbow (Appendix S3, Supporting information), but the observed BIC decrease suggests that 10–20 clusters would provide relevant summaries of the data. We selected $K = 14$ for the DAPC analysis as we have 15 sampled populations (according to mt lineage or geographic origin; see above), but we discarded population ZA due to lack of amplification for numerous loci. DAPC was then used to infer the relationships between the 14 clusters. Eight principal components (71% of the total variance) were retained, as determined by the α-score. The α-scores allow the definition of the number of principal components achieving the best discrimination without overfitting. Eight discriminant functions were retained to capture the maximum amount of variability contained in our data set.

Defining the reproductive mode

When we obtained entire individuals, the sperm morphology was verified. Sperm morphology is indicative of androgenesis in *Corbicula* clams; sexuals possess

monoflagellate sperm while androgenetic individuals produce biflagellate sperm (Ishibashi *et al.* 2003). The superficial parts of the body mass containing the diffuse gonads were excised, sheared and incubated in a collagenase solution 1 mg/mL at 37 °C until complete tissue dissolution. Samples were then centrifugated for 3 min at 600 *g*. The pellet was dropped on a slide, covered by a cover glass and observed under a phase contrast microscope (Leica Leitz LaborluxS) with immersion oil at 100X magnification. We determined the sperm morphology for all invasive lineages as well as for samples from Vietnam (Table 1). The reproductive mode of Japanese lineages was defined based on previous descriptions (Konishi *et al.* 1998; Glaubrecht *et al.* 2003).

Results

Genetic diversity, genetic relatedness and geographic distribution of invasive Corbicula

In the invaded range covering North and South America as well as Europe (Fig. 1), except in rare cases (see below; cytonuclear mismatch), four distinct *Corbicula* lineages were detected and no genetic variability was observed within each invasive lineage (Table 2). Interestingly, each of these four invasive forms appears more similar at the genetic level to some native populations than to any other invasive form (Figs 2 and 3).

It is noteworthy that all *Corbicula* individuals of American form A and European form R (from 23 distinct sampling sites; Table 1) are identical for all tested markers (Figs 1–3), indicating they belong to the same, probably clonal, lineage (called hereafter form A/R). Microsatellite data suggest a close relationship between form A/R and the estuarine sexual species *C. japonica* in Japan (Jp, KA) (Fig. 3) while mitochondrial data indicate a clustering with the androgenetic Japanese population KMT (Fig. 2). The Hawaiian population, based on its allelic combination, clusters with this group (Fig. 3).

The invasive South American form C and European form S are also considered a unique lineage (form C/S) because they share the same *COI* haplotype and probably the same multilocus genotype, although there are some missing genotyping data (some microsatellite markers did not amplify in some individuals) for form C individuals (see Appendix S2, and Fig. 3 with four points representing form C/S multilocus genotypes). Interestingly, based on *COI* sequences, form C/S clusters with native *C. fluminalis africana* from South Africa (Fig. 2).

Individuals of American form B and of European form Rlc (Table 1) constitute two other distinct invasive lineages, each fixed for one haplotype and one multilocus genotype (Figs 1–3, Tables 1 and 2). These two forms also show distinct relationships with Asian populations, depending on the marker used (Figs 2 and 3).

For the nuclear markers, we did not test for heterozygote excess in the invasive lineages as only one MLG was found in each form (Table 2). Indeed, all individuals harboured the same MLG (heterozygous at several loci). The genetic homogeneity within each lineage, however, confirms clonal propagation of *Corbicula* individuals in the invaded range.

Only the population from Hawaii showed polymorphism at the nuclear level, with each tested individual having a distinct MLG. Genetic divergences were also recorded in the 'mixed' forms found in Ohio River and in the 'intermediate' form found in the River Seine (Western Europe, see below).

Genetic diversity of native Corbicula

While there was no observed genetic variability within each invasive *Corbicula* lineage, a high genetic variation was detected in all native *Corbicula* populations, even at a very limited geographical scale, regardless of their locality or reproductive mode (Figs 1–3, Table 2). Indeed, both sexual and androgenetic lineages showed comparable genetic diversities in the native regions, studied here in 14 populations in Asia and 1 in Africa (Figs 1–3, Table 2). For example, 44 distinct *COI* haplotypes were detected in 172 specimens from Asia (16 haplotypes in sexuals, 28 in asexuals) compared with four in the whole invaded area (Fig. 1, Table 1). Microsatellite data further confirm the substantially higher genetic diversity found in the native range (see Figs 1 and 3). Allele number is significantly higher in native populations compared with invasive ones, in both the sexual and androgenetic *Corbicula* populations (Table 2; mean A, P-value = 0.002). Moreover, in contrast to the invasive populations, many different MLG were found in native populations (Fig. 1). We found mostly one MLG per individual (Fig. 1) in native sexuals and asexuals as reflected by the calculated genotypic richness R (often being equal to 1, Table 2) and the DAPC plot (Fig. 3).

Interestingly, the sexual lineages (*C. japonica* and *C. sandai*, Table 1), did not present a substantially higher genetic diversity than asexual androgenetic populations from the native region (Table 2, Figs 1–3).

Nuclear and mitochondrial captures

We found here that the invasive lineages of *Corbicula* were fixed for one mt haplotype and one MLG. However, in rare cases, we also found some invasive androgenetic individuals harbouring a 'mixed' nuclear

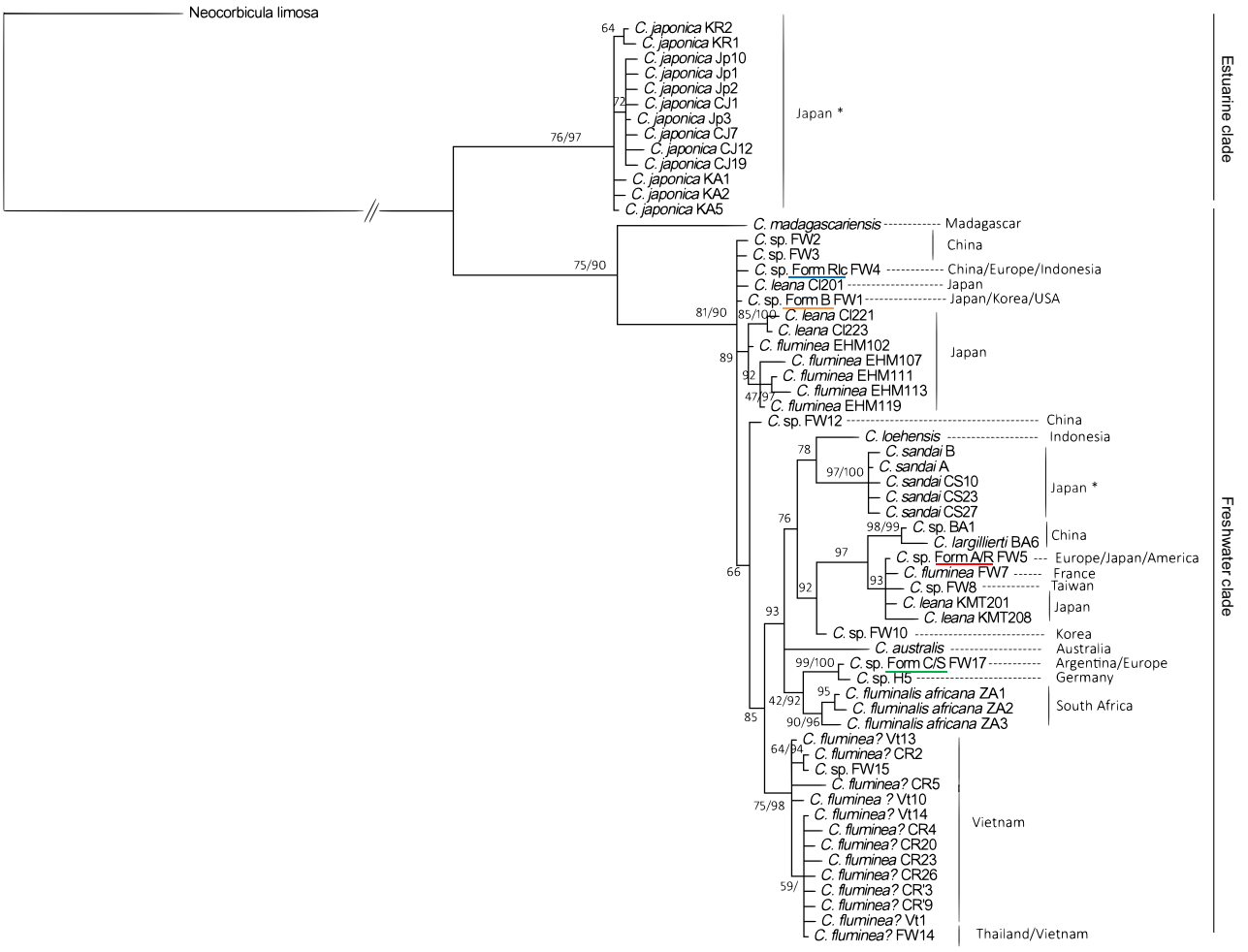

Fig. 2 Phylogenetic tree of *Corbicula* based on mt*COI* sequences (545 bp). The topology presented here is inferred by Bayesian Inference. Posterior probabilities and maximum-likelihood bootstrap values (for branches matching those inferred by ML) are indicated in that order. Sexual lineages are indicated by asterisks. The four invasive forms are colour-coded (form A/R in red, form B in orange, form C/S in green and form Rlc in blue).

genotype with microsatellite alleles of two distinct invasive lineages (or forms); in four individuals from Ohio River (mixed pattern between forms A/R and B, population Oh, Table 1) and in two 'intermediate' individuals from River Seine (mixed pattern between forms A/R and C/S, population I – Table 1). These mixed allelic patterns might result from nuclear hybridization events as demonstrated in other invasive populations of *Corbicula* (Pfenninger *et al.* 2002; Lee *et al.* 2005; Hedtke *et al.* 2011).

In addition, cytonuclear mismatches (Hedtke *et al.* 2008; Pigneur *et al.* 2011a) resulting from androgenetic mitochondrial capture were detected at some of the locations where different lineages occur in sympatry. The *COI* haplotype of form Rlc was associated with MLG of form A/R, and *COI* haplotype of form A/R was associated to MLG of form Rlc, in the Rivers Doubs and Gard (France), respectively.

Discussion

Genetic diversity in the invasive populations

While many invasive species present a high genetic diversity in their invasion area, some successful invasive populations exhibit a low genetic polymorphism, for example in the invasive asexual water hyacinth *Eichornia crassipes* (Zhang *et al.* 2010). In the genus *Corbicula*, the invasive lineages show an extremely low genetic diversity (at the studied markers) and are mostly fixed for one haplotype/genotype combination. This probably results from their androgenetic mode of reproduction. Indeed, there are now robust evidences that the invasive lineages of *Corbicula* found in Europe and America mostly reproduce asexually, and more specifically through androgenesis. First, biflagellate sperm was found in all studied invasive lineages of

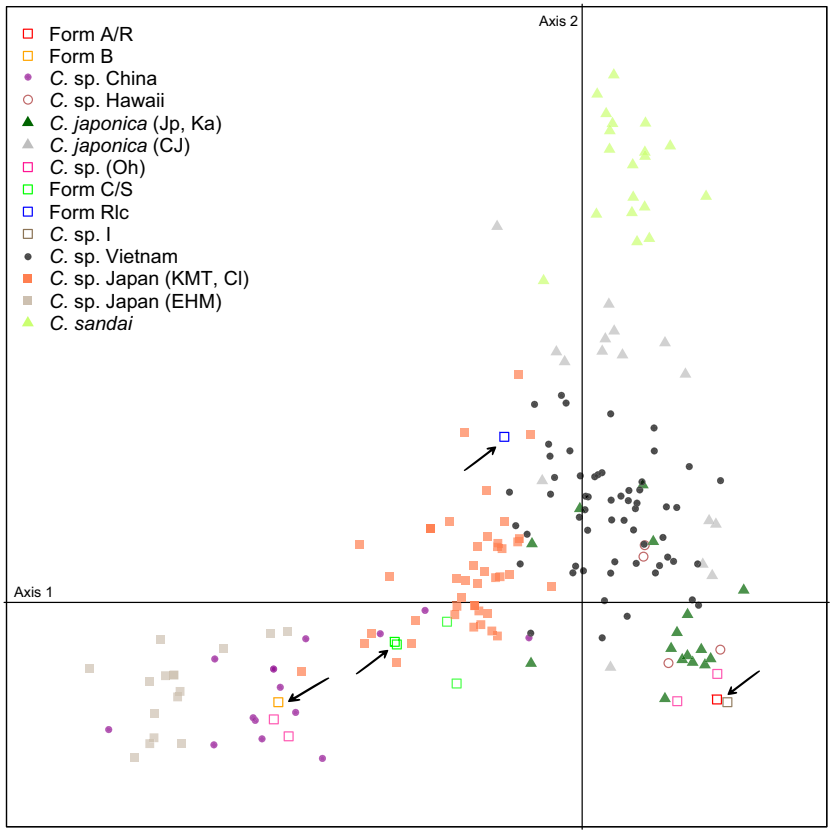

Fig. 3 Genetic diversity and genetic relationships in the genus *Corbicula* estimated through a discriminant analysis of principal components. Scatterplot of the first two principal components (first axis: 35% of total variance; second axis: 25% of the total variance) of the DAPC of 373 individuals genotyped at 10 microsatellite loci. Squares refer to androgenetic populations, triangles to sexual populations and circles to populations for which the reproductive mode is unknown but most probably androgenetic. Filled squares are the androgenetic populations found in the native region. For visual convenience, arrows locate the MLG of invasive lineages on the plot.

Corbicula, both in America and Europe (Lee *et al.* 2005; Pigneur *et al.* 2011a; present study). Second, several cases of cytonuclear mismatches have been recorded in the invasive populations where distinct lineages occur in sympatry (e.g. between forms R and S in Pfenninger *et al.* 2002 and Pigneur *et al.* 2011a). This phenomenon is attributed, in the genus *Corbicula*, to egg parasitism via androgenesis between distinct lineages (Hedtke *et al.* 2008; Hedtke & Hillis 2011; Pigneur *et al.* 2011a, 2012). We also detected several putative cases of "nuclear hybridization" between two distinct lineages (in River Seine and Ohio River). Similar genetic mixing events were also reported by Pfenninger *et al.* (2002) and Lee *et al.* (2005). Third, the absence of genetic diversity and the increased heterozygosity observed in the invasive lineages could also be considered as a 'sign of asexuality' (Hedtke *et al.* 2008). However, despite the absence of diversity at neutral genetic markers (mt DNA and microsatellites), genetic variance might be found at other loci (Roman & Darling 2007). Further work should, among others, rely on other markers to investigate this issue.

Sexual VS asexual populations: distribution and diversity

The present study examined the genetic diversity of native and invasive *Corbicula* populations with the aim of testing whether the native androgenetic populations were also weakly diversified. On the contrary, native androgenetic lineages showed a high genetic diversity, similar to that of sexual lineages (Table 2, Figs 1 and 3). Interestingly, the majority of alleles (100% for forms A/R, B and S/C; 91% for form Rlc) found in Europe and America are also present in the studied Asian populations. The elevated genetic diversity found in androgenetic lineages of *Corbicula* in the native region and the important allele sharing between the invaded range and Asia suggests that the reduced diversity found in the invasive populations could, among other reasons, result

from the introduction of few specimens or few genetic lineages from Asia. Furthermore, they could have encountered a strong founder effect or faced a short-lived bottleneck followed by a fast population expansion of each form, as is often the case for invasive organisms (Sax *et al.* 2005; Golani *et al.* 2007). Combined with their clonal mode of reproduction, this could have then resulted in the low genetic diversity observed in the invaded areas.

Three hypotheses might explain the elevated genetic diversity found in asexual *Corbicula* populations of the native region: (i) large effective population size; (ii) high mutation rate; or (iii) possible recent and/or multiple origin from sexuals (Shreve *et al.* 2011). The first two hypotheses cannot be confirmed here. The effective population size should be assessed based on the intraspecific nucleotide diversity and the mutation rate. However, we are lacking information about the mutation rate as it has, to date, never been estimated in *Corbicula*. The third hypothesis (recent and/or multiple origin from sexuals) seems highly plausible in the genus *Corbicula*. The recent radiation of asexual freshwater *Corbicula* taxa is confirmed by the short branch lengths separating them (Hedtke *et al.* 2008, 2011; Pigneur *et al.* 2011a; present study). This hypothesis is further supported in the present study by the substantial allele sharing between asexual and sexual *Corbicula* lineages both in the native and invasive regions (Fig. 3). Moreover, all the microsatellite markers used here were taken from a library designed only on invasive form A/R coming from the Meuse river (Belgium) (Pigneur *et al.* 2011b) while the 10 selected loci successfully amplified in almost every population/lineage of the globally distributed samples used in this study. This further confirms the recent origin of the different *Corbicula* lineages. Highly diverse asexual populations, as observed in the native region, could have arisen from frequent origins from sexuals as is commonly observed in mixed systems in which both sexual and asexual taxa cohabit (Simon *et al.* 2003; Adolfsson *et al.* 2010; Bode *et al.* 2010; Neiman *et al.* 2010). Androgenetic *Corbicula* clams are indeed found in sympatry with sexuals in several locations in Japan: with *C. sandai* in lake Biwa (Ishibashi & Komaru 2003; Etoundi E., personal observation) and with *C. japonica* in some estuaries (R. Kiso, Miyakoda) (E. Etoundi, personal observations). The observed incongruence between mitochondrial and nuclear phylogenies as observed by Hedtke *et al.* (2011) suggests that androgenetic lineages, after they originate from a sexual ancestor, may diverge by egg parasitism and subsequent genetic captures as well as hybridization/introgression events between the newly arisen asexuals. In the present study, we indeed observed such patterns of genetic captures and hybridization events, namely in rivers Doubs, Gard, Ohio and Seine (see Results section; *Nuclear and mitochondrial captures*).

Interestingly, the sexual *Corbicula* lineages are found only in particular areas in Japan (Park & Kim 2003; Von Rintelen & Glaubrecht 2006; present study) and probably in Indonesia (Glaubrecht *et al.* 2003). More specifically, *C. japonica* inhabits Japanese, Korean and possibly Chinese brackish waters (Yamada *et al.* 2014) while *C. sandai* is endemic to the Japanese freshwater Lake Biwa and its watershed (Houki *et al.* 2011). Asexual *Corbicula* are not only distributed across the global range of the genus but are also found in sympatry with sexual lineages. Such a relatively wide distribution of the asexual lineages of *Corbicula* compared to the restricted distribution of the sexual ones resembles the phenomenon of geographic parthenogenesis (Vandel 1928).

Distribution and phylogeography of invasive Corbicula lineages

The invasion pattern in both America and Europe confirms the hypothesis of a rapid spread of *Corbicula*, probably through clonality: only four lineages showing no genetic variability have been recorded across the invaded range. The form A/R found in America and Europe, however, cannot strictly be considered one 'clonal lineage' as form A seems triploid while form R includes both di- and triploids (Pfenninger *et al.* 2002; Hedtke *et al.* 2008; Skuza *et al.* 2009). Interestingly, while being found in the mt 'freshwater clade' close to the androgenetic Japanese population KMT, form A/R seems more closely related to the Japanese sexual form *C. japonica* (from the mt 'estuarine clade') on the basis of the microsatellites (Fig. 3). This incongruence could be related to ancient egg parasitism events as discussed above. The identical haplotype and genotype between forms A and R suggests that form R, sampled in Europe for the first time in the 1980s, derived directly from form A (Fig. 1) found in America since the 1920s, as already hypothesized by Kinzelbach (1991). Indeed, our results indicate an ancient invasion of a few clonal lineages from Asia towards America and a subsequent colonization of Europe without recurrent invasions. However, our data do not allow determining whether the introduction into Europe derived from North or South American populations (Fig. 1).

The same genetic link and invasion pathway could be suggested for form C (South America) and the form S (Europe) although the invasion record dates are closer for both forms: 1982 in the Rio de la Plata, Uruguay (Ituarte 1994) and probably 1984 in Weser River, Germany (Haesloop 1992). Interestingly, the *COI* haplotype of form C/S has, to date, not been recorded in Asian populations but instead clusters with haplotypes

from the studied South African population of *C. fluminalis africana*. An introduction from Africa to South America and then Europe could be suggested. However, it has not been possible to confirm this hypothesis with the microsatellite data and additional genetic data on *C. fluminalis sensu stricto* (Caucasus, Central Asia and Middle East; Korniushin 2004) would be needed to investigate this question.

The individuals of form B and form Rlc present closely related haplotypes. Hedtke *et al.* (2008) showed that form B would have a hybrid origin between two divergent species, one being a form A-like androgenetic ancestor. Via egg parasitism, an androgenetic ancestor of form A could have combined its genome with that of another lineage (either androgenetic or sexual), resulting in form B, which exhibits nuclear chromosomes from multiple lineages, along with the mitochondrial DNA of the second ancestor. Our mt data suggest that this second ancestor originates from mainland Asia: China, Korea or Vietnam (Siripattrawan *et al.* 2000; Hedtke *et al.* 2008). Moreover, the microsatellite alleles that form B does not share with the other invasive lineages were mainly detected in Chinese populations. The mt ancestor of form B could be the same one as for form Rlc as we observe only one nucleotide difference at the *COI* gene between both forms. Based on the microsatellite data, form Rlc seems related to Japanese and Vietnamese populations.

Invasive success of the clonal lineages

The intercontinental range expansion of the androgenetic *Corbicula* lineages, although linked to human activity, may also have been facilitated by some of their exclusive features. Androgenetic lineages of *Corbicula* are hermaphrodites and capable of self-fertilization (Kraemer *et al.* 1986). In addition to their r-reproductive strategy, selfing allows asexual *Corbicula* to quickly re-establish populations after massive bottlenecks or found new populations. Furthermore, introduced populations generally encounter strong genetic drift due to reduced effective population size, genetic bottlenecks and limited migration resulting in a loss of heterozygosity (Dlugosch & Parker 2008). Clonality may have favoured range expansion of androgenetic lineages, because heterozygous individuals would systematically produce heterozygous progeny, as shown by the high observed heterozygosity in invasive *Corbicula* lineages (Hedtke *et al.* 2008; present study). Therefore, these lineages would have benefitted from a reduced cost of dispersal, would not have suffered any heterozygosity loss during invasion and would have escaped inbreeding depression (Haag & Ebert 2004). Moreover, hybridization between different lineages or new cytonuclear associations occur in *Corbicula* and can lead to hybrid vigour with increased fitness. However, no fitness experiment of any *Corbicula* lineage has been conducted yet. Remarkably, the invasive form A/R is the most widespread and abundant in the invaded regions (e.g. Siripattrawan *et al.* 2000; Lee *et al.* 2005; Marescaux *et al.* 2010; Pigneur *et al.* 2011a), reaching densities up to almost 10,000 individuals/m² (Caffrey *et al.* 2011) and replacing form C/S in South America (C. Ituarte, personal communication) and possibly in Europe (E. Etoundi, personal observations). This lineage includes both diploid and triploid populations with triploidy being apparently predominant (Pfenninger *et al.* 2002; Lee *et al.* 2005; Hedtke *et al.* 2008; E. Etoundi, personal observations). Polyploidy could help broadly tolerant genotypes by masking deleterious mutations (Otto 2007), and therefore, polyploid individuals may be favoured during an invasion process. As all studied individuals of form A/R from 23 distinct populations show no genetic variability, the genus *Corbicula* is considered one of those 'genetix paradoxes' of invasive species. Although it is commonly recognized that invasiveness might also rely on plasticity and generalism, androgenesis offers new opportunities when novel genetic material – mitochondria or even nuclear chromosomes – is incorporated through egg parasitism within a distinct lineage (Pigneur *et al.* 2012). New hybrid genotypes or new cytonuclear associations are created and might provide a higher advantage over « nonhybrid » ones, although this is still to be demonstrated.

Finally, according to Roman & Darling (2007), 'molecular diversity might not predict invasion success'. While most current knowledge focuses on the level of genetic polymorphism of invasive species, further work should address the issue of mechanisms by which weakly diversified populations succeed in their invasion process. Androgenesis in *Corbicula* clams combines features of clonal reproduction and the ability of rare genetic material exchange (Pigneur *et al.* 2012). Associated with the other life history traits of invasive *Corbicula* lineages, it might have been a determinant mechanism that contributed to the invasiveness of undiversified populations of these clams.

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References

Adolfsson S, Michalakis Y, Paczesniak D *et al.* (2010) Evaluation of elevated ploidy and asexual reproduction as alternative explanations for geographic parthenogenesis in *Eucypris virens* ostracods. *Evolution*, **64**, 986–997.

Araujo R, Moreno D, Ramos MA (1993) The Asiatic clam *Corbicula fluminea* (Müller, 1774) (Bivalvia:Corbiculidae) in Europe. *American Malacological Bulletin*, **10**, 39–49.

Arnaud-Haond S, Belkhir K (2006) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Resources*, **7**, 15–17.

Barraclough TG, Birky CW, Burt A (2003) Diversification in sexual and asexual organisms. *Evolution*, **57**, 2166–2172.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) *GENETIX 4.02, Logiciel Sous Windows Pour la Génétique Des Populations*. Laboratoire Génome, Populations, Interactions, Université de Montpellier II, Montpellier, France.

Bode SNS, Adolfsso S, Lamatsch DK *et al.* (2010) Exceptional cryptic diversity and multiple origins of parthenogenesis in a freshwater ostracod. *Molecular Phylogenetics and Evolution*, **54**, 542–552.

Byrne M, Phelps H, Church T, Adair V, Selvakumaraswamy P, Potts J (2000) Reproduction and development of the freshwater clam *Corbicula australis* in southeast Australia. *Hydrobiologia*, **418**, 185–197.

Caffrey JM, Evers S, Millane M, Moran H (2011) Current status of Ireland's newest invasive species – the Asian clam *Corbicula fluminea* (Müller, 1774). *Aquatic Invasions*, **6**, 291–299.

Counts CL (1986) The zoogeography and history of the invasion of the United States by *Corbicula fluminea* (Bivalvia: Corbiculidae). *American Malacological Bulletin Special Edition*, **2**, 7–39.

DAISIE (2014) Delivering Alien Invasive Species Inventories for Europe. http://www.europe-aliens.org.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Folmer O, Black M, Hoeh W, Lutzand R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Glaubrecht M, von Rintelen T, Korniushin AV (2003) Towards a systematic revision of brooding freshwater Corbiculidae in southeast Asia (Bivalvia, Veneroida): on shell morphology, anatomy and molecular phylogenetics of endemic taxa from islands in Indonesia. *Malacologica*, **45**, 1–40.

Golani D, Azzurro E, Corsini-Foka M, Falautano M, Andaloro F, Bernardi G (2007) Genetic bottlenecks and successful invasions: the case of a recent Lessepsian migrant. *Biology Letters*, **3**, 541–545.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematics Biology*, **52**, 696–704.

Haag CR, Ebert D (2004) A new hypothesis to explain geographic parthenogenesis. *Annales Zoologici Fennici*, **41**, 539–544.

Haesloop U (1992) Establishment of the Asiatic clam *Corbicula cf. fluminalis* in the Tidal Weser River (N. Germany). *Archiv fur Hydrobiologie*, **126**, 175–180.

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Hedtke SM, Hillis DM (2011) The potential role of androgenesis in cytoplasmic–nuclear phylogenetic discordance. *Systematics Biology*, **60**, 87–109.

Hedtke SM, Stanger-Hall K, Baker RJ, Hillis DM (2008) All-male asexuality: origin and maintenance of androgenesis in the Asian clam *Corbicula*. *Evolution*, **62**, 1119–1136.

Hedtke SM, Glaubrecht M, Hillis DM (2011) Rare gene capture in a predominantly androgenetic species. *Proceedings of the National Academy of Sciences, USA*, **108**, 9520–9524.

Hillis DM, Patton JC (1982) Morphological and electrophoretic evidence for two species of *Corbicula* (Bilvalvia: Corbiculidae) in North America. *The American Midland Naturalist*, **108**, 74–80.

Houki S, Yamada M, Honda T, Komaru A (2011) Origin and possible role of males in hermaphroditic androgenetic *Corbicula* clams. *Zoological Sciences*, **28**, 526–531.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Ishibashi R, Komaru A (2003) Invasion of *Corbicula fluminea* into the Lake Biwa-Yodo river system. *Venus Japanese Journal of Malacology*, **62**, 65–70.

Ishibashi R, Ookubo K, Aoki M, Utaki M, Komaru A, Kawamura K (2003) Androgenetic reproduction in a freshwater diploid clam *Corbicula fluminea* (Bivalvia: Corbiculidae). *Zoological Sciences*, **20**, 727–732.

Ituarte CF (1994) *Corbicula* and *Neocorbicula* (Bivalvia: Corbiculidae) in the Parana, Uruguay, and Rio de La Plata Basins. *Nautilus*, **107**, 129–135.

Jombart T (2008) ADEGENET: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.

Kinzelbach R (1991) Die Körbchenmuscheln *Corbicula fluminalis*, *Corbicula fluminea* und *Corbicula fluviatilis* in Europa (Bivalvia: Corbiculidae). *Mainzer Naturwissenschaftliches Archiv*, **29**, 215–228.

Komaru A, Kawagishi T, Konishi K (1998) Cytological evidence of spontaneous androgenesis in the freshwater clam *Corbicula leana* Prime. *Development Genes and Evolution*, **208**, 46–50.

Komaru A, Kumamoto A, Kato T, Ishibashi R, Obata M, Nemoto T (2006) A hypothesis of ploidy elevation by formation of a female pronucleus in the androgenetic clam *Corbicula fluminea* in the Tone River Estuary, Japan. *Zoological Sciences*, **23**, 529–532.

Komaru A, Yamada M, Houki S (2013) Relationship between two androgenetic clam species, *Corbicula leana* and *Corbicula fluminea*, inferred from mitochondrial Cytochrome b and nuclear 28S rRNA markers. *Zoological Science*, **30**, 360–365.

Konishi K, Kawamura K, Furuita H, Komaru A (1998) Spermatogenesis of the freshwater clam *Corbicula* aff. *fluminea* Müller (Bivalvia:Corbiculidae). *Journal of Shellfish Research*, **17**, 185–189.

Korniushin AV (2004) A revision of some Asian and African freshwater clams assigned to *Corbicula fluminalis* (Müller, 1774) (Mollusca: Bivalvia: Corbiculidae), with a review of anatomical characters and reproductive features based on museum collections. *Hydrobiologia*, **2004**, 251–270.

Kraemer LR, Swanson C, Galloway M, Kraemer R (1986) Biological basis of behaviour in *Corbicula fluminea*. II. Functional morphology of reproduction and development and review of evidence for self-fertilization. *American Malacological Bulletin Special Edition*, **2**, 193–201.

Lee T, Siripattrawan S, Ituarte CF, Foighil DÓ (2005) Invasion of the clonal clams: *Corbicula* lineages in the New World. *American Malacological Bulletin*, **20**, 113–122.

Legendre P, Legendre L (1998) *Numerical Ecology*, 2nd edn. Elsevier, Amsterdam.

Lehtonen J, Jennions MD, Kokko H (2012) The many costs of sex. *Trends in Ecology and Evolution*, **27**, 172–178.

Librado P, Rozas J (2009) DNASP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Marescaux J, Pigneur L-M, Van Doninck K (2010) New records of *Corbicula* clams in French rivers. *Aquatic Invasions*, **5**, S35–S39.

Maynard Smith J (1978) *The Evolution of Sex*. Cambridge University Press, Cambridge.

McMahon RF (1982) The occurrence and spread of the introduced Asiatic freshwater clam, *Corbicula fluminea* (Muller), in North America: 1924–1982. *Nautilus*, **96**, 134–141.

Mouthon J (1981) Sur la présence en France et au Portugal de *Corbicula* (Bivalvia, Corbiculidae) originaire d'Asie. *Basteria*, **45**, 109–116.

Neiman M, Hehman G, Miller JT, Logsdon JM Jr, Taylor DR (2010) Accelerated mutation accumulation in asexual lineages of a freshwater snail. *Molecular Biology and Evolution*, **27**, 954–963.

Okamoto A, Arimoto B (1986) Chromosomes of *Corbicula japonica*, *C. sandai* and *C. (Corbiculina) leana* (Bivalvia: Corbiculidae). *Venus Japanese Journal of Malacology*, **45**, 194–202.

Otto SP (2007) The evolutionary consequences of polyploidy. *Cell*, **131**, 452–462.

Park J-K, Kim W (2003) Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment. *Molecular Phylogenetics and Evolution*, **29**, 529–539.

Park J-K, Lee JS, Kim W (2002) A single mitochondrial lineage is shared by morphologically and allozymatically distinct freshwater *Corbicula* Clones. *Molecules and Cells*, **14**, 318–322.

Pfenninger M, Reinhardt F, Strei B (2002) Evidence for cryptic hybridization between different evolutionary lineages of the invasive clam genus *Corbicula* (Veneroida, Bivalvia). *Journal of Evolutionary Biology*, **15**, 818–829.

Pigneur L-M, Marescaux J, Roland K, Etoundi E, Descy J-P, Van Doninck K (2011a) Phylogeny and androgenesis in the invasive *Corbicula* clams (Bivalvia, Corbiculidae) in Western-Europe. *BMC Evolutionary Biology*, **11**, 147.

Pigneur L-M, Risterucci A-M, Dauchot N, Li X, Van Doninck K (2011b) Development of novel microsatellite markers to identify the different invasive lineages in the *Corbicula* complex and to assess androgenesis. *Molecular Ecology Resources*, **11**, 573–577.

Pigneur L-M, Hedtke S, Etoundi E, Van Doninck K (2012) Androgenesis: a review through the study of the selfish shellfish *Corbicula* spp. *Heredity*, **108**, 581–591.

Posada D (2008) JMODELTEST: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Puillandre N, Dupas D, Dangles O, Zeddam JL, Barbin K, Silvain JF (2008) Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biological Invasions*, **10**, 319–333.

Qiu AD, Shi AJ, Komaru A (2001) Yellow and brown shell color morphs of *Corbicula fluminea* (Bivalvia:Corbiculidae) from Sichuan Province, China, are triploids and tetraploids. *Journal of Shellfish Research*, **20**, 323–328.

Quantum GIS Development Team (2013) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Renard E, Bachmann V, Cariou ML, Moreteau JC (2000) Morphological and molecular differentiation of invasive freshwater species of the genus *Corbicula* (Bivalvia, Corbiculidea) suggest the presence of three taxa in French rivers. *Molecular Ecology*, **9**, 2009–2016.

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology and Evolution*, **22**, 454–464.

Sax DF, Stachowicz JJ, Gaines SD (2005) *Species Invasions: Insights Into Ecology Evolution, and Biogeography*. Sinauer, Sunderland, Massachusetts.

Schön I, Martens K, Van Dijk P (2009) *Lost Sex*. Springer Publications, Berlin.

Shreve SM, Mockford EL, Johnson KP (2011) Elevated genetic diversity of mitochondrial genes in asexual populations of Bark Lice ('Psocoptera': *Echmepteryx hageni*). *Molecular Ecology*, **20**, 4433–4451.

Simon JC, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biological Journal of the Linnean Society*, **79**, 151–163.

Siripattrawan S, Park JK, Foighil DÓ (2000) Two lineages of the introduced Asian freshwater clam *Corbicula* occur in North America. *Journal of Molluscan Studies*, **66**, 423–429.

Skuza L, Łabęcka AM, Domała J (2009) Cytogenetic and morphological characterization of *Corbicula fluminalis* (O.F. Müller, 1774) (Bivalvia: Veneroida: Corbiculidae): Taxonomic status assessment of a freshwater clam. *Folia Biologica – Krakow*, **57**, 177–185.

Sousa R, Antunes C, Guilhermo L (2008) Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. *Annales de Limnologie. International Journal of Limnology*, **44**, 85–94.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, USA*, **97**, 5948–5953.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Vandel A (1928) La parthénogenèse géographique. Contribution à l'étude biologique et cytologique de la parthénogenèse naturelle. *Bulletin Biologie France Belgique*, **62**, 164–281.

Von Rintelen T, Glaubrecht M (2006) Rapid evolution of sessility in an endemic species flock of the freshwater bivalve *Corbicula* from ancient lakes on Sulawesi, Indonesia. *Biology Letters*, **2**, 73–77.

Yamada M, Ishibashi R, Toyoda K, Kawamura K, Komaru A (2014) Phylogeography of the brackish water clam *Corbicula japonica* around the Japanese archipelago inferred from mitochondrial COII gene sequences. *Zoological Sciences*, **31**, 168–179.

Zhang Y-Y, Zhang D-Y, Barrett SCH (2010) Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. *Molecular Ecology*, **19**, 1774–1786.

L.M.P. and K.V.D. designed research. E.E., L.M.P. and J.M. performed research and analysed data. E.E., L.M.P. and K.V.D. wrote the study. D.C.A. and N.Y. contributed samples and new analytical tools.

Data accessibility

COI haplotypes: Genbank Accession KC211240-89 and complete list in online Appendix S1. Sequence alignment file and tree file: Dryad doi: 10.5061/dryad.9cn66.

Microsatellite data (multilocus genotypes): online Appendix S2.

Sampling locations and voucher numbers: Table 1.

Code and input files for DAPC using adegenet: Dryad doi:. 10.5061/dryad.9cn66.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 COI haplotype designation (Code) and localities of Corbicula spp. sequences included in the phylogenetic analysis.

Appendix S2 Microsatellite dataset (10 loci).

Appendix S3 Distribution of BIC in relation to number of clusters considered.