

The Genetics of the Golden Mussel (*Limnoperna fortunei*): Are Genes Related to Invasiveness?

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Abstract Successful invasive species often share some ecological traits, such as rapid growth and rapid sexual maturation, both of which are characteristic of *Limnoperna fortunei*. In addition, phenotypic plasticity, i.e., the capability to express different phenotypes as a response to diverse environmental challenges, may play a fundamental role in the geographic expansion of many invasive species, including the golden mussel. Little is known about the genetics of *L. fortunei*, but the first transcriptome for *L. fortunei* has recently been sequenced and gene–environment relationships that are likely associated with the successful invasions of this species have begun to be elucidated. Over 24,000 transcripts have been functionally annotated, and results suggest the expansion of the gene families’ heat shock protein 70 and cytochrome P450. This may indicate that *L. fortunei* has a broad genetic repertoire that confers it an advantage to deal with stressors presented in new locations. Several other key genes such as byssus proteins, immune system-related genes, and antioxidant enzymes have been characterized and are now available for gene

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expression studies. A genome project is being carried out in Brazil to characterize the entire set of genes and gene families of *L. fortunei*. This will expand our current knowledge of the genetic characteristics of the invasion helping to forecast new invasion events and develop biotechnology-based strategies to control the infestation.

Keywords *Limnoperna fortunei* · Golden mussel · Genetics · Phenotypic plasticity · Transcriptome · Adaptation

Phenotypic Plasticity

Around 5 t of ballast water are transported annually around the globe discharging more than 10,000 alien species in new environments every day. From this total, only a small fraction ends up establishing and colonizing a new environment (Carlton and Geller 1993; David and Gollasch 2008). What makes some alien species become invasive and others not?

Some ecological traits are well-known to be related to success in colonizing a new environment, such as short life spans, early sexual maturation, and high reproductive rates (Lockwood et al. 2007; Davis 2009), all of which are characteristic of *Limnoperna fortunei* (Karatayev et al. 2007; Uliano-Silva et al. 2013). Nonetheless, such traits may not suffice for successful colonization of a new region if the alien species is faced with a number of new stressors to which it is not adapted. The genetic substrate of invasive populations, upon which natural selection operates, could be of primary importance for the success, or lack thereof, of a biological invasion (Cox 2002). Among the components of this genetic substrate, genetic plasticity is essential insofar as it is responsible for the environmental tolerance of the invading species while it struggles to occupy a new and challenging environment (Franks and Munshi-South 2014). Phenotypic plasticity is the ability of a species to express multiple alternative phenotypes from a single genotype under a variety of different environment conditions (Stearns et al. 1991). Evolutionary studies have shown that phenotypic plasticity confers some advantages, such as the maintenance of genetic diversity, higher adaptation rates, and reduction of the bottleneck effect when species are facing the challenges of a transitional environment (Stearns et al. 1991).

Shortly after invading South America (Pastorino et al. 1993) and Japan (Kimura 1994), *L. fortunei* became a major nuisance for many industrial facilities, as well as a conspicuous component of local fauna, which fostered studies on this mussel (see Preface in this volume). In recent years, evidence has accumulated on the ecological and economic harm caused by this species (Magara et al. 2001; Uliano-Silva et al. 2013; Boltovskoy and Correa 2015), granting it the status of an “ecosystem engineer” (Darrigran and Damborenea 2011). Successful invasions are thought to be largely associated with the ability of *L. fortunei* to colonize waterbodies with a wide range of environmental conditions (Karatayev et al. 2007; Oliveira et al. 2011; Uliano-Silva et al. 2013). This may suggest that the golden mussel’s success has

benefited from the ability to express alternative phenotypes to cope with the many environmental challenges of the Asian and South American lotic and lentic bodies of water colonized (Uliano-Silva et al. 2013). However, the genetics of *L. fortunei* was not investigated until late 2013, when its first transcriptome was sequenced, assembled, and annotated.

First Transcriptome Survey for *L. fortunei*

Uliano-Silva et al. (2014) performed the first transcriptome survey of *L. fortunei*. A transcriptome is the total set of genetic transcripts in a given organism, which can vary with intracellular and external environmental conditions. The transcriptome reflects the genes that are being actively expressed at a given time. In this study (Uliano-Silva et al. 2014), only mRNA was sequenced, covering the genes expressed in the tissues of the gills, adductor muscle, digestive gland, foot, and mantle. Using next-generation sequencing technology (Roche 454 GS Junior), 84,063 partial gene sequences were sequenced and assembled, where 1351 were complete full-length genes, and more than 24,000 expressed transcripts were functionally annotated. This extensive material has provided information that can be used to investigate the gene–environment relationships of *L. fortunei* during the colonization process (Uliano-Silva et al. 2014).

Invading and colonizing new environments present major challenges and is stressful for organisms (Lee 2002). A range of gene families is known to become highly expressed at times of cellular stress helping to avoid homeostatic imbalance (Evans and Hofmann 2012). Examples of such gene families are the antioxidant enzymes, which neutralize or repair damage caused by free radicals in the cell (Sies 1997), the molecular chaperones that prevent protein denaturation under heat and several other cellular stressful settings (Clark and Peck 2009), and the family of cytochrome P450, which are phase 1 biotransformation enzymes that transform harmful xenobiotics to a more polar molecule facilitating its excretion from the cell (Teunissen et al. 1992).

Molecular Chaperones, HSP70

One of the first gene families closely investigated in *L. fortunei* comprises the HSP70 chaperones. These genes have recently been shown to be important in the Pacific oyster *Crassostrea gigas* (Zhang et al. 2012). The sequenced genome of this bivalve, also an exotic species in many areas of the world, including southern South America, where it is cultured for human consumption (Melo et al. 2010), has 88 copies of the HSP70 gene. This is significantly more than previously found for the same gene in other species (e.g., 39 in sea urchins, 17 in humans; Zhang et al. 2012). Furthermore, the expression of HSP70 genes were induced at least 15-fold in

oysters exposed to several stressors, including heat and metal pollution. Exposure to heat and air increased up to 2000-fold the expression of five *C. gigas* HSP70s. This gene expansion and their notable induction were attributed to the remarkable ability of this intertidal species to cope with severe stress when exposed to air during low tides (Zhang et al. 2012).

The importance of this gene family to cope with stressful situations in the oyster *C. gigas*, suggests that it may also be important for the invasive success of *L. fortunei*. The transcriptome of the golden mussel has at least 55 different isoforms of HSP70, which is markedly higher than in humans and sea urchins. However, the transcriptome characterization of gene families is not a fail-safe approach, and it may underestimate the number of different isoforms of HSP70 present in the genome of the golden mussel.

Preliminary analysis of *L. fortunei* HSP70s supports the hypothesis that this gene family is indeed related to successful invasions. Analysis of mollusc HSP70 phylogeny showed that two *L. fortunei* HSP70s isoforms are evolutionarily related to the expansion observed in *C. gigas* (Uliano-Silva et al. 2014). These results also showed that all the other *L. fortunei* HSP70s are phylogenetically related to several other HSP70 isoforms that are expressed under a variety of circumstances in other bivalves.

The fact that *L. fortunei* has an extensive repertoire of HSP70s indicates that the challenge of invading new environments can be facilitated by the modulation of these anticellular stress genes (Uliano-Silva et al. 2014).

Cytochrome P450

Another group of genes that are worth investigating in relation to the ability to withstand stressful environmental challenges is the cytochrome P450 (CYP) gene family. These monooxygenase enzymes are chiefly involved in catalyzing the biotransformation of xenobiotics and hydrophobic compounds into more polar forms facilitating their excretion from the cell and are present in several other biochemical pathways (Teunissen et al. 1992). Each isoform is known to catalyze specific reactions. For example, the isoform CYP3A participates in the biosynthesis of cholesterol and steroid hormones, and is responsible for metabolizing about 80% of all man-made drugs (Li et al. 2008). CYP2A is involved in the biotransformation of polychlorinated biphenyls (PCBs) (Fernandez-Salguero and Gonzalez 1995), once widely employed in various industrial processes and now banned in most countries. Several studies have reported the role of cytochromes in the maintenance of homeostasis in bivalves exposed to chemical compounds (Mello et al. 2012), and domestic sewage (Bainy et al. 2000).

After having annotated the genes through transcriptome sequencing, work on *L. fortunei* has centered on investigating whether the cytochrome P450 profile would allow it to invade, settle, and adapt to specific habitats. Transcripts representing the 24 CYP isoforms of *L. fortunei* were combined with the CYP gene sequences for all

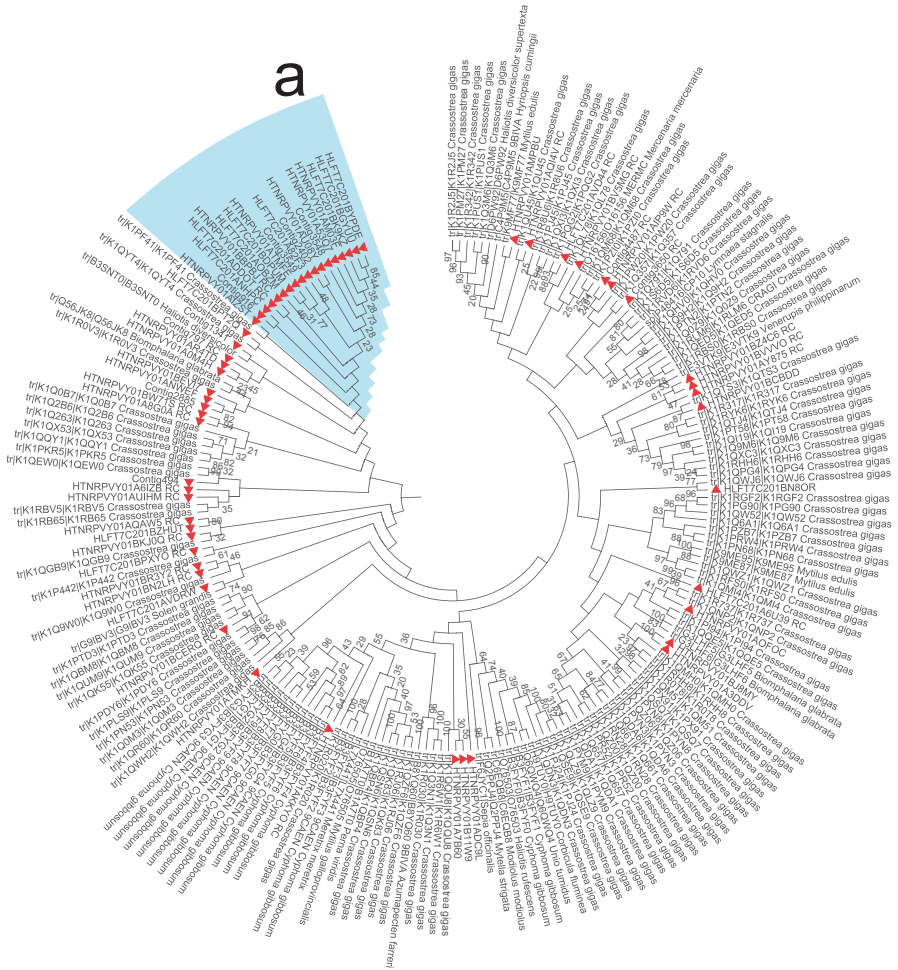


Fig. 1 Consensus phylogenetic tree of Mollusca cytochrome P450 family members. All 66 cytochrome P450 sequences described in the transcriptome of *L. fortunei* were combined with all 143 cytochrome P450 gene sequences available for the 19 molluscs in the UNIPROT database for the alignment and construction of the phylogenetic tree. The tree was built using the maximum likelihood method and bootstrapping (100 pseudoreplicates, values less than 30% are not shown). Sequences of *L. fortunei* are marked with red triangles. Detail (a) highlights the possible expansion of the CYP3A genes found in *L. fortunei*

molluscs available online to construct a phylogenetic tree (Fig. 1). The figure shows that there are *L. fortunei* CYPs phylogenetically related to several *C. gigas* CYP isoforms throughout the tree, thus confirming the possible similarity of the CYP profile in the genomes of both species. However, a possible source of bias in this analysis is that the CYP sequences of *C. gigas* are overrepresented in this molluscan phylogeny, because the genome of *C. gigas* is one of only two bivalve genomes to have been sequenced. Interestingly, *L. fortunei* has a set of CYP3As that do not relate

phylogenetically to any CYPs of other bivalves, but they are strongly associated with each other (Fig. 1, detail a). This suggests an expansion in the CYP3A gene family in *L. fortunei* that has not yet been described for any other bivalve. If genome sequencing confirms this expansion, the next step will be to elucidate whether or not this CYP profile, more robust than for other species, allows *L. fortunei* to cope with the stress of challenging environments, such as the acid waters of the Amazon system (Oliveira et al. 2010).

Other Genes Potentially Important for Controlling the Spread and Impacts of *L. fortunei*

In addition to gene families related to resilience under cellular stress, there are other important genes in the context of invasion dynamics of the golden mussel. A salient trait of *L. fortunei* is its ability to attach to hard objects using its byssal threads (see Chapter “Distribution and Colonization of *Limnoperna fortunei*: Special Traits of an Odd Mussel” in this volume). The byssal plaque has strong crosslinks between neighboring proteins, with a high content of DOPA, a modified amino acid, and metal atoms, ensuring firm adhesion even in the presence of water (Ohkawa et al. 2001; Lee et al. 2011; see Chapter “Control of *Limnoperna fortunei* Fouling: Antifouling Materials and Coatings” in this volume). Two byssus proteins, Mepf1 and Mepf2, were found in the transcriptome of *L. fortunei*; their expression patterns are a promising topic of research that may help in the search of methods to mitigate fouling in industrial facilities (Uliano-Silva et al. 2014).

The immune system is another key factor allowing bivalves to survive environmental challenges. While viral and fungal infectious diseases represent a serious threat to shrimp farming worldwide, cultures of *C. gigas* seem immune to these problems. The oyster thrives in aquaculture facilities in southern Brazilian where waters are highly contaminated with viruses (human adenovirus, noroviruses, hepatitis A, and JC Polyomavirus) and fecal coliform bacteria (Souza et al. 2012). The transcriptome of *L. fortunei* shows the presence of at least eight genes involved in the signaling pathway of toll-like receptors (Uliano-Silva et al. 2014) indicating that *L. fortunei* possesses precursors of an adaptive immune system as shown for other invertebrates (Hibino et al. 2006; Miller et al. 2007; Philipp et al. 2012). This would confer an advantage when invading environments with a variety of contaminants.

Limnoperna fortunei is more resistant to chemical control than other fouling mussels, such as *Dreissena polymorpha* (Cataldo et al. 2003; see Chapters “Parallels and Contrasts Between *Limnoperna fortunei* and Species of *Dreissena*” and “Chemical Strategies for Control of the Golden Mussel (*Limnoperna fortunei*) in Industrial Facilities” in this volume). It thrives under a wide range of conditions (Karatayev et al. 2010); but one of the few catastrophic events that seems to strongly depress its numbers, occasionally wiping out entire populations, is the “de-quadra,” an extensive anoxic event that occurs after periodic floods in the Pantanal wetland of Brazil (Oliveira et al. 2010). These anoxic episodes are responsible for

massive kills, but some local organisms have special adaptations to cope with them. The fish *Piaractus mesopotamicus*, for example, has an enhanced basal activity of the peroxide-depredate enzyme, GPx, that helps it support oxidative stress (Cunha Bastos et al. 2007). Records of massive kills of *L. fortunei* in association with dequadas in the Pantanal wetland may indicate that the mussel does not have a robust antioxidant system. This assumption is supported by the apparent lack of expansions of antioxidant gene families in the transcriptome of *L. fortunei* (Uliano-Silva et al. 2014), as also noted for the genome of *C. gigas* (Zhang et al. 2012). The profile of the antioxidant genotype of *L. fortunei* needs further investigation, but if the above assumptions prove correct, the antioxidant system may be an important target for the development of control tools against *L. fortunei* biofouling (see Chapter “Control of *Limnoperna fortunei* Fouling by Oxygen Deprivation” in this volume).

Concluding Remarks

Preliminary results on the transcriptome of *L. fortunei* indicate that the mussel’s invasive success may be intimately linked to its phenotypic plasticity (Uliano-Silva et al. 2014). However, the ultimate goal of this approach is acquiring adequate knowledge of *L. fortunei* genetics through the sequencing of its entire genome. The assessment of coding regions and the number of genes and gene families of a species, as revealed by transcriptome sequencing, may be incomplete because it only characterizes genes expressed in the organism at the time of collection of the corresponding tissue samples. The only reliable way to characterize genes and gene families, other transcripts (e.g., interference RNA, micro RNA), as well as various noncoding regions, is through the sequencing and assemblage of the species’ genome. The genome can also characterize the “taxonomically restricted genes” (TGRs), which represent 10–20% of the genes of a species and are not obscured by similarities with phylogenetically close organisms.

Phenotypic plasticity is ultimately dependent upon the genotype of a species. The range of gene families and their expression is what will confer the organism the capability to adapt to stressful environments. The goal of work currently underway is to relate this genetic information with the spread of *L. fortunei* outside of its native range. Assembling the genome of *L. fortunei* will expand our current knowledge of the genetic characteristics of biological invasions, help to forecast new invasion events, and aid in the development of biotechnology-based strategies to control infestations. Genome sequencing of the oyster *C. gigas* has shown the potential of this approach to clarify the relationship between the genotype and the life-habits of a species. Zhang et al. (2012) concluded that the genome of *C. gigas* is about 637 Mb in size. Our preliminary results show that the genome of *L. fortunei* is slightly larger, ~800 Mb (unpublished data). The next step is to determine how much of this represents novel genes, and how much of it is noncoding DNA.

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