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ORIGINAL PAPER

Repeated reduction in parasite diversity in invasive populations of *Xenopus laevis*: a global experiment in enemy release

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Abstract The introduction of species to multiple continents creates natural experiments suited to the evaluation of ecological hypotheses. For the *Enemy Release Hypothesis* (ERH), which postulates that the success of invasive populations hinges upon release from the effects of their natural enemies, assessments of parasite loss during invasion across independent geographical replicates are scarce. This study is the first to test the ERH for a globally invasive amphibian, *Xenopus laevis*, a successful invader on four continents with a well-described parasite fauna. In this study, the metazoan parasite communities of *X. laevis* from 20 invasive and 27 native sites in five countries and three continents were compared. An overall pattern of reduced parasite diversity in invasive *X. laevis* was not yet countered by acquisition of novel parasites. Invasive *X. laevis* harboured impoverished parasite communities that were distinct from those of native *X. laevis* from undisturbed habitats. Conversely, parasite communities from native *X. laevis* from disturbed habitats were similar to those from the invasive range. Accompanying parasites were common in the native range and included both generalists with indirect and specialists with direct life cycles. Our findings emphasise that parasite loss is characteristic of the invasion process of *X. laevis* and possibly contributes to its success as a global invader. The ERH is supported in terms of metazoan parasites as natural enemies, irrespective of the geographical origin, climatic conditions and invasion history of the host populations. This study also draws attention to parasites that co-invade with their hosts as invaders in their own right.

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Introduction

In the Anthropocene, the world's biota is experiencing modification at unprecedented rates (Bar-On et al. 2018; Ceballos et al. 2017). The increasing magnitude of human-mediated animal, plant and pathogen translocation to novel environments has led to growing interest in the discipline of invasion science (Richardson and Ricciardi 2013). Since the inception of modern invasion ecology following Charles Elton's seminal book (1958), several hypotheses have been developed to explain the disproportionate success of invasive species (Jeschke 2014). Opportunely, the natural experiment arising from the global distribution of certain invasive species, such as rats and Australian acacias, has presented conservationists with a unique opportunity to test evolutionary and ecological hypotheses across independent geographical replicates (Morand et al. 2015; Richardson et al. 2011).

Oft cited among the hypotheses in invasion ecology is the *Enemy Release Hypothesis* (ERH), which ascribes the increased fitness of invasive species to a release from the effects of natural enemies, such as co-evolved parasites, during the process of co-invasion (Keane and Crawley 2002; Torchin and Mitchell 2004). The ERH is empirically supported across a wide range of invasive taxa, particularly in terms of parasite loss, which holds true for the majority of invasive species in their non-native ranges (Heger and Jeschke 2014; Lui and Stiling 2006; Torchin et al. 2003; Torchin and Mitchell 2004). However, the validity of the ERH as a unifying theory in invasion ecology suffers, among other concerns, from a lack of studies conducted on a global scale (Blackburn and Ewen 2017; Lester et al. 2015; Prior and Hellmann 2015; Prior et al. 2015; Schultheis et al. 2015). For example, in invasive amphibians, the only two studies which have investigated parasite loss to date were conducted on two species of tree frog, *Eleutherodactylus coqui* and *Osteopilus septentrionalis*, that are both native in and invasive to Central America (Marr et al. 2008; Ortega et al. 2015). In fact, comparatively few studies have investigated the ERH in globally distributed animals from any class, notable exceptions being the repeated parasite loss demonstrated in both the European house sparrow and the European green crab (Marzal et al. 2011; Torchin et al. 2001).

In the light of this, the globally invasive frog, *Xenopus laevis* Daudin, 1802 (Anura: Pipidae), with its multiple invasive populations on four continents, is eminently suited to test the ERH on a global scale (Measey et al. 2012). Its spread from southern Africa to other continents was initiated in the early 1930s, when it was widely adopted as a biological pregnancy assay and later as a model animal for research and education (Gurdon and Hopwood 2000; Shapiro and Zwarenstein 1934; van Sittert and Measey 2016). The global range expansion of *X. laevis* has not been halted since, with climate change and an inherent adaptability to novel environments boosting their invasive potential in many regions (Ihlow et al. 2016; Rödder et al. 2017; van Sittert and Measey 2016). Furthermore, as a domestic exotic in southern Africa, the invasiveness of *X. laevis* is not just confined to populations outside of its native range (Measey and Davies 2011; Measey et al. 2017). Since the onset of trade in this frog, *X. laevis* has been translocated in the native range in large numbers (van Sittert and Measey 2016), further expanding its range without direct human mediation by moving overland or via farm dams and artificial waterways (de Villiers and Measey 2017; Fouquet and Measey 2006; Measey 2004, 2016; Measey et al. 2012).

Moreover, the fact that its parasite fauna has been well studied, makes *X. laevis* the ideal model to test the ERH in terms of parasites as natural enemies. Since the description of its first associated parasite (Cohn 1906), over 20 metazoan parasite species have been associated with it in its native range (Avery 1971; Beverley-Burton 1963; Cosgrove and Jared 1974; Crous and du Preez 1997; Dick 1959; du Preez et al. 1996; Elkan and Murray 1952; Ferguson and Appleton 1988a, b; Fischthal and Thomas 1968; Harris and Tinsley 1987; Héritier et al. 2015; Jackson and Tinsley 1995a, b, 1997, 1998, 2001; King and van As 1992, 1997; 2000; Kruger and du Preez 2015; Macnae et al. 1973; Manter and Pritchard 1964; Moravec and Cosgrove 1982; Nigrelli and Maraventano 1944; Pritchard 1964; Prudhoe and Bray 1982; Southwell and Kirshner 1937; Svitin et al. 2018; Theunissen et al. 2014Thurston 1967; Thurston 1970; Tinsley and Jackson 1995, 1998; Tinsley and Sweeting 1974; van der Lande and Tinsley 1976; Vercammen-Grandjean

1960; Wade 1981, 1982). Likewise, the parasitic fauna of the established invasive populations, although not as well studied as in the native range, nonetheless have been surveyed on three continents. Hitherto, two full parasitological surveys have been conducted in California and Chile (Castillo et al. 2017; Kuperman et al. 2004). A third investigation into the parasites of *X. laevis* in Portugal remains unpublished (Rodrigues 2014).

Few host-parasite systems lend themselves to inform a global perspective on the fate of co-evolved parasites during the process of invasion. This study aimed to address this gap by testing the ERH on geographical replicates of metazoan parasite communities of a globally distributed amphibian. To this end, this study included full parasitological surveys of *X. laevis* across the whole of the native range and in the invasive population in Western France to enhance the existing data available from the invasive populations in Chile, California and Portugal (Castillo et al. 2017; Kuperman et al. 2004; Rodrigues 2014). Specifically, we aimed to address the questions of (1) whether invasive *X. laevis* populations exhibited loss of metazoan parasites, both in terms of species richness and infection levels, (2) what factors caused parasites to accompany *X. laevis* to the invasive range, (3) whether these accompanying parasites attained as high infection levels in the invasive range as in the native range and (4) how the metazoan parasite community compositional dissimilarities correlate with the geographical origin of *X. laevis* populations.

Methods

Host and parasite collection in France

In June 2017, a total of 43 adult *X. laevis*, 17 males and 26 females, were collected by the eradication programme in baited funnel traps from six sites across the invasive range of *X. laevis* in Western France (Fig. 1b). After five weeks of experimental breeding, where the live animals were kept separately by collection site, the frozen corpses of the frogs were made available for parasitological analysis. Although laboratory conditions can be stressful for hosts, the metazoan parasites of *X. laevis* that have been examined in this regard all have the ability to survive even longer periods of laboratory maintenance (Elkan and Murray 1952; Jackson and Tinsley 1988; Thurston 1970; Tinsley 1972; Tinsley 1996; Tinsley and Sweeting 1974; Tinsley and Wynne Owen 1979), meriting the inclusion of this survey in this comparative study. All frogs were screened for parasites approximately 2 weeks *post mortem*. Before dissection, the thawed frogs were measured and the epidermis, lateral line, eyes, buccal cavity, Eustachian tubules and nostrils were examined for external parasites. Thereafter, the body was slit open longitudinally and the alimentary tract, kidney, excretory bladder, gall bladder with bile ducts, liver, lungs, heart and reproductive organs were removed and examined separately for internal parasites using a stereomicroscope. Helminths were collected, counted, fixed in warm ethanol and stored in 70% ethanol.

Parasite identification in the French population

Since freezing can be damaging to the internal structures of soft-bodied parasites, traditional morphological methods could not be used to identify the recovered parasites. Rather, the specimens were tentatively identified to morphospecies based upon site of infection and general body structure. These identities were confirmed through molecular techniques with the DNA barcoding gene, *COI*, by using two specimens per morphospecies as representative of the whole population. DNA was extracted from two specimens per morphospecies (one from a core site and one from a peripheral site) using the PCRBIO Rapid Extract PCR Kit (PCR Biosystems Ltd., London, United Kingdom). The *COI* amplicons were obtained using the forward primer 'L-CO1p' (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and the reverse primer 'H-Cox1p2' (5′-TAAAGAAAGAACATAATGAAAATG-3′) (Littlewood et al. 1997). The thermocycling profile proposed by Verneau et al. (2009) was implemented. Sequences were obtained on an ABI3500XL sequencer using BigDye® Terminator v3.1 Cycle Sequencing. Specifically, DNA products were sequenced in both directions using the PCR primer pair, yielding a sequence of approximately 450 base pairs. Sequences were assembled, edited using Geneious 9.0 software and compared with existing *COI* sequences on GenBank to confirm species identity (Héritier et al. 2015; Waeschenbach et al. 2017). Sequences were submitted to the GenBank database under the accession numbers MK342937–40.

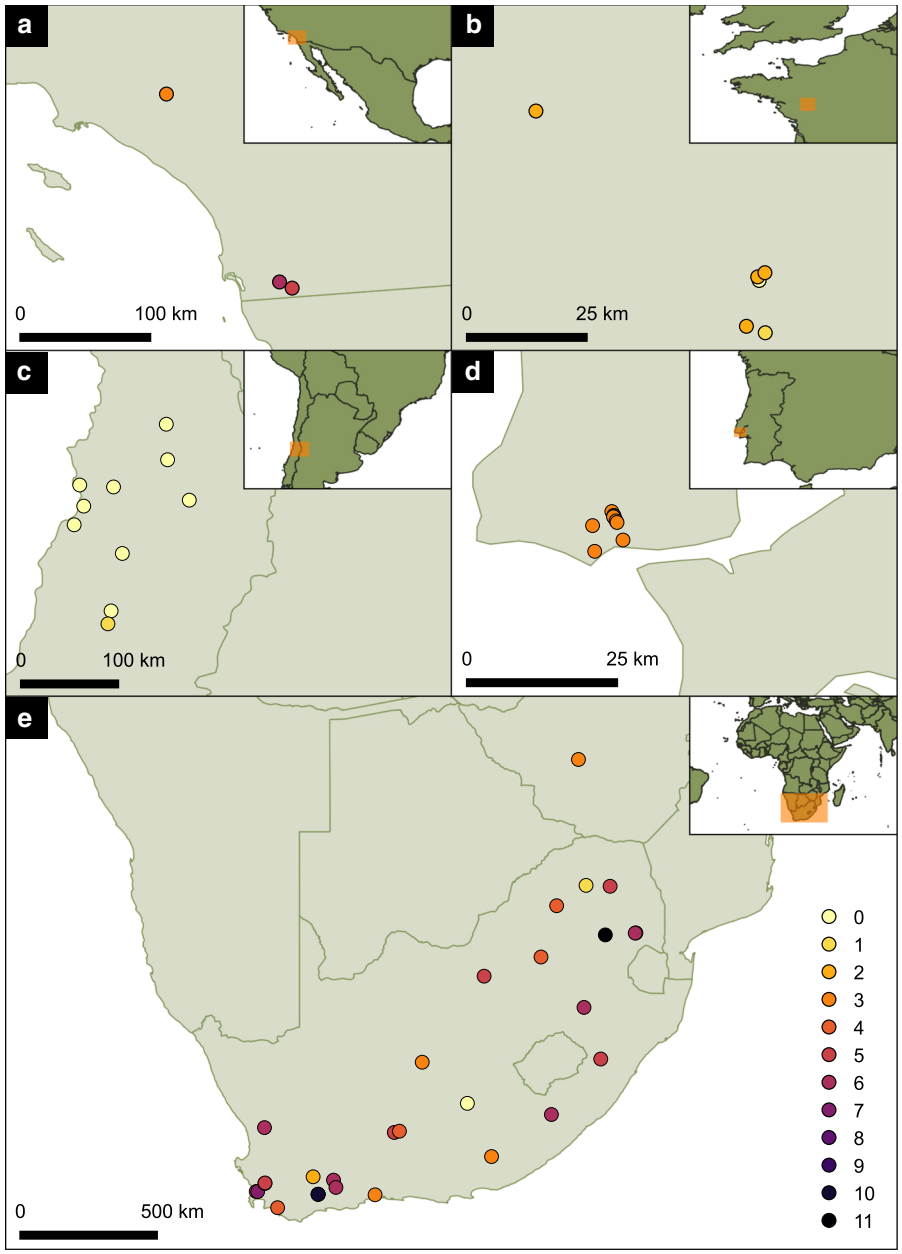

Fig. 1 Recovered parasite species richness at 47 sites where full parasitological surveys of *Xenopus laevis* were conducted. This includes data from three previously published works from the invasive range, namely three sites in California, North America (Kuperman et al. 2004) (**a**), nine sites in Chile, South America (Castillo et al. 2017) (**b**) and eight concatenated sites in Oeiras, Portugal, Europe (Rodrigues 2014) (**d**). Newly generated data from 27 sites from across the native range in Southern Africa (**e**) and six sites from Western France, Europe (**c**) are also included. The parasite species richness at a site is indicated by its position on the colour scale, ranging from zero (light yellow) to eleven (black). All maps are displayed according to the Mercator projection

Host and parasite collection in South Africa

A total of 172 adult *X. laevis* were collected from March 2017 to February 2018 in baited funnel traps from 27 sites across the native range in Southern Africa, specifically including collection localities from the known distribution of all the mitochondrial

lineages (de Busschere et al. 2016; Furman et al. 2015) (Fig. 1e). All frogs were sacrificed within a month of collection according to internationally accepted standard operating procedures. Anaesthesia in 6% ethyl-3-aminobenzoate methanesulfonate (MS222) (Sigma-Aldrich Co., St. Louis, Missouri, USA) was followed by euthanasia through cutting the spine and destroying the brain. Subsequent host measurements and parasite screening and collection were performed immediately *post mortem* using the same methods as for the hosts and parasites in France. All parasites were preserved in 70% ethanol.

Parasite identification in South Africa

Morphological species identification was sufficient for the majority of the parasites recovered in South Africa, since the parasites of *X. laevis* are well-described in the native range. In addition, the parasites were removed from the hosts whilst still alive and could be optimally fixed for morphological studies. For this reason, molecular techniques were only employed for the identification of some of the larval nematodes and digenean metacercariae. Extraction and sequencing followed exactly the same procedure as for the parasites from France. The larval nematodes were distinguished from one another and species on GenBank with the help of the *COI* gene, for which amplicons were obtained with the forward primer 'LCO1490' (5′-GGTCAACAAATCATAAAGATATTGG-3′) and the reverse primer 'HCO2198' (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) and the following thermocycling profile: initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and elongation at 72 °C for 2 min, terminated by one cycle of elongation at 72 °C for 7 min. The digenean metacercariae were told apart by the complete internal transcribed spacer (*ITS*) gene region, which was amplified by the forward and reverse primers 'D1' (5′-AGGAATTCCTGGTAAGTGCAAG-3′) and 'D2' (5′-CGTTACTGAGGGAATCCTGGT-3′) (Galazzo et al. 2002) with the following thermocycling profile: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and elongation at 72 °C for 2 min, terminated by one cycle of elongation at 72 °C for 5 min.

Parasitological surveys from the literature and clarification of parasitological parameters

Three full parasitological surveys of invasive *X. laevis*, conducted in California (Kuperman et al. 2004), Chile (Castillo et al. 2017) and Portugal (Rodrigues 2014), were included for comparison (Fig. 1a, c, d). Comparable locality-specific data does not exist for the native range, therefore only the results of our parasitological survey were included, along with our results from France. Results from sites in the same aquatic systems (such as dams on the same property connected by one river) were concatenated. In total, 47 sites were included in the comparison (refer to the table in Appendix S1 in the Supporting Information for detailed information on all sites). Only metazoan parasites, including mites, leeches and helminths, were included in the analyses, owing to the fact that freezing of hosts, such as in France, will make it impossible to recover protozoan parasites. For each site, parasite species richness (number of parasites in all hosts from one site) and the per-site prevalence of each parasite species (percentage of hosts from one site infected with a given parasite species) were calculated, sensu Bush et al. (1997). Other parasitological parameters, namely mean intensity and mean abundance (Bush et al. 1997) could not be calculated from the information available in all the source publications and were only calculated for the newly generated data from France. For further analyses, summed parasite prevalence (the sum of the prevalences of each of the parasite species at a site) and mean parasite prevalence (prevalences averaged across all parasite species present at a site) were also calculated, following Torchin et al. (2003). Summed prevalence gives an indication of the potential impact of the parasitism on the host population, as it is a measure of the unweighted cumulative extent of the parasitism hosts experience at a site (Torchin et al. 2003). Finally, for each of the parasite species present in the African hosts, frequency of occurrence across South Africa (percentage of sites where the parasite occurs) and mean species prevalence (per-site prevalence averaged across all sites in South Africa, excluding those with zero prevalence) were calculated.

Statistical analyses

Host sample size often strongly influences the number of parasite species collected at a site (Engemann et al. 2015; Luque and Poulin 2007). To determine whether the unbalanced sampling effort at the 47 sites, ranging from one individual in Zimbabwe to 132 in Dulzura Creek, California, could potentially confound our results by correlating with the recovered species richness at these sites, the Spearman's rank correlation coefficient was calculated.

To resolve whether hosts from the invasive and native ranges experienced similar levels of parasitism, the 27 native localities were compared with the 20 invasive localities in terms of parasite species richness and the summed and mean parasite prevalence with the Wilcoxon–Mann–Whitney test.

The effect of several factors on the likelihood of parasites to follow *X. laevis* out of Africa was evaluated separately. Only the parasites from the native range which could be identified to the point where sufficient life history information could be obtained, were included for analysis. The effect of parasite taxonomical class (Acari, Cestoda, Digenea, Hirudinea, Monogenea, or Nematoda), type of life cycle (direct, or indirect), life stage when present in *X. laevis* host (all stages, adult, cyst, or larval) and presence in the Western Cape province, where most of the invasive *X. laevis* originated from (de Busschere et al. 2016), were assessed though the Fisher's exact test of independence. The Wilcoxon–Mann–Whitney test was employed to test the effect of host specificity (high to low: that is, recorded from a single host species, a single host genus, or multiple host genera), mean species prevalence across sites, excluding zero prevalence sites, and frequency of occurrence in South Africa on the presence or absence of a parasite in invasive populations.

We specifically selected two parasites that were the most common in both in the native and invasive ranges to examine whether parasites can attain similar infection levels in native and invasive populations. The Student's *t* test was preferred to test the effect of geographic origin on per-site prevalence, since the response variables were normally distributed for each group with equal variance.

To investigate the parasite community composition of the two ranges, two methods were utilised—firstly to test for a significant difference in community composition based upon the geographic origin of the hosts and then to visually interpret the dissimilarities. Prior to the analyses, per-site species prevalence data were Hellinger transformed (Legendre and Gallagher 2001), utilising the package 'vegan' in R (Oksanen et al. 2018). Since some sites did not share parasite species, a dummy parasite species was added to each site at a per-site prevalence level of 50%. This was done to avoid a situation of undefined dissimilarity indices between sites (see also Locke et al. (2012) and Warburton et al. (2016)). This technique is especially appropriate in cases where assemblages are impoverished for biological reasons, as is the case with invasive animals and their parasite communities (Clarke et al. 2006). Bray–Curtis distances, which are not only sensitive to the presence or absence of species, but also to differences in prevalence of specific species between sites, were utilised to measure compositional dissimilarity of parasite communities between sites (Ricotta and Podani 2017). The Bray–Curtis is a semi-metric dissimilarity index and therefore better suited to impoverished communities where species prevalence is not normally distributed. An analysis of similarity (ANOSIM) was conducted in the R package 'vegan' (Oksanen et al. 2018) with 999 permutations based upon the Bray–Curtis distances to test whether there was indeed a significant difference in community composition between the native and invasive ranges. Non-metric multidimensional scaling (NMDS) was also employed utilising the 'vegan' R package to visualise whether the parasite communities from each of the sites fell into clearly separated geographical groups based upon compositional dissimilarity (Oksanen et al. 2018). The wrapper function 'metaMDS' was exploited with 20 random starts to compute both the Bray–Curtis distances in an initial step and subsequently the solution of the ordination to visualise the parasite community dissimilarity. Ellipses were computed according the standard deviation around the centroid (weighted mean) for each group. The parasite species richness was overlaid onto the two-component ordination space, assuming a non-linear relationship a priori, via the fitting of a generalised additive model produced in the function 'ordisurf' (Oksanen et al. 2018). The generalised additive model estimated whether there might be a significant relationship between the observed clustering and variation in species richness between the sites (Marra and Wood 2011).

All statistical analyses were performed in the built-in package 'stats' in the program R *version 3.4.4* (R Core Team 2018), unless mentioned otherwise. Summary statistics were computed in the R package 'Rmisc' (Hope 2013), or alternatively in 'dplyr' (Wickham et al. 2017). Results were visualised through the package 'ggplot2' (Wickham 2016).

Results

Parasitological survey in France and South Africa

The *X. laevis* from France ranged in snout-urostyle length from 25.2 to 99.3 mm (mean = 69.3 mm, SD ± 14.08). From these 43 frogs, two parasite species were morphologically and genetically identified. The parasite from the bladder was the monogenean *Protopolystoma xenopodis* Price, 1943, from the Polystomatidae and the cestode inhabiting the intestine was similarly confirmed to be *Cephalochlamys namaquensis* Cohn, 1906, from the Cephalochlamydidae. Both species were widespread across the region, hailing from both northern and southern peripheral sites, as well as core sites close to the introduction site, with a mean species prevalence of 19% and mean intensity of two worms per host for *P. xenopodis* across four sites and a mean species prevalence of 63% and mean intensity of four worms per host for *C. namaquensis* across five sites (Table 1).

The frogs collected from South Africa were similar in size to those from France, ranging in snout-urostyle length from 38.3 to 110.2 mm (mean = 71.5, SD ± 15.66). From a total of 172 frogs collected from 27 sites, 21 different metazoan parasites species were recovered. These parasites represented six different taxonomic classes, namely Acari, Hirudinea, Digenea, Monogenea, Cestoda and Nematoda. Parasite species richness varied considerably, ranging from zero parasite species in the frogs from a swimming pool near Colesberg, Northern Cape, to 11 parasite species in the frogs from dams in a pristine mountain stream near Dullstroom, Mpumalanga (Fig. 1e). The seven sites which hosted less than four parasite species in total all originated from recently disturbed or newly established habitats. These habitats included swimming pools, ornamental garden ponds, urban recreational dams, drainage from abattoirs and crop irrigation systems, temporary mountain streams, dams downstream of informal settlements and artificial ponds in botanical gardens. On the contrary, the nine host populations with parasite species richness of six or more hailed mostly from natural or permanent artificial water bodies in natural environments, such as farm dams and their connecting rivers or dams and pools in mountain streams. Some populations with six or more parasite species were sampled in ornamental garden ponds, but from sites in the vicinity of large natural water bodies in undisturbed areas. The two most common parasite species by far were *P. xenopodis* and *C. namaquensis*, both present in 25 of the 27 sites (92.6%). The rest of the parasite species were much rarer across South Africa, only present in ten or less of the sites (maximum 37.0%). The mean species prevalence of *P. xenopodis* across all sites, including sites with zero prevalence, was higher than in France at 56%. In the case of *C. namaquensis*, the same value was 65%.

In the Chilean invasive *X. laevis*, only one parasite species, identified as a nematode larva of the genus *Contracaecum*, has been reported at a mean species prevalence of 3.4% from 179 hosts from 10 sites (Fig. 1c) (Castillo et al. 2017). The Californian *X. laevis* parasite communities, collected from 230 hosts from three sites, were much more diverse and represented parasites native to both South Africa and California (Kuperman et al. 2004). Parasite species richness ranged from three to six at the three sites, with a total of seven parasite species across sites (Fig. 1b) (Kuperman et al. 2004). All three parasite communities included three South African parasites, namely the two monogeneans, *Gyrdicotylus gallieni* Vercammen-Grandjean 1960 (mean species prevalence of 12%) and *P. xenopodis* (mean species prevalence of 47%), and the cestode, *C. namaquensis* (mean species prevalence of 47%) (Kuperman et al. 2004). The Portuguese invasive *X. laevis*, represented by 80 hosts from two streams in Oeiras, harboured South African *P. xenopodis* at a mean species prevalence of 55% and two other parasites that were native to the invasive range (Fig. 1d) (Rodrigues 2014).

Effect of sampling effort

Despite great variation in sampling effort between the sites, parasite species richness was not significantly correlated with sample size (Spearman's rank correlation, $r_s = 0.15$, $n = 47$, $P = 0.31$). This did not mean

Table 1 Distribution and level of infection of the two parasites of *Xenopus laevis* from its invasive range in Western France

Locality	Number of frogs screened	Prevalence (%)		Abundance		Intensity	
		P. xenopodis	*C. namaquensis*	*P. xenopodis*	*C. namaquensis*	*P. xenopodis*	*C. namaquensis*
Site 1	8	38	88	0.9	3.3	2 [3] (1–3)	4 [1] (1–11)
Site 2	10	–	70	–	1.0	–	1 [1] (1–2)
Site 3	10	20	40	0.2	2.0	1	5 [4] (2–10)
Peripheral sites	28	18	64	0.3	2.0	2 [1] (1–3)	3 [2] (1–11)
Site 4	5	–	–	–	–	–	–
Site 5	4	25	100	0.8	1.5	3	2 [2] (1–2)
Site 6	6	33	83	0.3	5.7	1	7 [3] (1–18)
Core sites	15	20	60	0.3	2.7	2 [1] (1–3)	4 [2] (1–18)
All sites	43	19	63	0.3	2.2	2 [1] (1–3)	4 [2] (1–18)

The population is represented by 43 frogs from six sites. Sites 1 to 3 are towards the edge of the invasive range (periphery) and sites 4 to 6 are close to the original introduction site (core). Mean intensity of infection is given with median intensity in square brackets and minimum and maximum values in parentheses. Mean values averaged across sites include sites with zero prevalence

"–" Denotes absent infection level values due to absence of a parasite species at a specific collection site

that a full account was given of all the species at each site, but this result cancelled out variation in sample size as a possible confounding factor. Therefore, we did not make use of species richness estimators to correct for potential inaccuracies in subsequent analyses (Engemann et al. 2015).

Parasitism of hosts in native and invasive ranges

Of the 21 metazoan parasite species recorded from hosts in the native range, only three parasite species successfully accompanied *X. laevis* during the process of invasion. In addition, a total of seven new parasite species, which were native to the different invasive ranges, colonised invasive *X. laevis* upon arrival. Parasite species richness was significantly higher in hosts from the native range (mean = 4.7 species per population, SD ± 2.35) in comparison to those from the invasive ranges (mean = 1.4 species per population, SD ± 1.79) (Wilcoxon–Mann–Whitney test, $P < 0.001$) (Fig. 2). This was also the case for summed parasite prevalence (Wilcoxon–Mann–Whitney test, $P < 0.001$), where hosts in the native range (mean = 254%, SD ± 130.5) experienced greater cumulative effects of parasitism than in the invasive range (mean = 49%, SD ± 60.5). Similarly, mean parasite prevalence was significantly higher in the native (mean = 56%, SD ± 24.1) than in the invasive range (mean = 20%, SD ± 25.7) (Wilcoxon–Mann–Whitney test, $P < 0.001$).

In opposition to the general trend of parasite loss from the native to the invasive ranges, great variation in parasite species richness could be observed within the native range (from zero parasites to 11) and among the various invasive ranges (from zero parasites to six) (Fig. 1a–e). Notably, the parasite species richness at the native sites did not vary with relation to climatic region or biomes, with high richness sites being fully interspersed by lower richness sites across the range (Fig. 1e).

Characteristics of accompanying parasites

Of the 13 South African parasites species that were sufficiently identified for the distinguishing of traits, only three managed to co-invade with their host, namely *P. xenopodis*, *C. namaquensis* and *G. gallieni*. Taxonomic class was a significant indicator of whether a species would accompany the host in the process of invasion (Fisher's exact test, $P = 0.005$), because all the monogenean and cestodean parasites were present in at least one of the invasive populations and none of the mites, leeches, digeneans or nematodes managed co-invasion. Likewise, parasites with

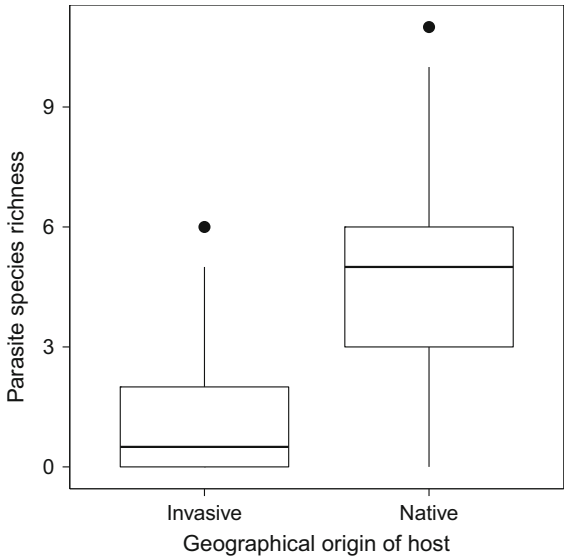

Fig. 2 Metazoan parasite species richness of *Xenopus laevis* populations in the invasive range is lower than in the native range. Minimum, first quartile, median, third quartile and maximum species richness values are shown for 20 invasive sites from Europe and North and South America and 27 native sites from Africa. Outliers are indicated by black dots

direct life cycles were significantly over-represented in the accompanying parasite species pool (Fisher's exact test, $P = 0.04$). On the other hand, the level of hosts specificity was not a significant predictor of co-invasion (Wilcoxon–Mann–Whitney test, $P = 0.10$). However, it is interesting to note that the co-invading parasites with higher host specificity possessed a direct life cycle, whilst the co-invading parasite with low host specificity possessed an indirect life cycle. The life stage of the parasite when infecting adult *X. laevis* had no effect on its presence in the invasive range (Fisher's exact test, $P = 0.07$). Neither were the non-accompanying parasites significantly absent in the Cape host population (Fisher's exact test, $P = 0.16$). However, presence in the Cape host population was a prerequisite for co-invasion. The parasite species that did not accompany their hosts tended to be those that were present in fewer of the host populations across the native range (median = 17%) as compared to those that did manage the co-invasion (median = 93%) (Wilcoxon-Mann–Whitney test, $P = 0.03$). Conversely, mean species prevalence across all hosts was not significantly different between accompanying (median = 60%) and non-accompanying parasites (median = 54%) (Wilcoxon–Mann–Whitney test, $P = 0.57$).

Susceptibility of invasive hosts to parasitism

The two most prevalent parasite species in both the native and invasive ranges were *P. xenopodis* and *C. namaquensis*. The per-site prevalence of these species in the sites where they were present was compared between the native and invasive ranges to determine whether parasites can attain similar infection levels across the distribution of *X. laevis* (Fig. 3). In the case of *C. namaquensis*, there was no significant difference in the percentage of hosts infected in the native (mean = 65%, SD $\pm$ 35.1) as compared to in the invasive range (mean = 25%, SD $\pm$ 35.4) (Student's t-test, $t = -0.583$ $P = 0.57$). However, the opposite was true for *P. xenopodis*, seeing that per-site prevalence was significantly higher in the native (mean = 56%, SD $\pm$ 31.3) versus the invasive range (mean = 16%, SD $\pm$ 21.0) (Student's t-test, $t = -2.984$, $P = 0.006$).

Parasite community dissimilarity analyses

The global ANOSIM indicated that the overall parasite community composition among sites was significantly different when taking into account the geographical origin (native versus invasive range) of the hosts (ANOSIM, $R = 0.41$, $P = 0.001$). Qualitative visualisation of parasite community dissimilarity between the native and invasive ranges through NMDS yielded a stable solution (stress = 0.13) and agreed with the results of the ANOSIM in that sites did cluster together based upon the geographic origin of the hosts (Fig. 4). This is clearly illustrated by the non-overlapping ellipses that encircled all sites that occurred within one standard deviation of the centroid of each of the geographic groups. The percentage of variance in species richness that was explained by the statistically significant response surface fitted by the generalised additive model was 94% ($F_{8.03,9} = 72.29$, $P < 0.001$). This confirmed that the highest species richness scores tended to be associated with the native range and lower species richness with the invasive range. Two notable exceptions in the native range are Colesberg and Polokwane, where the hosts harboured zero and one parasites species respectively. These sites did not cluster with the rest of the native sites. This

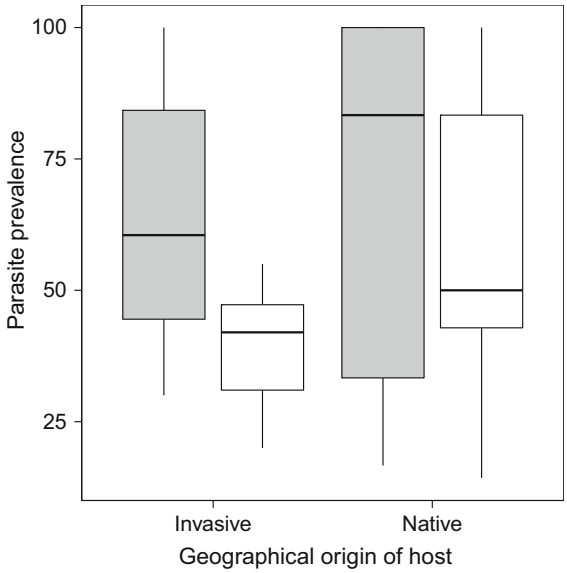

Fig. 3 Per-site prevalence of two common parasites of *Xenopus laevis* is higher in the native range than in the invasive range for a monogenean parasite, but does not differ significantly in the case of a cestodean parasite. The minimum, first quartile, median, third quartile and maximum prevalence values of the cestode, *Cephalochlamys namaquensis*, across 8 sites in the invasive and 25 sites in the native range are indicated in grey. The same values for the monogenean, *Protopolystoma xenopodis*, also across 8 sites in the invasive and 25 sites in the native range, are indicated in white

relationship also indicated that native sites with less parasite species tended to cluster more closely with invasive sites. Specifically, impoverished communities in the native range were almost identical in community composition to the four French sites that harboured both *P. xenopodis* and *C. namaquensis*. In contrast, invasive sites with acquired parasites, such as those from Chile, California and Portugal, tended to be more dissimilar to native sites.

Discussion

This study is the first to demonstrate that a globally invasive amphibian species underwent repeated loss of metazoan parasites during the process of invasion. Only three parasites accompanied *X. laevis* on its invasion pathway and seven parasites colonised it upon arrival across the various invasive ranges, in contrast to the 21 parasite species that were recovered from *X. laevis* in the native range. In addition, decreased cumulative effects of parasitism and decreased levels of infection by certain parasites in the invasive range suggest that there might also be release from some of the negative effects of parasitism, lending support to the ERH in terms of release from metazoan parasites. Parasite loss from the native to the invasive range is a given in most organisms (Heger and Jeschke 2014; Lui and Stiling 2006; Torchin et al. 2003) and colonisation by new parasites from the invasive range typically cannot make up for this loss (Torchin et al. 2003). Specifically, this is also the case for the only two other amphibians that have been assessed in a similar manner, albeit on a regional scale, namely the Puerto Rican tree frog, *E. coqui* (Marr et al. 2008), and the Cuban tree frog, *O. septentrionalis* (Ortega et al. 2015).

In opposition to the two amphibian species evaluated to date, this pattern of metazoan parasite loss in *X. laevis* is observed across different pathways of range expansion, on different continents and in different climatic regions. In most of the invasive populations, *X. laevis* was introduced into the wild after many generations of laboratory cultivation (Crayon 2005; Lobos et al. 2013; Measey et al. 2012; van Sittert and Measey 2016; Weldon et al. 2007). This invasion history is reflected by the fact that the two most common accompanying parasites, *P. xenopodis* and *C. namaquensis*, can both survive for at least a year in captive hosts (Jackson and Tinsley 1988; Tinsley 1996). In its native range, *X. laevis* is known to be one of the first aquatic vertebrates to populate new habitats, which they frequently reach via overland migration, or via jump dispersal with the aid of farms dams or artificial waterways (de Villiers and Measey 2017; Measey 2016; Measey and Channing 2003; Measey et al. 2017). The results from the present study indicate that these pioneer populations harbour distinct parasite communities with lowered parasite species richness that are similar to those in invasive populations elsewhere, particularly in France. In addition, parasite loss occurs in all of the climatic regions where invasive *X. laevis* is found, be it temperate, Mediterranean or subtropical.

Rather, despite the shared loss of parasites across continents and modalities of expansion, the mechanisms behind the loss can probably not be ascribed to a single factor. In the majority of cases, it is probably an artefact of long periods of captivity. After subsequent release into the wild, parasite loss may be due to

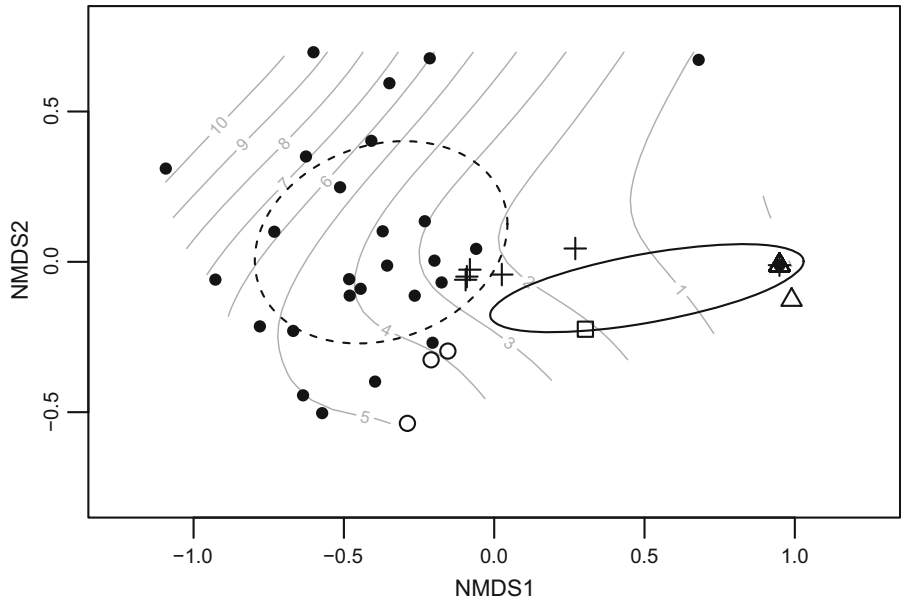

Fig. 4 Nonmetric multidimensional scaling of compositional dissimilarity reveals that metazoan parasite communities from native (Southern Africa) and invasive (California, Chile, France, Portugal) *Xenopus laevis* populations cluster separately due to enemy loss in invasive hosts. Included are 27 sites from Southern Africa (filled circles), three sites from California (open circles), nine sites from Chile (open triangles), six sites from France (crosses) and one site from Portugal (open square). Two native sites are fully interspersed with the invasive sites, namely Polokwane, top right corner, and Colesberg, which clusters with the Chilean sites. The fitted smooth response surface (grey contour lines) corresponds to species richness at each site. The ellipses encircle all sites that fall within one standard deviation of the centroid of the invasive (solid line) and native ranges (dotted line)

habitat unsuitability for the parasite cycle, specifically in terms of loss of intermediate hosts or unfavourable environmental conditions. For example, *P. xenopodis* relies on specific temperature optima for egg production (Jackson and Tinsley 1988) and *C. namaquensis* on the availability of a suitable copepod as intermediate host (Ferguson and Appleton 1988a; Thurston 1967). On the other hand, it might be a result of greater investment in immunological defence on the part of the host during range expansion, especially in the native range. For instance, dispersing *X. laevis* differ in some respects from *X. laevis* from established habitats. In France, *X. laevis* populations from the range edge exhibit lowered investment in reproduction and increased stamina, which might enhance their dispersal ability (Courant et al. 2017; Louppe et al. 2017). Immunological defence against parasites in *X. laevis* has received little attention to date and might not increase fitness per se in the light of the low pathogenicity of its parasites (Tinsley 1996), but cannot be ruled out as a potential factor.

With this in mind, it is interesting to note that the three accompanying parasites, namely the monogeneans *P. xenopodis* and *G. gallieni* and the cestode *C. namaquensis*, reveal some characteristics that might have facilitated their co-invasion. Not only are all three parasites present in the Cape, where the majority of *X. laevis* was exported from (van Sittert and Measey 2016; Weldon et al. 2007), they are also present in significantly more of the screened native populations than the parasites that did not transfer. Their likelihood to co-invade along with *X. laevis* was not significantly influenced by their mean prevalence in the native range, a likely consequence of the magnitude of the repeated, enduring export of *X. laevis* (van Sittert and Measey 2016). Since invaders generally do not experience such high levels of propagule pressure, it makes sense why accompanying parasites in *X. laevis* do not need high prevalence in the native range to facilitate co-invasion, as opposed to the trend in many other co-invading parasites (Torchin et al. 2003). Equally important, the majority of the accompanying parasites of *X. laevis* have direct life cycles, a trait that

has long been linked to a greater likelihood to manage co-invasion (Kuperman et al. 2004; Torchin and Mitchell 2004). However, our results show that parasites with complex life cycles can also be involved in co-invasion, in agreement with the findings of a recent review (Lymbery et al. 2014). Host specificity of the accompanying parasites reveals a similar dichotomy. Contrary to the findings of the majority of studies reviewed by Heger & Jeschke (2014), our results demonstrate that the tight link between the host and the accompanying host-specific monogenean parasites promotes co-invasion, rather than suppresses it, as in the case of the highly host-specific *P. xenopodis* and *G. gallieni*. On the other side of the spectrum, the only parasite of *X. laevis* that has ever been recorded from hosts that are not from the genus *Xenopus*, namely *C. namaquensis*, also managed co-invasion (Dollfus 1968; Mettrick 1963).

Loss of parasite species is not the only factor that contributes to the distinctness of the parasite communities of invasive hosts. At least one parasite, *P. xenopodis*, displays lowered prevalence in invasive populations of the host. Coupled with the fact that the summed prevalence, an indication of the cumulative effect of parasitism, is also lower in the invasive range compared to the native range, this hints at a form of release from the effects of parasitism in invasive populations (Torchin et al. 2003). However, this must be stated with great caution (Prior and Hellmann 2015; Prior et al. 2015). Not only might these values be somewhat inaccurate due to the unevenness of sampling effort in this study (Jovani and Tella 2006), but lowered levels of parasitism might not directly lead to increased invasive success, especially in the case of *X. laevis* that possesses a host of other traits that contribute to its invasiveness (Rödder et al. 2017). The acquisition of parasites upon establishment can also completely counter the effects of parasite loss given enough time (Kołodziej-Sobocińska et al. 2018; Schultheis et al. 2015). Eventually, acquired parasites may even contribute to the population regulation of the invasive *X. laevis* populations, since their association with the host is much more recent, which may translate to higher virulence than in the case of co-evolved parasites (Dunn et al. 2012; Ricklefs 2010).

All things considered, this study demonstrates that there is an overall pattern of metazoan parasite release, both with regards to species richness and prevalence, in *X. laevis* during invasion that is not yet countered by acquisition of new parasites. This process is repeated across continents and even in the ever-changing landscape of the native range. In line with two similar studies on the protozoan parasites of birds and the metazoan parasites of crustaceans (Marzal et al. 2011; Torchin et al. 2001), the repeated loss of metazoan parasites across independent geographical replicates observed in the present study supports the ERH, irrespective of continent, climate, mechanism of parasite loss, or invasion history. The assessment of co-invading parasites is rarely considered in control programmes, unless these parasites have high pathogenicity. However, the role of parasites in biological invasions should not be underestimated, because both host and accompanying parasite may fundamentally modify ecosystems and trophic interactions in invaded ranges (Amundsen et al. 2013; Dunn et al. 2012; Roy and Lawson Handley 2012). In the long run, we must realise that the accompanying parasites of *X. laevis* are also invaders in their own right. Predicting the future occurrences of these parasites is an important step in the control of *X. laevis* as an invasion package.

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Author contributions All authors were involved in initial conception, study design and data collection, as well as in editing and revision of the manuscript. A.L.S. generated the molecular data, performed the statistical analyses, prepared the tables and figures and led the manuscript writing.

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