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Genetic evidence of multiple introductions and mixed reproductive strategy in the peregrine earthworm *Pontoscolex corethrurus*

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Abstract

Pontoscolex corethrurus is a well-known invasive earthworm in tropical zone which is believed to have originated from the Guayana Shield in South America and was described as parthenogenetic. A recent phylogenetic study revealed four cryptic species in the *P. corethrurus* complex (L1, L2, L3 and L4), among them L1 was particularly widespread and was proposed as *P. corethrurus sensu stricto*. Here, our aims were to investigate the genetic variation of *P. corethrurus* L1 in its presumed native and introduced ranges and to examine its reproductive strategy. An extensive dataset of 478 cytochrome oxidase I gene (COI) sequences, obtained in specimens sampled all around the world, revealed a weak COI haplotype diversity with one major haplotype (H1) present in 76% of the specimens. Analyses of the genetic variation of 12 L1 populations were done using both nuclear (226 AFLP profiles) and mitochondrial (269 COI sequences) genetic information. The high AFLP genotype diversity at the worldwide scale and the fact that no genotype was shared among populations, allowed to reject the 'super-clone' invasion hypothesis. Moreover, a similar level of mean genetic diversity indices were observed between the introduced and native ranges, a pattern explained by a history of multiple introductions of specimens from different parts of the world. At last, occurrence of identical AFLPs genotypes (i.e. clones) in several population confirmed asexual reproduction, but recombination was also revealed by gametic equilibrium analysis in some populations suggesting that *P. corethrurus* L1 may have a mixed reproductive strategy.

Introduction

Many species have been reported as having extremely widespread distributions. Among these cosmopolitan species, earthworms are poorly represented except for a few taxa. Because of the limited dispersal ability of earthworms it is even striking that out of approximately 5358 described species according to the online Drilobase database, about 120 species are considered peregrine i.e. widely ranging, often owing to human actions (Blakemore 2009; Csuzdi 2012; Hendrix et al. 2008). Over the past centuries, global commerce has favored transport of temperate more than tropical earthworm species but Hendrix et al. (2008) alerted that the emergence of new tropical invasive species is likely. Insights into the invasiveness of earthworms in tropical regions may be gained by examining the specific attributes responsible for the success of the most well-known tropical peregrine earthworm, *Pontoscolex corethrurus* (Müller 1857).

This pan-tropical earthworm is believed to have originated from the Guayana Shield in South America (Righi 1984) which includes Guyana, Suriname, French Guiana, most of Southern Venezuela, as well as parts of Colombia and Brazil. A recent phylogenetic study using two mitochondrial (cytochrome c oxidase I and 16S-rDNA genes) and two nuclear (internal transcribed spacer 2 and 28S-rDNA) markers, in addition to a large-scale multilocus sequence, revealed the high complexity of the genus *Pontoscolex* (Taheri et al. 2018a). The commonly treated as a unique entity *P. corethrurus* was shown to correspond to a complex of four distinct species (i.e., defined as L1, L2, L3 and L4). Two highly divergent genetic lineages (L1 and L4) were already described by Cunha et al. (2014) , using sequences of two mitochondrial genes and Amplified Fragment Length Polymorphism (AFLP) markers, in three populations of the São Miguel Island in the Azores archipelago. Taheri et al. (2018a) revealed that *P. corethrurus* L1 was the most widespread lineage in the world. It was present in the location where this species was first described by Fritz Müller in 1857 (Müller 1857), suggesting that L1

72 corresponds to *P. corethrurus sensu stricto*. Most published studies in the past referencing "*P.*
73 *corethrurus*" have probably dealt with the *P. corethrurus* L1 species, although the risk to have
74 investigated another lineage is not null. *P. corethrurus* was described as an endogeic
75 earthworm, tolerant to a wide range of biotic and abiotic environmental conditions (review in
76 Taheri et al. 2018b), and reproducing parthenogenetically, although the possibility of sexual
77 reproduction could not be ruled out (Dupont et al. 2012; Gates 1973). The mode of
78 parthenogenesis in *P. corethrurus* is not well known. It is generally accepted that
79 parthenogenetic earthworms are automictic (i.e. mode of parthenogenesis that retains meiosis)
80 with a premeiotic doubling of the chromosome number, followed by regular meiosis which
81 restore diploidy in the following egg (Diaz Cosin et al 2011). Because replicated chromosomes
82 pair prior to meiosis I, the offspring are genetically identical to the mother (such as in apomictic
83 parthenogenesis with suppression of meiosis), and heterozygosity is maintained (Simon et al.
84 2003, Lutes 2010). In other types of automictic parthenogenesis, diploidy is restored by
85 duplication or fusion of the female gamete and it rapidly leads to complete homozygosity
86 (Simon et al. 2003).

87 Several peregrine earthworm species are known to have a parthenogenetic reproduction
88 often accompanied by polyploidy, in addition to some other advantageous traits such as high
89 fecundity, small size, resistant cocoons, wide and rapid dispersal, feeding diet plasticity
90 (reviewed in Fernandez et al. 2011a) and resistance to drought (Bartz, Brown, Decaëns &
91 Lapied personal observations) and to waterlogged soils (Bartz personal observation). Two main
92 features allow parthenogenetic species to invade and establish in new habitats faster than
93 sexually reproducing species; (i) the potential increase of the population in numbers per
94 generation is double that of an amphimictic species and (ii) the ability of a single individual to
95 establish a new colony (Cuellar 1977). However, the lack of recombination makes asexual
96 lineages prone to accumulate deleterious mutations that limit their evolutionary potential.

Mixed reproductive systems can combine the advantages of both sexual and asexual reproduction in terms of genetic load reduction, fixation of beneficial mutations, and adaptation to fluctuating environments (Bazin et al. 2014). In earthworms, mixed reproductive systems, combining parthenogenesis and amphimixis, is only known in the peregrine species *Aporrectodea trapezoides.* Although the most abundant clone of *A. trapezoides* was showed to be purely parthenogenetic, two pseudogametic (i.e. parthenogenetic reproduction requiring sperm to stimulate embryogenesis without incorporating the parental DNA) populations and two populations where amphimictic and parthenogenetic forms coexist were discovered (De Sosa et al. 2017; Fernandez et al. 2011b).

Population genetics tools can contribute significantly to the understanding of the reproductive strategies of peregrine species (e.g. Dupont et al. 2007). The elucidation of genetic structure patterns and variation within and among populations in distant locations has also the potential to provide insights into the geographic origin, migration pathways and colonization history (e.g. single *vs* multiple introductions) of peregrine species (Sakai et al. 2001). For instance, molecular markers have proved to be powerful tools for inferring the evolutionary history of *A. trapezoides* (Fernandez et al. 2011a; Fernandez et al. 2016) which has Palearctic origin but a current worldwide distribution. Analyzing two mitochondrial and two nuclear markers, Fernandez et al (2011a) revealed a relatively high clonal diversity in this species whereas one clone was shared by almost one third of the sampled individuals and was widely distributed. Such "super-clones" are thought to be "general-purpose genotypes" (Lynch 1984) which show a broad ecological tolerance resulting from interclonal selection in temporally variable environments (Vorburger et al. 2003).

To date, only two studies focused on *P. corethrurus* population genetics and were of limited geographical scale. Dupont et al (2012) investigated the genetic variability of *P. corethrurus* in its presumed native range using AFLPs. Among the six populations from French

122 Guiana, a higher level of genetic diversity was found in populations originating from the most
123 disturbed sites while a higher number of clones was observed in the other populations. In the
124 São Miguel Island in the Azores archipelago, Cunha et al. (2014) found a higher intra-lineage
125 genetic diversity in the population of *P. corethrurus* L1 living in pineapple greenhouses, most
126 probably because of repeated introductions. Here, our aim was to achieve a large-scale
127 population genetics study of *P. corethrurus* L1, investigating samples coming from its
128 presumed native and introduced ranges, and using cytochrome oxidase I (COI) mitochondrial
129 gene, in addition to AFLPs markers. The genetic variability and the genetic differentiation of
130 the different *P. corethrurus* L1 populations were studied in order to test the following
131 hypothesis: (i) a "super-clone" has invaded most of the introduced areas, (ii) the clonal diversity
132 is lower in the introduced range than in the native one because mutations did not have time to
133 accumulate, and (iii) this species has a mixed reproductive strategy.

2. Methods

2.1. Mitochondrial DNA analysis

We first assessed genetic diversity and its distribution among *P. corethrurus* L1 by re-analyzing the extensive global dataset of cytochrome oxidase I (COI) sequences published in Taheri et al (2018a). Of the 662 COI sequences in Taheri et al (2018a) study, 478 belonged to *P. corethrurus* L1 specimens coming from a total of 49 locations belonging to five biogeographical realms (Genbank accession and information on the samples in supplementary data Table S1). In order to investigate the relationships among the different L1 haplotypes, a haplotype network was constructed based on derived haplotypes in Network v. 5.0.0.1, using median joining calculations (Bandelt et al. 1999).

For the analysis of genetic diversity at the population level, we selected 12 populations with a minimum sampling size of 9 individuals (Table 1, 269 COI sequences); 5 populations came from the presumed native range of the species (i.e. the Guayana Shield) and more precisely from French Guiana [Cayenne (CAY), Mitaraka (MIT), Pararé A (PARA), Pararé B (PARB) and Pararé C (PARC)] and 7 other populations came from the presumed introduced range represented by north and south of Brazil [Caxiuana (CAX)], Belem (BEL), Joinville (JNL), Orleans (ORL)], Mexico [Tlalcotlen (TLC)], Gabon [La Lopé (LOP)] and Thailand [Chachoengsao (CHG)]. For these populations, number of polymorphic sites, number of haplotypes, haplotype (gene) diversity and nucleotide diversity (Pi; Nei 1987) were calculated, using DnaSP v. 5 software (Librado and Rozas 2009). The historical stability of populations was assessed with a test of neutrality (Tajima's D, Tajima 1989) that also served as indicator of population expansion in DnaSP. We quantified the amount of genetic differentiation of population groups using a hierarchical Analysis of Molecular Variance (AMOVA) implemented in Arlequin V3.5 (Excoffier & Lischer, 2010). In this analysis, the COI data set was partitioned at three levels: groups of populations from native *versus* introduced range,

among-populations within groups and among all populations. One thousand random permutations were used to infer the significance of the variance components.

2.2. Nuclear DNA analysis using AFLPs

2.2.1 AFLP procedure

The genetic variation of the 12 previously described populations was also investigated using AFLP markers. DNA extraction was performed as described in Taheri et al (2018a). AFLP analysis of 226 specimens was carried out according to Vos et al. (1995) with a few modifications: approximately 50 ng/µl of purified genomic DNA of each specimen was digested with two digestive enzymes. The first digestion was done in 10µl with Taq1 (20U, New England BioLabs, Ipswich, MA, USA (NEB)), Buffer Taq1 (10X, NEB), BSA (1mg.ml-1, NEB) and incubated for 1h30 at 65°C by thermal cycler (T100TM, BIO-RAD Laboratories Inc., Foster city, CA, USA). The second digestion of the DNA from the last step was done in 15µl with a solution of: EcoRI (20U, NEB), Buffer EcoRI (10X, NEB), BSA (1mg.ml-1, NEB) and incubated for 1h30 at 37°C. We verified DNA digestion quality by gel electrophoresis and negative controls were run at each time. Adapters were ligated using 50pmoles μl^{-1} of double-stranded Taq1 adapter (Taq top 5'-GACGATGAGTCCTGAC and Taq bottom 5'-CGGTCAGGACTCAT, Eurofins Genomics, Germany) and 5pmoles µl-1 of double-stranded EcoRI adapter (Eco top 5'-CTCGTAGACTGCGTACC-3' and Eco bottom 5'-AATTGGTACGCAGTCTAC-3', Eurofins Genomics, Germany), T4 DNA ligase buffer (10X, Promega, Madison, WI, USA), ATP (10mM, NEB), BSA (1mg.ml-1, NEB), T4 DNA ligase (3U, Promega). Samples with a total volume of 50µl (prepared solution + digested DNA from previous step + adjusted water) was then incubated for 3h at 37°C. Digestion-ligation production of 20µl was diluted with 40µl of AE buffer (QIAGEN Sciences, Maryland, USA). Then each dilution was divided in 3 separate samples of 15µl each. Pre-selective PCRs

contained 5pmoles of E01 primer (GACTGCGTACCAATTCA) and 5pmoles of T01 primer (GATGAGTCCTGACCGAA), MgCl2 (25mM, Promega), dNTPs (10mM, InvitrigenTM, Life technologies, Carlsbad, CA, USA), GoTaq buffer (5X, Promega), DNA Taq polymerase (5U, Promega), adjusted with water to have a total volume of 50µl in each sample (added to diluted ligation product). The PCR pre-selective was done by an initial denaturation step at 94°C for 2min, followed by annealing setup of 30 cycles containing; 94°C for 30s, 56°C for 1 min and 72°C for 60s, and finally elongation setup of 72°C for 10 minutes. After this setup, the three sub samples were reassembled in one, and then 1µl of each sample was diluted in 19µl of AE buffer (QIAGEN). Selective PCR reactions contained 5µl of pre-amplified DNA with 15µl of a solution containing MgCl2 (25mM, Promega), dNTPs (10mM, Invitrigen), GoTaq buffer (5X, Promega), 5pmoles.µl-1 T32 primer (5'-GATGAGTCCTGACCGAAAC-3') or 5pmoles.µl-1 T38 primer (5'-GATGAGTCCTGACCGAAACT-3'), DNA Taq polymerase (5U, Promega) and one primer combination E32-FAM was used (5'-GACTGCGTACCAATTCAA-3'). A touchdown thermal cycling (PTC-100) started with denaturing setup of 94°C for 2 min, following by 9 cycles; 94°C for 30s, 65°C for 30s with 1°C diminution per cycle, 72°C for 60s, following by 26 cycles containing 94°C for 30s, 56°C and 72°C during 1min and finally 72°C for 10 minutes. After each setup DNA solutions were centrifuged by ROTANTA 460R (Hettich Lab Technology, Tuttlingen, Germany). Amplified products were mixed with formamide (Hi_DiTM, Applied Biosystems, Foster city, CA, USA (AB) and a Genescan^{TM}-500LIZ^{TM} (AB) size standard (9.5µl of formamide and 0.5 µl of GenescanTM-500LIZ^{TM} for 2µl of amplified product). Fragments were separated on an ABI PRISM^{TM} 3130 Genetic Analyzer (platform INSERM, Henri Mondor hospital, Créteil). Raw data were visualized, and the fragments manually scored using Genemapper V5 (Applied Biosystem) software. Processed data were exported as presence/absence matrix.

209 Genetic markers, including AFLPs, can be prone to genotyping errors with various
210 potential sources (Bonin et al. 2004). Therefore, we estimated the genotyping error rate of our
211 dataset by re-genotyping and blind scoring of 54 randomly chosen individuals. The error rate
212 was calculated by mismatch error rate based on the formula proposed by Bonin et al. (2004),
213 multiplied by the total number of markers.
214
215 *2.2.2 Genetic diversity analysis*
216 Genetic diversity statistics, including number of different genotypes by considering the
217 'error rate' calculated from replicates (i.e. 1.16), genotype diversity, gene diversity, proportion
218 of variable markers and the frequency down-weighed marker value (DW, Schonswetter et al.
219 2005) that indicates the rarity of each marker within the dataset were calculated using AFLPdat
220 program (Ehrich 2006). Genotype and gene diversity were calculated based on Nei's formula
221 (Nei 1987). Similar genotypes within locations were removed and further analyses on *P.*
222 *corethrurus* L1 were carried out on a dataset without clones.
223
224 *2.2.3 Multilocus gametic disequilibrium*
225 In order to evaluate the evidence for recombination, measures of multilocus gametic
226 disequilibrium were calculated and tested for significance with 500 randomizations in
227 Multilocus software (http://www.bio.ic.ac.uk/evolve/software/multilocus/), using 3 different
228 methods. First, we performed a character compatibility analysis. In a data set with a bi-allelic
229 marker with presence or absence (1 or 0) at each locus such as AFLPs, the presence of all four
230 possible combinations of alleles at two different loci (e.g. 0/0, 1/0, 0/1, 1/1) is an indirect signal
231 of recombination. In contrast, a hierarchical data structure (e.g. 0/0, 0/0, 1/1, 1/1) fits to a model
232 of mutations that are vertically transmitted and accumulated within a clonal lineage. The
233 proportion of compatible pairs of loci was thus computed to probe the predominant mating

system in the populations. Moreover, the index of association (I_A) and an alternative measure of index of association that is less sensitive to the number of loci ($\bar{r}_d$, Agapow and Burt 2001) were computed. We further tested for gametic disequilibrium based on the distribution of allelic mismatches between pairs of genotypes over all loci using an exact test implemented in Arlequin. To adjust for multiple comparisons, the SGoF method (Carvajal-Rodriguez et al. 2009) as implemented in the software Myriad (http://myriads.webs.uvigo.es/MyriadsReadme.htm) was applied.

2.2.4 Genetic structure among populations

Analysis of molecular variance (AMOVA) was conducted in Arlequin V3.5 in the same way as for the COI dataset. In addition, an unbiased estimate of differentiation among populations, $\theta^{(II)}$ was obtained using the Bayesian method proposed by Holsinger et al. (2002) and implemented in the software Hickory v1.1. The data were run with the default parameters using the f-free model.

To illustrate the relationships among populations, a split network was constructed using the software Splitstree version 4.1.4.6 (Huson and Bryant 2006) on AFLP profiles. We used the distance-based Neighbor-Net (N-net) method for construction of networks. The N-net provides good visualization of the data when it presents complex evolutionary steps or reticulate relationship among genotypes (Huson and Bryant 2006). The networks were constructed based on Nei's distance (GD) matrix between populations calculated with a Bayesian method using AFLP-Surv version 1.0 (Vekemans et al. 2002) with non-uniform distribution by assuming deviation from Hardy-Weinberg equilibrium; F_{is} values were estimated by Hickory software using the full model (f = 0.033, Holsinger et al. 2002). Analyses were done with 1000 permutations and 1000 bootstrap values.

3. Results

3.1 COI genetic variation

An extensive global dataset of COI sequences obtained in L1 specimens was used to assess the distribution of mitochondrial genetic diversity among populations. Thirteen COI haplotypes were inferred from 478 sequences within 49 locations. COI haplotype network is shown in Figure 1. Haplotype one was shared by most individuals i.e., 364 out of 479, followed by haplotype two with 64 individuals. Five haplotypes (i.e., H3, H7, H8, H9, and H10) were exclusively found in the presumed native range while six haplotypes were found exclusively in the presumed introduced range (H4, H5, H6, H11, H12 and H13). In 5 populations (PARA, PARB, PARC, ORL and LOP), no polymorphic site was observed. In the native range, the highest values of COI genetic diversity indices were obtained in the MIT population (Table 1) with 10 polymorphic sites and a haplotype diversity of 0.693. In the introduced range, the highest COI genetic diversity was observed in CAX (11 polymorphic sites, haplotype diversity of 0.561). No difference of mean haplotypic diversity was observed between the native (mean gene diversity = 0.24, SD = 0.46) and the introduced (mean gene diversity = 0.26, SD = 0.32) ranges (2-tailed test, $t = -0.07$, $p = 0.943$).

The null hypothesis of neutrality was rejected due to significant negative value in the BEL population indicating a recent population expansion in this location. Positive and significant Tajima's D tests were obtained in the CAY and MIT populations indicating population sub-structure in these sites. AMOVA analysis showed that 64 % of the genetic variation was partitioned among all populations, 25 % was attributed to differences among populations within groups, and 11 % of the variation was due to differences between native and introduced groups (Table 2). The genetic differentiation among the native and introduced

283 groups (Φ_{CT}) was not significant while the genetic differentiation among all populations was
284 important (Φ_{ST} = 0.361, P<0.001).

285

286 **3.2 AFLPs genetic variation**

287 A total of 394 AFLPs loci were scored and among them 318 were polymorphic. The
288 difference between the number of individuals analyzed using AFLPs and the number of
289 different AFLP genotypes in the dataset indicates the number of clones in each population. High
290 number of clones (85%) were found in LOP location (Gabon) with 17 similar genotypes out of
291 20 individuals (Table 1). Important proportion of clones was also observed in CHG (Thailand)
292 with 10 similar genotypes out of 35 individuals (29%) and in CAX (Brazil) with 4 similar
293 genotypes out of 18 individuals (22%). In five populations i.e., CAY, PARC, BEL, JNL, and
294 TLC, no clones were found. The highest rates of rare alleles were observed in CAY, MIT, CAX
295 and LOP with DW values of 1804, 830.7, 708.9 and 692.7, respectively. The smallest DW
296 values were found in JNL and PARA with 354.1 and 362.2 values respectively.

297 Further analyses were done on data set without clones, reducing the dataset from 226
298 profiles to 185. The highest gene diversity was found in JNL, CAY and PARC (0.168, 0.165
299 and 0.163 respectively) while the lowest values were obtained in LOP, PARA, PARB and ORL
300 (0.012, 0.052, 0.083 and 0.087 respectively, Table 1). No difference of mean gene diversity
301 was observed between the native (mean gene diversity = 0.12, SD = 0.01) and the introduced
302 (mean gene diversity = 0.11, SD = 0.01) ranges (2-tailed test, t = 0.269, p = 0.793).

303 All indices of multilocus gametic disequilibrium were congruent (Table 1). All
304 populations showed significant gametic disequilibrium and character compatibility (except
305 LOP but the analysis couldn't be correctly carried out in this population because an extremely
306 low polymorphism) as expected in an asexually reproducing parthenogenetic species. However,
307 the values of these indices were highly different among populations. Thus, the TLC population

showed a particularly high value of index of association ($I_A = 42.52$) while the lowest significant value was obtained in JNL ($I_A = 2.23$).

The genetic differentiation Φ_{ST} measured by AMOVA analysis was 0.364; 64 % of the variation was partitioned within populations, 31 % was attributed to differences among populations within groups, and 5 % of the variation was due to differences between the native and introduced groups. All three hierarchical levels were significant with $P<0.05$ (Table 2) but the genetic differentiation among groups was low ($\Phi_{CT} = 0.052$, $P = 0.001$). A similar level of genetic differentiation among populations was obtained using the f-free model with Hickory software: $\theta^{(II)} = 0.342$. Relationships between populations are illustrated by the Neighbor-Net results obtained from Nei's genetic distances (Fig. 2, supplementary data Table S2): LOP and CAY were the most differentiated to each other and to the other locations while the sample from Asia (CHG) was genetically close to the populations from South America. Moreover, all populations from the native range were grouped together.

Discussion

The "super-clone" hypothesis

Despite the very extensive geographical range of *P. corethrurus* in the tropical and sub-tropical zone, and the limited potential for natural dispersal of earthworms (Costa et al., 2013), our sampling of Africa, Asia, South America and Central America and various islands revealed a low global mitochondrial haplotypic diversity with a total of 13 COI haplotypes and, among them, three major haplotypes (H1, H2 and H3). These haplotypes were not very divergent: H1 was only separated from H2 by four mutations and by six mutations from H3. The haplotype H1 was observed in all continents and was in high frequency in the introduced populations. All populations from the presumed native range showed the H1 haplotype and it was the sole haplotype in three of them (PARA, PARB and PARC). The haplotype H2 was also frequent in both native and introduced ranges but was absent from Africa while the haplotype H3 was only observed in two populations (MIT and CAX). These results could suggest that H1 and H2 correspond to "super-clones" such as those observed in aphids. For instance, population studies of the parthenogenetic aphid *Myzus persicae* revealed that some genotypes are widespread both in time and space (Vorburger et al. 2003). It was proposed that these predominant genotypes possess general-purpose genotypes, i.e. genotypes with broad ecological tolerances as a result of interclonal selection in temporally variable environments (Lynch 1984). In the case of *P. corethrurus*, a successful invasive earthworm species that has a pan-tropical distribution, this postulate was appealing and was tested through our population genetic study using AFLP markers. Although clones were observed in several populations and sometimes in high frequency, no AFLP profile was shared among populations. For instance, the LOP population that had the highest number of clones, had a high DW value, meaning that the AFLP genotypes within this population were highly different from the other genotypes of the total dataset. Overall, AFLP results allow to reject the hypothesis that a "super-clone" was responsible for

347 the success of *P. corethrurus* invasion. The most likely factors that may be suggested to explain
348 the lack of COI haplotype diversity in *P. corethrurus* populations are a slow evolutionary rate
349 of the COI gene (i.e. constraint on the ability of the mitochondrial genome to accumulate
350 mutations) or, more likely, genetic drift associated with historical events, such as recent
351 population bottlenecks or founder effects (e.g. López-Legentil & Turon, 2007). The fourfold
352 higher effective population size for the nuclear genome compared to mitochondrial loci, which
353 results in genetic drift being four times weaker for nuclear loci may explain the discrepancy
354 between the mitochondrial genetic depletion and the relatively high genetic variability of the
355 nuclear loci.

356 ***Genetic variation in introduced versus native range***

357 The number of COI haplotypes was slightly higher in the presumed introduced range
358 per comparison with the presumed native range (9 and 7 respectively), and comparison of mean
359 haplotypic diversity values between populations from the native and introduced ranges showed
360 no significant difference. This is explained by the fact that the COI haplotypic diversity was
361 highly variable among populations. Within the native range, several populations did not show
362 any COI polymorphism while other populations displayed some of the highest values of COI
363 diversity of the dataset (Table 1, Fig 1). Similar patterns of discordant genetic diversity among
364 populations from the native range was observed with the nuclear markers; AFLPs gene diversity
365 was two to three times higher in CAY and PARC populations per comparison with PARA and
366 PARB. The congruence between AFLPs and mitochondrial data suggests that the low COI
367 polymorphism of this species is not due to a marker-specific effect but most probably due to
368 population and species history.

369 Strong genetic drift effects are expected when populations are founded by a small
370 number of colonists (Sakai et al. 2001). Our data for *P. corethrurus* fit this theoretical
371 expectation in the LOP population where an extremely low AFLP genetic diversity was

observed, congruent with the lack of COI polymorphism (Table 1). Except the LOP population, which was probably very recently founded, levels of genetic variation measured from AFLP data in introduced populations were comparable to those found in populations from the presumed native range (Table 1). For instance, the JNL population (Brazil) showed high gene diversity (0.168) comparable to those of CAY (0.165). Moreover, no enhanced genetic differentiation, except for the specific case of LOP, was observed in the introduced range although repeated founder effects are likely to increase genetic divergence among populations (Slatkin 1977). The absence of reduced genetic diversity in most of the introduced range is also certainly a result of *P. corethrurus* rapid population expansion (e.g. Zenger et al. 2003). The signature of population expansion is, for instance, still visible in the population of Belem where a negative and significant Tajima's D value was obtained. Moreover, it is noteworthy that the native range of the species is not known with certainty. In particular, the case of Northern Brazilian sites (CAX and BEL) is tricky. We hypothesized that they correspond to the introduced range because they do not belong to the Guayana Shield but they might also correspond to the native area.

Our results suggest that successive introduction from multiple sources has enhanced genetic diversity in not only some introduced localities but also in the presumed native range. Significant sub-structures were indeed revealed by positive and significant Tajima's D value in two populations of the native range (i.e. CAY and MIT). A previous study has already showed that the suburban environment of Cayenne, where the CAY population was sampled, is prone to constant introduction of individuals (Dupont et al. 2012). In the locality of Mitaraka (MIT population) situated in the Amazonian primary forest where human activity is extremely limited, the explanation could be completely different. In this locality, specimens were collected in five different sub-localities (supplementary data Table S1) separated by a maximum of 3 km. This distance is therefore probably sufficient to detect sub-structure using AFLP markers,

highlighting the low active dispersal of the species. At the opposite, in more disturbed sites, passive dispersal linked to human activities is probably the predominant mode of spread (Dupont et al. 2012). In South America, successive introduction and rapid expansion through passive dispersal could be occurring since humans arrived and dispersed in Amazonia (Cunha et al. 2016).

The mixed reproductive strategy of Pontoscolex corethrurus sensu stricto

P. corethrurus has several life history traits that predispose it to invasiveness (Taheri et al. 2018b). Among them, the ability of a single individual to establish a population is an important characteristic of several invasive species. (Dybdahl and Drown 2011). The important number of clones revealed in some populations by the AFLP analysis highlights the prominence of parthenogenetic reproduction in this species. For instance, in LOP only 15% of the AFLP genotypes were different. The importance of asexual reproduction is also confirmed by the high levels of gametic disequilibrium (Table 1) that were observed in several populations such as TLC (I_A = 42.52; $\bar{r}_d$ = 0.39), CAX (I_A = 22.34; $\bar{r}_d$ = 0.12), PARB (I_A = 18.51; $\bar{r}_d$ = 0.21) and ORL (I_A = 13.37; $\bar{r}_d$ = 0.16).

Although asexual reproduction provides an immediate demographic advantage, it is expected that in the long term, asexual populations deteriorate in fitness due to mutation accumulation or the inability to cope with changing environment (Lynch et al. 1993; Muller 1932). Thus, asexual lineages depend on occasional sexual recombination to add variation to their genomes, purge deleterious mutations, and adapt to changing environments. A mixed strategy of asexual reproduction with low level of sex combines the best of both strategies (Combosch and Vollmer 2013; Hurst and Peck 1996). It has been previously suggested that *P. corethrurus* was capable of such a mixed reproductive strategy (Dupont et al. 2012; Gates 1973;

421 Taheri et al. 2018b) and this hypothesis was tested here using the AFLP genotypes. We showed
422 that low values of gametic disequilibrium estimators in some populations (e.g. $I_A = 2.23$; $\bar{r}_d =$
423 0.01 and $I_A = 2.54$; $\bar{r}_d = 0.01$ in JNL and PARC populations respectively, Table 1) associated
424 to an absence of clones revealed the occurrence of recombination within these populations.
425 Meiotic recombination occurs during amphimictic reproduction and automictic
426 parthenogenesis. Because recombination occurs between sister chromosomes in the premeiotic
427 doubling of chromosome happening before the regular meiosis of the automictic
428 parthenogenesis (i.e. the type of parthenogenesis described in earthworms), gametic
429 disequilibrium is not expected in this case. Although we cannot exclude that another type of
430 automictic parthenogenesis occurs in *P. corethrurus*, our results seem to support the hypothesis
431 previously proposed of a plastic reproduction strategy in *P. corethrurus*.

432

433 **Conclusion**

434 Successive founder effects and demographic bottlenecks are most probably responsible
435 of the low COI genetic diversity revealed in our worldwide scale study. Although mtDNA
436 contain historic genetic mutations and is suitable for resolving taxonomic uncertainties, AFLP
437 markers are more appropriate for inferring contemporaneous genetic patterns. Comparing
438 results obtained using both kind of markers revealed that the founder event in LOP was
439 probably more recent than in the other populations where COI polymorphism was also null
440 (PARA, PARB, PARC and ORL). A major question in invasion biology is how newly founded
441 and subsequently isolated populations overcome the detrimental effects of low genetic
442 diversity, the so-called 'genetic paradox' (Allendorf and Lundquist 2003; Roman and Darling
443 2007). The establishment success of newly founded *P. corethrurus* populations may be due to
444 recurrent introductions from multiple sources, accumulation of new mutations and high level
445 of intrinsic phenotypic plasticity (i.e. Taheri et al. 2018b), in addition to a mixed reproductive

446 strategy. Asexual reproduction improves *P. corethrurus* ability to reach high abundance (e.g.
447 Taheri et al. 2018b) and is a particularly important trait in the first stage of the invasion, when
448 sexual partners are rare. During establishment and expansion of the population, events of sexual
449 reproduction will then promote the genetic diversity. Such a mixed reproductive strategy is a
450 way to purge the genome of deleterious mutations (i.e. no clonal population senescence after
451 many generations), elegantly defining a mode of escape to Muller's ratchet (Muller 1932).

452

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591

592 **Figure legends**

593

594 **Fig. 1:** Locations of the *P. corethrurus* L1 specimens used in the study and COI haplotype
595 network based on 478 sequences in 49 locations; with the name of the countries where samples
596 were collected in bold (for details about each site, see Table S1). Each colour represents a
597 different haplotype and pie charts in the map display the relative frequencies for a given locality
598 of each of the haplotypes. The white small circles indicate the presence of islands that could
599 not be visualized otherwise. The location of the 12 populations used in the population genetics
600 analysis are indicated by their code in the map. Codes of populations from the native range (i.e.
601 Guayana Shield) are underlined. In the network, connecting lines show mutational pathways
602 among haplotypes. Only one mutation separates the haplotypes, unless the number of mutation
603 is indicated.

604

605 **Fig. 2:** Neighbor-Net networks done on AFLP profiles with Splitstree based on Nei's genetic
606 distance matrix for 12 populations of *P. corethrurus* L1. Populations from the presumed native
607 range are in black while populations from the introduced range are in blue. The country of
608 origin of each population is indicated by the following codes : GUY – French Guiana, BRA –
609 Brazil, MEX – Mexico, GAB – Gabon, THA – Thailand.

610

611

Table 1: Overall genetic variability in 12 *P. corethrurus* L1 populations, based on 318 polymorphic AFLP markers and 633 bp fragment of the COI gene.

		Native range					Introduced range						
	Sites	CAY	MIT	PARA	PARB	PARC	CAX	BEL	JNL	ORL	TLC	LOP	CHG
AFLP	N_{AFLP}[a]	17	20	19	8	13	18	21	22	24	9	20	35
	VM%[b]	52.284	31.472	21.574	22.335	46.701	48.223	41.878	51.523	22.589	28.173	2.030	34.518
	DW (from means)[b]	1804	830.7	362.2	406.1	516.6	708.9	570.7	354.1	426.1	467.2	692.7	471.1
	NoH[c]	17	17	16	7	13	14	21	22	21	9	3	25
	Genotype diversity[d]	1	0.979	0.965	0.964	1	0.935	1	1	0.978	1	0.416	0.973
	Gene diversity[d]	0.165	0.112	0.052	0.087	0.163	0.138	0.132	0.168	0.083	0.113	0 .012	0 .110
	PCLP[d]	**0.91**	**0.97**	**0.99**	**1.00**	**0.94**	**0.96**	**0.93**	**0.88**	**0.98**	**0.99**	1.00	**0.96**
	I_A[d]	**6.78**	**6.82**	**11.91**	**18.51**	**2.54**	**22.34**	**12.77**	**2.23**	**13.37**	**42.52**	1.29	**11.98**
	$\bar{r}_d$[d]	**0.03**	**0.06**	**0.15**	**0.21**	**0.01**	**0.12**	**0.08**	**0.01**	**0.16**	**0.39**	0.21	**0.09**
	LD (%)[d]	8.66	14.78	8.32	9.67	8.49	16.04	19.84	4.06	34.17	19.64	0.00	20.12
COI	N_{COI}[a]	12	64	16	16	15	12	15	17	13	9	20	60
	NPS[e]	6	10	0	0	0	11	6	5	0	6	0	1
	Number of haplotype	2	4	1	1	1	4	2	2	1	2	1	2
	Haplotype diversity	0.530	0.693	0	0	0	0.561	0.133	0.221	0	0.500	0	0.381
	P_i (per site)[f]	0.00503	0.00671	0	0	0	0.00426	0.00126	0.00174	0	0.00474	0	0.00060
	Tajima's D[g]	2.294*	2.762**	-	-	-	-1.080	-1.983*	-0.744	-	1.566	-	0.999

[a]The number of individuals that have been genotyped using AFLPs or sequenced for COI are given by N_{AFLP} and N_{COI} respectively. [b] Percentage of variable markers (VM%) and Frequency-down-weighted marker values (DW) were computed using the AFLP dataset with clones. [c] The number of different AFLP genotypes in the population is given by NoH. [d] For the computation of genotype diversity, gene diversity, proportion of compatible locus pairs (PCPL), indexes of association (I_A and $\bar{r}_d$) and gametic disequilibrium (LD), clones were removed from the AFLP dataset; significant values (P<0,002) are shown in bold. [e] NPS correspond to the number of COI polymorphic sites. [f] P_i correspond to the COI nucleotide diversity.[g] For Tajima's D, significance is given by: * P<0.05, **P<0.01.

Table 2: Analysis of molecular variance (AMOVA) of COI and AFLP (in italics) genotypes for 5 populations from the presumed native range and 7 populations from the introduced range of *P. corethrurus*.

	Variance components	**% of total variation**	**P-value**	**Φ statistics**
Among groups (native *versus* introduced)	0.142	11.22	0.194	$\Phi_{CT} = 0.112$
	1.49	*5.16*	*0.001*	$\Phi_{CT} = 0.052$
Among populations within groups	0.316	24.91	0.000	$\Phi_{SC} = 0.281$
	9.08	*31.26*	*0.000*	$\Phi_{SC} = 0.330$
Among all populations	0.809	63.87	0.000	$\Phi_{ST} = 0.361$
	18.47	*63.58*	*0.000*	$\Phi_{ST} = 0.364$

Figure1:

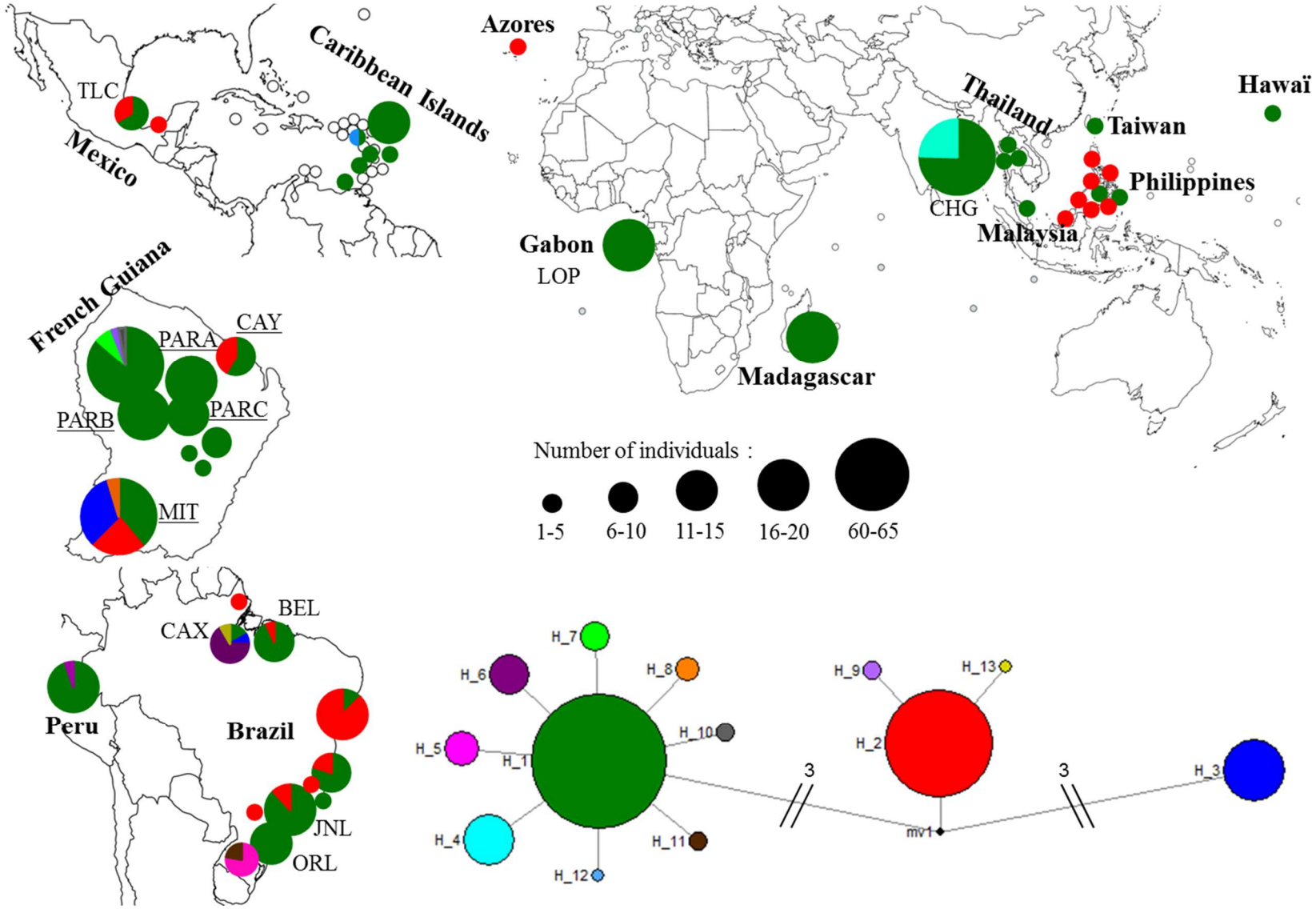
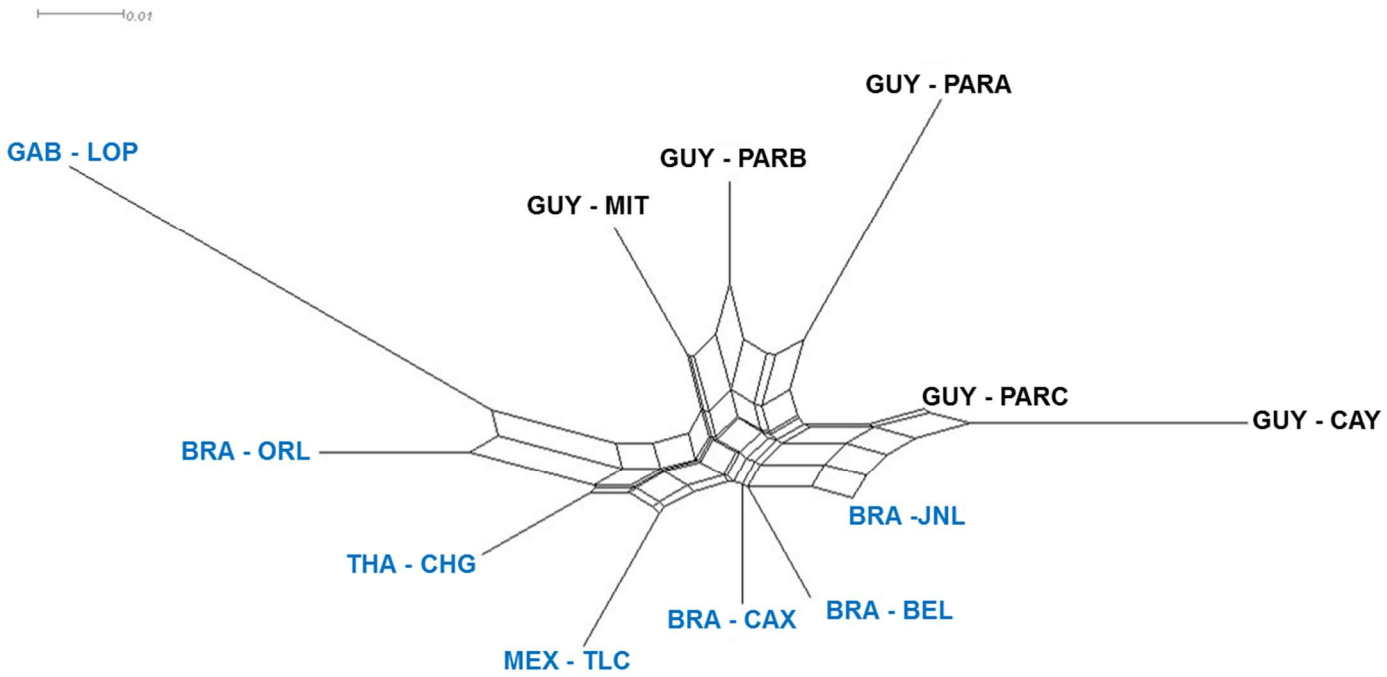

Azores
Caribbean Islands
TLC
Mexico
Thailand
Hawaï
Taiwan
Philippines
CHG
Malaysia
Gabon
LOP
Madagascar
French Guiana
CAY
PARA
PARB
PARC
MIT
Number of individuals :
1-5
6-10
11-15
16-20
60-65
BEL
CAX
Peru
Brazil
JNL
ORL
H_7
H_6
H_8
H_10
H_5
H_1
H_4
H_11
H_12
H_9
H_13
H_2
H_3
3
3
mv1

Figure 2


0.01
GUY - PARA
GAB - LOP
GUY - PARB
GUY - MIT
GUY - PARC
GUY - CAY
BRA - ORL
BRA -JNL
THA - CHG
BRA - CAX
BRA - BEL
MEX - TLC