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INVASIVE SPECIES - REVIEW

Ascidians as models for studying invasion success

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Abstract During the past three decades, coastal marine waters have become among the most invaded habitats globally. Ascidians are among the most notorious invaders in these ecosystems. Owing to their rapid spread, frequent population outbreaks, and associated negative ecological and economic impacts, invasive ascidians have become a global problem. Thus, the study of ascidian invasions has become a prominent area of invasion biology. Here, we review current knowledge and conclude that ascidians are good models for studying invasion success in the marine realm. Firstly, we summarize the reconstruction of invasion pathways or colonization histories and associated negative impacts of invasive ascidians, and address the urgent need to clarify ambiguous taxonomy of ascidians. Secondly, we discuss factors that underlie or facilitate invasion success of ascidians, including vectors of introduction and spread, environmental changes, biological traits, and possible genetic issues. Finally, we summarize current science-based policies and management solutions that are in place to prevent and control spread of invasive ascidians. We conclude by highlighting key research questions that remain to be answered, and propose future research to investigate mechanisms of invasion success in the marine realm using ascidians as model systems.

Introduction

Over the past three decades, the introduction and spread of marine non-indigenous species (NIS) has become common owing to human activities such as increased shipping associated with global trade and aquaculture (Cohen and Carlton 1998; Levings et al. 2002; Ruiz and Carlton 2003; Carlton and Ruiz 2005). Shipping activities associated with global trade facilitate the dispersal of marine NIS both attached to vessel hulls and carried within ballast water (Ruiz and Carlton 2003; Sylvester et al. 2011; Briski et al. 2013). The rapid development of aquaculture is responsible for the introduction of large numbers of NIS for farming (e.g., De Silva et al. 2009; Lin et al. 2015). Also, some of these species were introduced inadvertently as contaminants or "fellow travelers" with aquaculture species, and/or on equipment transferred among local water bodies (Naylor et al. 2001; De Silva et al. 2009; Lin et al. 2015). In addition, the live seafood/bait and aquarium trades have also become important vectors for biological invasions of marine NIS (e.g., Rhyne et al. 2012). Mediated by these vectors, it is estimated that thousands of species are transported within and between oceans on any given day

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(Carlton and Gellar 1993). With scant evidence that invasions are leveling off, it is not surprising that the number of introduced NIS and the frequency of NIS population outbreaks are increasing (Rilov and Crooks 2009).

A number of ascidians (or sea squirts; Phylum Chordata, Class Ascidiacea) represent notorious invaders in marine ecosystems. Invasive ascidians have caused significant ecological and economic damage to invaded habitats (see section "Invasion history and impacts" for more detail). Owing to their global spread, frequent population outbreaks, and associated negative ecological and economic impacts, invasive ascidians have become a global problem (Lambert 2007). Ascidians have received significant attention, particularly over the past decade. In consequence, three international conferences have been held to address numerous problems caused by ascidian invasions (see special issues in Journal of Experimental Marine Biology and Ecology, volume 342, issue 1, 2007; Aquatic Invasions, volume 4, issue 1, 2009, and volume 6, issue 4, 2011). Invasive ascidians have become one of the focal hotspots in invasion biology (e.g., Lambert 2007; Bullard and Carman 2009).

Large research efforts have been made to understand causes and consequences of ascidian invasions, including invasion sources (e.g., Dupont et al. 2010; Rius et al. 2012), post-establishment spread and associated vectors (e.g., Dijkstra et al. 2007), dispersal dynamics (e.g., Bock et al. 2011; Zhan et al. 2012), and management of invasions (e.g., McKindsey et al. 2007). In particular, recent investigations in several model species of ascidians such as *Ciona intestinalis* species complex and *Botryllus schlosseri* species complex have provided deep insights in dispersal dynamics and factors responsible for geographical distributions (Zhan et al. 2010, 2012; Bock et al. 2012). Collectively, invasive ascidians have become promising models for studying invasion success in marine ecosystems. The use of these model species facilitates answers to both fundamental questions such as roles of factors responsible for invasion success (see section "Factors underlying invasion success" for more detail) and applied topics such as policies and management solutions for prevention of biological invasions in marine ecosystems (see section "Current policy, rapid response and control of spread" for more detail).

Many published reviews have focused on particular topics related to ascidian invasions, such as global species diversity (Shenkar and Swalla 2011), model species (*C. intestinalis*) for ecological and evolutionary studies (Procaccini et al. 2011), ecological and/or economic impacts to invaded habitats (Aldred and Clare 2014), and ecology and natural history (Lambert 2005; Bullard and Carman 2009). Here, we aim to synthesize the latest research progress into a systematic and comprehensive review, concluding that invasive ascidians are ideal models for studying invasion success.

We first summarize invasion histories and negative impacts for some well-known invasive ascidians, and stress the need for clarifying ambiguous taxonomy. We then evaluate factors underlying invasion success, including the availability of multiple vectors for introduction and spread, environmental changes that can promote rapid spread, biological traits, and genetic patterns/mechanisms directly and/or indirectly responsible for invasion success. As one of the major applications of fundamental research, we summarize and discuss current science-based policies and management solutions that could prevent and control spread of invasive ascidians. Finally, we conclude by highlighting key research questions outstanding and propose future studies to investigate invasion success using ascidians as models in marine invasion biology. In this review, we define NIS as those that historically have never occurred in a particular region and differentiate them from invasive species, which we define as high impact species.

Invasion history and impacts

Taxonomy: a call for further investigation using genetic methods

Clarification of taxonomy represents a key prerequisite step to answer many basic questions in invasion biology of ascidians including "who are invaders?", "where are they from?", and "what effects do they cause in invaded habitats?" However, our knowledge of a majority of marine species is woefully inadequate (e.g., Fautin et al. 2010). Owing to the lack of systematic, biogeographical, and/or historical data, we cannot develop a simple list of which species are non-indigenous in many regions of world's seas (Carlton 2009; Geller et al. 2010). The lack of systematic knowledge and available keys for species identification is a serious problem in ascidians (e.g., Shenkar and Swalla 2011 and references therein), leading to difficulties in accurate evaluation of invasion histories (see detail in section "Invasive ascidians and their invasion history") and species-specific, negative impacts (see detail in section "Negative impacts"). Thus, systematic knowledge, particularly with respect to accurate species identification, is crucial to clarify causes and consequences of ascidian invasions in marine ecosystems.

Ascidiacea, comprising approximately 3000 described species found in all marine habitats, is the largest and most diverse class of the subphylum Tunicata (also known as Urochordata) (Shenkar and Swalla 2011). The number of species of Ascidiacea may be underestimated (Appeltans et al. 2012), mainly due to limited collections from areas including South and Central America, northern Canada, Alaska, and the tropical western Pacific Ocean (Shenkar and Swalla 2011). Following the initial classification (Lahille 1886), the taxonomy of the class Ascidiacea has been highly debated, mainly owing to unclear/unavailable taxonomic keys for many species. The currently accepted classification by most taxonomists is a three-order division based on the structure of the adult branchial sac (Monniot et al. 1991): Aplousobranchia (simple branchial sac), Phlebobranchia (vascular branchial sac), and Stolidobranchia (folded branchial sac). Such order-level classification also corresponds to molecular phylogeny reconstructed based on the small subunit ribosomal DNA (SSU rDNA, Fig. 1).

A huge challenge remains to resolve many ascidian species to lower taxonomic levels, such as species based on traditional morphology. This problem mainly stems from the presence of available taxonomic keys, species complexes, and/or high geographical morphological variation (e.g., Stefaniak et al. 2009; Zhan et al. 2010; Bock et al. 2012). Recent studies demonstrate that many invasive ascidians are actually species complexes containing morphologically cryptic but genetically distinct species (e.g., Zhan et al. 2010; Bock et al. 2012; Pérez-Portela et al. 2013). This diversity occurs even in model species such as *C. intestinalis* (Zhan et al. 2010) and *B. schlosseri* (Bock et al. 2012). While several highly invasive species such as *C. intestinalis* spA (=*Ciona robusta*, Brunetti et al. 2015) and spB (=*C. intestinalis*, Brunetti et al.

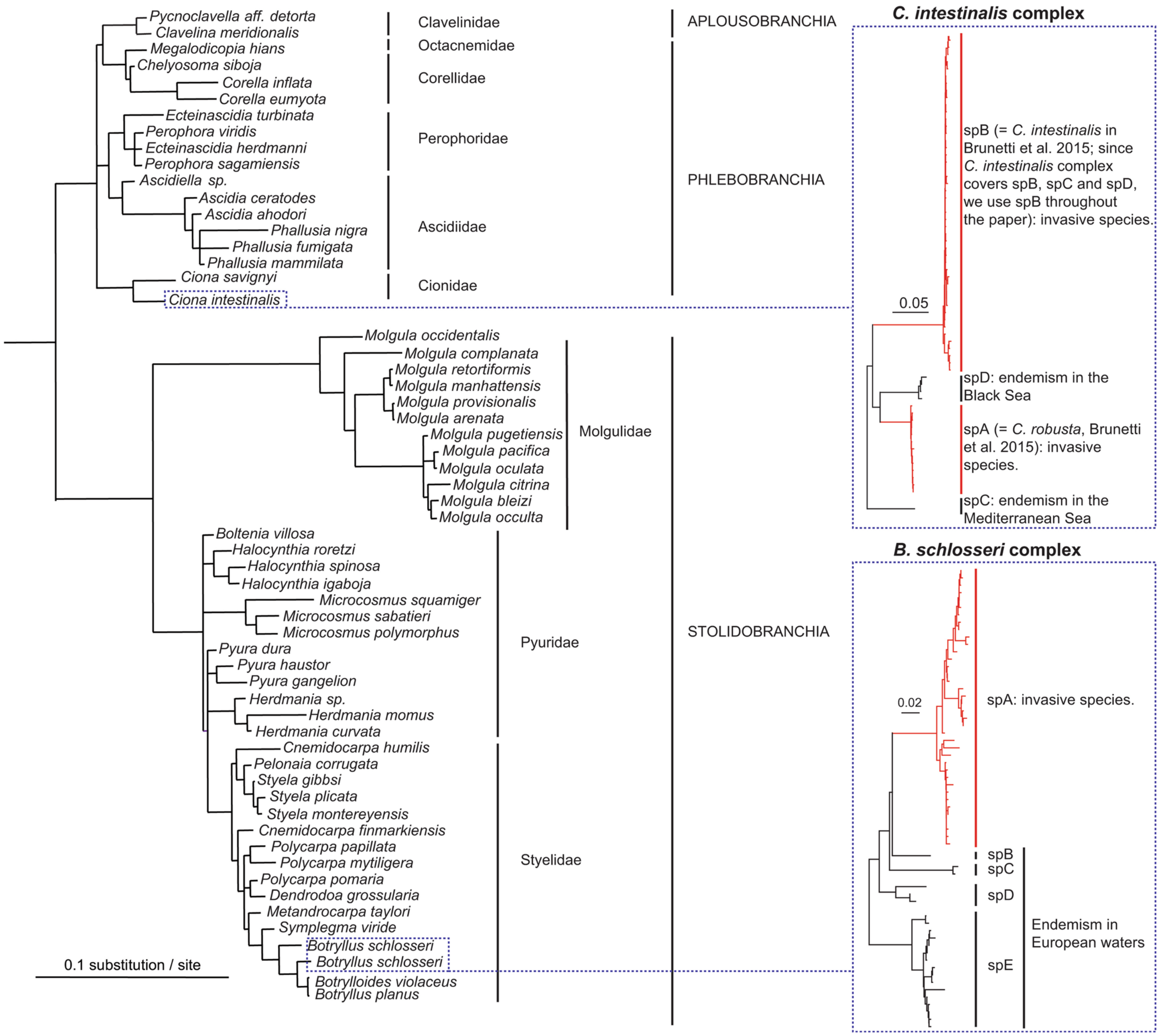

Fig. 1 Phylogeny of ascidians inferred from the small subunit ribosomal DNA (SSU rDNA, modified from Tsagkogeorga et al. 2009) and phylogenies reconstructed based on mitochondrial genes for the two model ascidians: *Ciona intestinalis* species complex (modified from Zhan et al. 2010) and *Botryllus schlosseri* species complex (modified from Bock et al. 2012)

2015) and *B. schlosseri* spA have disjunct global distributions, other members of these species complexes largely remain restricted to their native ranges (Zhan et al. 2010; Bock et al. 2012). Such findings suggest that endemic species may go extinct if eradication actions are taken without detailed taxonomic clarification. More importantly, these studies call for combining morphological and genetic approaches to solving the problem of ambiguous taxonomy in ascidians. Genetic identification can strongly enhance, and in some cases would appear to have many advantages over, traditional morphology (Stefaniak et al. 2009; Geller et al. 2010). In addition, the use of genetic data has facilitated deep understanding of both fundamental questions, such as invasion histories, invasion sources and factors responsible for introduction and post-establishment spread (e.g., Estoup and Guillemaud 2010), and more applied topics such as development of effective management strategies and sustainable science-based policies (see review by Geller et al. 2010).

Invasive ascidians and their invasion history

Human-mediated introductions of invasive ascidians have been occurring with an increasing frequency (Lambert and Lambert 2003; Mead et al. 2011). Thus far, coasts of all continents except Antarctica have been reported invaded by ascidians (see Supplementary material 1). Although Antarctica has not been reported as a recipient continent, several invasive ascidians are derived from the Antarctic biogeographical province, such as *Corella eumyota* (Lambert 2004). Some invasive ascidians from the Northern Hemisphere have successfully colonized the Southern Hemisphere (Supplementary material 1). In terms of the number of introduced ascidians, one of the most affected and well-surveyed areas is the coast of California. Thus far, more than 15 NIS have successfully established, ten of which arrived within the last two decades (Lambert and Lambert 2003; Bullard et al. 2007a). In less explored areas such as South Africa, nine introduced and nine cryptogenic ascidian species have been recorded (Rius et al. 2014b).

Based on available literature, we identified 80 ascidian species which were clearly reported as “introduced” or “non-indigenous” (Supplementary material 1). Due to ambiguous taxonomy and/or poor historical records, many ascidians remain cryptogenic species in areas where they have been recently detected (see review by Shenkar and Swalla 2011). When these 80 non-indigenous ascidians were grouped based on solitary versus colonial growth forms, the number of species between the two groups was remarkably similar, with 39 and 41 species of the former and latter, respectively (Supplementary material 1). The number of non-indigenous ascidians in the three orders was 23 for Aplousobranchia, 18 for Phlebobranchia, and 40 for Stolidobranchia (Supplementary material 1).

The majority of listed species invaded relatively small geographical ranges (Supplementary material 1), though some may have larger but unknown distribution ranges owing to undersampling, identification problems, or language barriers. Several species such as *Botrylloides violaceus*, *B. schlosseri*, *C. intestinalis*, *Didemnum vexillum*, *Microcosmus squamiger*, and *Styela clava* have successfully colonized a wide geographical range (Supplementary material 1). We summarize the known invaded ranges and invasion histories of these six representative highly invasive ascidians in Table 1.

Even for well-known, highly invasive ascidians, the native/invaded ranges and invasion histories remain uncertain (but see *M. squamiger*, Rius et al. 2012), mainly owing to ambiguous taxonomy (Table 1). For example, the first detection date of *B. violaceus* can be either 1945 (collection made in 1939; van Name 1945; unconfirmed) or the 1970s (Fay and Johnson 1971; confirmed), with confusion with *Botrylloides diegensis* on the west coast of North America preventing clear determination. *D. vexillum* poses an even more difficult problem: It has been problematic to identify this invader, to determine how many species are involved, and to clarify its invasion history, mainly owing to relatively few available diagnostic characters and high morphological variability among populations collected from different environments/areas (Lambert 2009; Stefaniak et al. 2009). *D. vexillum* has been mis-identified as at least five native species locally, and researchers have used several different names, such as *Didemnum* spA, *D. vexillum*, and *Didemnum vestum* (Lambert 2009; Stefaniak et al. 2009). Recently, the taxonomic issue has been alleviated using genetic markers based on global samples. Coupled with morphological evidence, *D. vexillum* seems to be the most appropriate name for this ascidian species (Lambert 2009; Stefaniak et al. 2009).

Negative impacts

Similarly to invasion history, accurate evaluation of species-specific, negative impacts caused by invasive ascidians has been obscured by ambiguous taxonomy, though the level of ambiguity varies among species. In summary of the known common negative impacts among species, introduction and spread of invasive ascidians has caused significant ecological and economic damage to recipient habitats. Ecologically, invasive ascidians usually affect benthic communities owing to their strong competitive abilities. Many studies clearly demonstrated that invasive ascidians decreased species richness and changed biodiversity of invaded habitats (see review by Aldred and Clare 2014). Once non-indigenous ascidians become established in new

Table 1 Invasion histories and vectors of the six representative highly invasive ascidians

Species/common name	Native range	Invasion history	Vectors	Major references
Botrylloides violaceus/Violet tunicate	Northwest Pacific, most likely Japan	Owing to taxonomic confusion, invasion history remains largely unclear: Invaded areas include the coasts of Oceania such as Australia, Europe such as Italy, UK, Ireland, and the Netherlands, and both coasts of North America	Major vectors: hull fouling, aquaculture transfers; other vectors/means: ballast water, rafting of fragmented colonies, fellow travelers with large crustaceans	Zaniolo et al. (1998), Dijkstra et al. (2007), Gittenberger (2007), Minchin (2007), Bernier et al. (2009), Pérez-Portela et al. (2009) and Callahan et al. (2010)
Botryllus schlosseri complex/Golden star tunicate	Generally considered of European origin	Species complex (see Fig. 1 for more detail). SpA colonized New Zealand in 1922, Australia in 1928, South California in 1960s, Gulf of Maine in 1978, Prince Edward Island in 2001. New records of established populations are constantly being added, such as in Australia, Tasmania, South Africa, Chile, and Argentina	Major vectors: hull fouling, aquaculture transfers, floating debris in ballast tanks; other vectors/means: rafting of fragmented colonies, fellow travelers with eelgrass, algae, etc.	Stoner et al. (2002), Carver et al. (2006), Ben-Shlomo et al. (2006), López-Legentil et al. (2006), Ben-Shlomo et al. (2010), Briski et al. (2011) and Bock et al. (2012)
Ciona intestinalis complex/Vase tunicate	Unknown due to ambiguous taxonomy	Species complex (see Fig. 1 for more detail). Its invasion history was obscured by its ambiguous taxonomy, such as confusion between *C. intestinalis* and *C. savignyi*. Since the early 1910s, spA (=*C. robusta*) has occupied temperate seas including Mediterranean Sea, south European and South American Atlantic coasts, and Pacific Ocean, while *C. intestinalis* spB is found in North Atlantic waters	Major vectors: ballast water, hull fouling, aquaculture transfers; other vectors/means: larvae advected by water currents	Lambert and Lambert (1998), Carver et al. (2003), Blum et al. (2007), Caputi et al. (2007), Lambert (2007), Nydam and Harrison (2007, 2010), Zhan et al. (2010) and Procaccini et al. (2011)
Didemnum vexillum	Likely native to the northwestern Pacific Ocean	Known as *Didemnum* spA in many publications. Owing to ambiguous taxonomy and high morphological variability in different environments, its invasion history remains largely unresolved	Major vectors: hull fouling, aquaculture transfers; Other vectors/means: drifting on floating debris	Herborg et al. (2009), Lambert (2009), Stefaniak et al. (2009) and Ordoñez et al. (2015)

Table 1 continued

Species/common name	Native range	Invasion history	Vectors	Major references
Microcosmus squamiger	Australian origin	It has been detected worldwide. Most populations in invaded areas were reported in the past 50 years, such as on the Mediterranean Sea in the 1960s and west coast of North America in 1980s. Genetic studies have shown that this species was firstly introduced to the Mediterranean Sea and South Africa, followed by sequential introductions in California and, more recently, the NE Atlantic Ocean	Major vectors: fouling on hulls and sea chests	Naranjo et al. (1996), Turon et al. (2007), Rius et al. (2008), Rodriguez and Ibarra-Obando (2008) and Rius et al. (2012)
Styela clava/Clubbed tunicate	Coastal areas of Japan, Korea, China, and Siberia	In North America, it was detected in the late 1920s in California and then spread to both Pacific and Atlantic coasts. In Europe, it was first reported as *S. mammiculata* in 1953 in the UK and subsequently expanded its range north to Denmark and south to Portugal, and the Mediterranean Basin. In Oceania, it has colonized the coasts of Australia in 1999 and New Zealand in 2005	Major vectors: fouling on hulls and sea chests, larvae in ballast water; other vectors/means: adults attached to drifting subjects	Buizer (1980), Lambert (2003), Davis and Davis (2004), Wonham and Carlton (2005), Minchin et al. (2006), Davis and Davis (2008) and Dupont et al. (2010)

environments, they may overgrow and out-compete native species and finally become dominant members of communities (Lambert and Lambert 2003; Bullard et al. 2007a; Aldred and Clare 2014). In some cases, population explosions of invasive ascidians reduce the abundance of other benthic species, resulting in transformation of community structure (Castilla et al. 2004; Rius et al. 2009). For example, invertebrate species richness in San Francisco Bay was negatively correlated with the abundance of a highly invasive ascidian, *C. intestinalis*, while other species were excluded or became rare in *C. intestinalis*-dominated communities (Blum et al. 2007). Moreover, invasive ascidians can affect ecological processes by reducing benthic–pelagic coupling. For example, dense mats formed by *D. vexillum* may prevent planktonic predators from foraging effectively from benthic communities (Lengyel et al. 2009; Mercer et al. 2009). In addition, animal-mediated modification of benthic habitats can influence both biotic and abiotic properties of invaded regions. For example, dense mats of *D. vexillum* can serve as physical barriers which can influence geochemical cycling of nutrients/elements and exchange of dissolved oxygen, leading to indirect, knock-on changes in benthic communities (e.g., Mercer et al. 2009).

Economically, the most reported negative impacts are associated with the aquaculture industry. Usually, invasive ascidians reduce harvest and increase production costs owing to competition with and biofouling on cultured species (Robinson et al. 2005; McKindsey et al. 2007; Adams et al. 2011; Fitridge et al. 2012; Fletcher et al. 2013). For example, up to a 50 % loss in shellfish harvest followed the invasion and population growth of *S. clava* in eastern Canada (Colautti et al. 2006). In addition, invasive ascidians compete with cultured species for food by reducing plankton from the water column and space by biofouling in aquaculture facilities (Petersen 2007; Ramsay et al. 2008; Lutz-Collins et al. 2009; Adams et al. 2011; Rius et al. 2011). A conservative estimate for the control of biofouling species—including many ascidians in aquaculture—ranges from 5 to 10 % of production costs, equivalent to US $1.5–3 billion per year (Fitridge et al. 2012). Owing to the largely negative effects, governments implement control and/or eradication programs, which incur significant costs. For example, the cost associated with eradication of *D. vexillum* in Shakespeare Bay, New Zealand, was as high as $650,000 ($NZD), though the effort failed to eliminate the species (Coutts and Forrest 2007). In 2005, $1,000,000 ($CAD) was allocated by the Canadian Government and Prince Edward Island Aquaculture Industry to research and monitoring of invasive ascidians in Atlantic Canada (Department of Fisheries and Oceans Canada 2005).

Factors underlying invasion success

Blackburn et al. (2011) proposed a unified framework for biological invasions that incorporates both distinctive stages for species moving between native and invaded habitats, and barriers between stages that serve to reduce overall invasion success. Differences in biological traits and genetic characteristics among NIS, the vectors that spread them, and environmental conditions of donor and recipient regions can define the magnitude of spread for NIS. Researchers have sought to understand causative factors associated with invasion success in many aquatic invaders, notably in invasive ascidians. Though some hypotheses have been successfully tested to explain invasion success, many questions remain poorly understood and require further exploration. Based on existing evidence, the availability of multiple human-mediated vectors (see detail in section "Natural versus vector-mediated dispersal"), unique biological (see detail in section "Biological characteristics") and/or genetic characteristics (see detail in section "Genetic patterns recovered by invasion genetics"), as well as environmental changes caused by human activities and global climate changes (see detail in section "Environmental changes"), may facilitate ascidians to overcome barriers at different stages and successfully invade new habitats (Fig. 2). Collectively, deep investigations into the roles of these factors, as well as their potential interactions, on invasion success make invasive ascidians good models to evaluate the generality of obtained results in invasion biology.

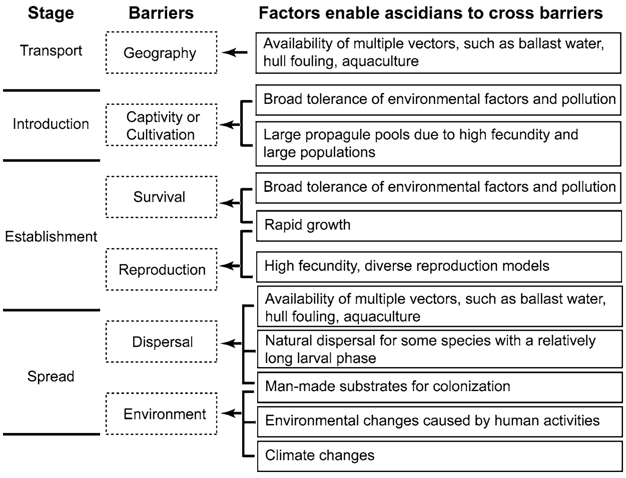

Fig. 2 Application of Blackburn et al.'s (2011) model to define invasion barriers and stages for ascidians. Factors that may enable ascidians to cross barriers at invasion stages are listed in the *right boxes*

Natural versus vector-mediated dispersal

Compared with many marine species with long planktonic larval phases, the life history of ascidians offers a great opportunity to discern between artificial (passive) and natural (active) dispersal. After a relative short planktonic larval phase (usually minutes to several days; Svane and Young 1989; Bingham and Young 1991; Marshall and Keough 2003), larvae metamorphose and settle down to become sessile adults. Due to short-lived tadpole larvae, ascidians do not naturally disperse far, usually just several meters or even less, especially in colonial species (Ayre et al. 1997). Large-scale dispersal can thus only be attributed to human-mediated transfers, resulting in widespread geographical distributions that we observed presently. Consequently, ascidians represent a rather unique system to study how and to what degree human-mediated versus natural dispersal contribute to geographical distributions of invasive species in the marine realm (e.g., Teske et al. 2014; Teske 2014).

Natural dispersal

While natural dispersal can still occur in several species, including relatively long-lived larvae advected by marine currents (e.g., 1–5 days for *C. intestinalis*) and rafting of fragmented colony parts in colonial species (e.g., *B. violaceus*), it seems likely that natural dispersal only influences fine-scale or local spread (Dijkstra et al. 2007; Bernier et al. 2009; Zhan et al. 2012). Indeed, ascidians have been found on some large crustaceans such as rock crabs (*Cancer irroratus*) and American lobsters (*Homarus americanus*). The observations suggest that ascidian “fellow travelers” attached on live species can spread and possibly colonize new locations (Bernier et al. 2009). Once an ascidian species becomes established, it often spreads locally and regionally via “stepping-stone” introductions associated with a variety of human-mediated vectors including movement of contaminated boats and equipments (Lambert 2007).

Human-mediated vectors

Human-mediated vectors that facilitate the introduction and spread of ascidians include ballast water (Svane and Young 1989), hull fouling (Coutts et al. 2003), aquaculture (Bernier et al. 2009), and recreational and fisheries watercraft (Darbyson et al. 2009a, b; Locke et al. 2009). Owing to varying biological and reproductive characteristics among ascidian species, the importance of each of these vectors varies widely, especially between solitary and colonial species (see detail in Table 1). For distant dispersal, ships’ hulls and sea chests represent major vectors transporting juvenile and/or adult ascidians (Godwin 2003; Lambert and Lambert 2003; Coutts and Dodgshun 2007; Coutts et al. 2010). In general, ballast water is not considered a principal vector for distant dispersal, mainly due to the short phase of free-swimming larvae. However, sediment, substrate, and/or the internal surfaces of ballast tanks may harbor ascidians (Rocha 2002; Briski et al. 2011). Given a high level of fecundity, long reproduction seasons, and extremely large populations in many major ports, a large number of larvae must be pumped into ballast tanks. Even if a small proportion of larvae pumped into ballast tanks can survive and are later released into recipient habitats, they may seed new populations and become new invasion sources. Despite the fact that viable ascidian larvae have been found in ballast water samples (e.g., Chu et al. 1997), it remains unknown whether ascidian larvae can survive, metamorphose, and mature during long voyages in ballast tanks and whether mature adults can reproduce in ballast tanks. Consequently, the direct and indirect roles of ballast water in spread of ascidians remain unclear and require further investigation.

In addition to hull fouling and ballast water, stock translocations and transfers of associated farming equipment are important vectors for transfers of ascidians (Lambert 2007). For example, *B. violaceus* and *D. vexillum* may have been introduced to the Gulf of Maine when Pacific oysters (*Crassostrea gigas*) were imported for aquaculture (Dijkstra et al. 2007). Transfers of mussels were considered responsible for introductions of *S. clava*, *B. violaceus*, and *B. schlosseri* throughout Prince Edward Island, Canada (Locke et al. 2007). Moreover, local and regional dispersal may be facilitated by pleasure craft and/or commercial vessels, which may be largely responsible for secondary spread to adjacent ports and harbors (Murray et al. 2011). For example in the southern Gulf of Saint Lawrence, recreational boating appears to be a more important vector for spread of ascidians than commercial fishing boats (Darbyson et al. 2009a).

In summary, the availability of multiple vectors facilitates ascidians to overcome barriers of geography and captivity/cultivation at the stages of transport and introduction, respectively (Fig. 2). Human-mediated dispersal, in combination with possible natural dispersal, helps ascidians overcome the dispersal barrier at the stage of spread (Fig. 2). The use of multiple spread/dispersal means, as well as unique life history traits mentioned above, makes ascidians good models to test many hypotheses regarding dispersal dynamics and geographical distributions and further evaluate how and to what degree these factors contribute to invasion success. There is little doubt that overcoming dispersal barriers involves an interaction between human-mediated vectors and species-specific biological characteristics. Below we discuss possible biological characteristics responsible for the introduction and rapid spread of invasive ascidians.

Biological characteristics

Broad tolerance of environmental conditions

Ascidians generally possess relatively broad tolerance of environmental conditions (Shenkar and Swalla 2011). Salinity and water temperature are among the most important environmental factors in marine ecosystems. Most ascidian species survive in salinities between 25 and 40 ‰, with only a few species surviving below 25 ‰ or above 44 ‰ (Lambert 2005; Gab-Alla 2008). However, some species, especially highly invasive ones, can survive a broader range of salinities, such as *C. intestinalis* at 12–40 ‰ (Therriault and Herborg 2008). Furthermore, *C. intestinalis* can withstand short-term exposure to salinity <11 ‰ (Dybern 1967; Therriault and Herborg 2008), making it the most tolerant ascidian species to low salinity reported thus far. Laboratory studies demonstrated that *B. schlosseri* and *B. violaceus* survived salinities at 14–38 and 20–38 ‰, respectively (Epelbaum et al. 2009).

Aside from salinity, ascidians can also tolerate and survive a wide range of temperatures (Lambert 2005; Rius et al. 2014b). For example, laboratory experiments showed that *B. schlosseri* and *B. violaceus* could survive water temperature at 10–25 and 5–25 °C, respectively (Epelbaum et al. 2009). Similarly, *C. intestinalis* can survive at water temperature as high as 35 °C (Dybern 1965). Generally, mortality of adults increases when water temperature is lower than 10 °C, but in Atlantic Canada *Ciona* populations have survived for several months at ~−1 °C (Carver et al. 2003; Therriault and Herborg 2008). In addition, invasive ascidians can tolerate rapid temperature changes. For example, *Didemnum* species can tolerate water temperatures ranging from <1 to >24 °C, with daily changes of up to 11 °C (Valentine et al. 2007).

Besides tolerance to a large range of temperature and salinity, some ascidian adults have a high level of tolerance to pollution, including heavy metals such as mercury, iron, and copper (Beiras et al. 2003). A recent study demonstrated that tolerance varied among developmental stages: Fertilization and larval development were the most sensitive stages to abiotic factors and early development could not be completed under prevailing conditions where adults occurred (Pineda et al. 2012). Tolerance of pollution and key environmental factors may facilitate invasive ascidians to live in fast-changing environments during transportation and introduction stages, such as when entrained in ballast tanks. In addition, these characteristics may facilitate invasive ascidians to rapidly adapt to different environments in recipient habitats (i.e., to cross the barrier at the establishment stage, Fig. 2), although the mechanism(s) of rapid local adaptation remain unclear in many species.

Rapid growth and diverse reproduction

Rapid growth rate and relatively high fecundity may also contribute to invasiveness of ascidians (Yamaguchi 1975; Lambert 2007). For example, *C. intestinalis* complex and *Ciona savignyi* can reach sexual maturity in 2 months (Yamaguchi 1975; Nomaguchi et al. 1997; Carver et al. 2003; Rius et al. 2014a). *C. intestinalis* can produce gametes continually as long as water temperature is suitable, and each mature individual can potentially spawn once daily over the spawning period and release approximately 500 eggs per day (Carver et al. 2003). Compared with solitary species, colonial ascidians generally have a lower level of fecundity, even though a mature colony can release 7–20 well-developed tadpole larvae per week (Milkman 1967; Lambert 2005). Rapid growth and high fecundity enable invasive ascidians to overcome both survival and reproduction barriers at the establishment stage (Fig. 2). In addition, an extremely high level of fecundity, coupled with large populations, can produce large propagule pools that can be introduced to distant locations through multiple vectors, and/or disperse naturally to neighboring regions.

Reproductive modes are diverse in ascidians. Self-fertilization was reported in some ascidians such as *C. eumyota* in its invaded ranges (Dupont et al. 2007). Such reproduction strategy enables invaders to initiate a new population from a limited number of individuals, to survive genetic bottlenecks, and to rapidly adapt to local environments (Cohen 1996; Dupont et al. 2007). Besides sexual reproduction, colonial ascidians such as *B. schlosseri* and *B. violaceus* can increase colony size through the asexual production of zooids and disperse asexually through fragmentation (Lambert 2005; Bullard et al. 2007b). Diverse reproduction modes provide a short-term strategy by which invasive ascidians can escape the negative demographical consequences of small population size during biological invasions (i.e., reproduction barrier at the establishment stage, Fig. 2).

In summary, the unique biological characteristics of ascidians facilitate overcoming barriers during biological invasions, such as broad tolerance of environmental factors/pollutions for overcoming the captivity/cultivation and survival barriers at the introduction and establishment stages, and high fecundity and diverse reproduction models for crossing the reproduction barrier at the establishment stage (Fig. 2). In addition, the common and contrasting biological traits in different invasive ascidians make them a good system to test whether the contribution of biological traits to invasion success is species-specific or a general pattern.

Genetic patterns recovered by invasion genetics

In invasion genetics, approaches common to the field of population genetics, phylogeography, and phylogenetics are used to answer questions in invasion biology. Since the publication of the classic volume on “The Genetics of Colonizing Species” (Baker and Stebbins 1965), much has been learned by investigating genetic and evolutionary mechanisms underpinning biological invasions (Rius et al. 2015b; Barrett 2015; Bock et al. 2015). Breakthroughs (see examples highlighted in Table 2) include the knowledge that reduced genetic diversity in newly established invasive populations is not commonplace (Kolbe et al. 2004; Roman and Darling 2007; Dlugosch and Parker 2008), that novel ecological contexts can drive contemporary post-establishment adaptive evolution (Lee 2002; Bossdorf et al. 2005; Prentis et al. 2008; Vandepitte et al. 2014), and that intra- and interspecific hybridization can result in the formation of particularly virulent invasive genotypes (Ellstrand and Schierenbeck 2000; Kolbe et al. 2004; Rius and Darling 2014). Studies of invasive ascidians have mirrored many of these advances (Table 2) and are poised to contribute to our understanding of the genetic causes and consequences of biological invasions.

Intraspecific genetic diversity within populations

At an elementary level, invasion genetics studies have characterized within-population genetic variation, most readily measured with neutral loci (Table 2). Some invasive ascidian populations were shown to contain only modest levels of polymorphism. This is, for example, the case of *C. eumyota*, a solitary ascidian originating from the southern hemisphere that was recently introduced to the English Channel (Lambert 2004). Using 12 microsatellite markers, Dupont et al. (2007) investigated genetic diversity maintained in native and introduced *C. eumyota* populations. Irrespective of their geographical origin, surveyed populations exhibited extremely reduced variation, with 40 % of markers recovered as monomorphic. This finding was attributed to the interplay between recent bottlenecks and self-fertilization, two factors known to increase genome-wide homozygosity. While the finding of uniformly reduced population genetic diversity is not common for studies of invasive ascidians, the results of Dupont et al. (2007) advanced intriguing evolutionary questions. For example, does self-fertilization provide any long-term benefits to invasive ascidian populations? To what extent might this mode of reproduction contribute to the gradual unmasking of deleterious recessive mutations, with negative fitness consequences?

On the other extreme, invasive ascidian populations that are unvaryingly genetically diverse have also been reported, as in the case of *M. squamiger*, *S. clava,* or *Styela plicata* (e.g., Dupont et al. 2009; David et al. 2010; Rius et al. 2012). Indeed, this has been a comparatively much more common finding. The increased polymorphism in surveyed populations was attributed to a lack of bottleneck upon introductions (David et al. 2010) and/or to post-introduction gene flow from original gene pools and/or from admixture with genetically differentiated gene pools (Rius et al. 2012 but see Ordoñez et al. 2013). These studies provided an indication that ascidian invasions are often associated with heightened “propagule pressure” (i.e., large inocula) scenarios. In the majority of ascidian invasions, the pattern recovered can vary between these two extremes because numerous factors can affect diversity. As mentioned above, the reproductive biology, invasion history, or local availability of natural and human-mediated vectors of dispersal should vary in their relative contribution on a species- and population-specific basis. In agreement with this expectation, many genetic studies on invasive ascidians have revealed a discontinuous distribution of genetic variation, with genetically depauperate populations detected in the vicinity of highly polymorphic ones (e.g., Zhan et al. 2010, 2012; Goldstien et al. 2011; Lejeusne et al. 2011; Darling et al. 2012; Pérez-Portela et al. 2012).

While neutral loci are informative with regard to the demographical dynamics of ascidian populations and propagule pressure, neutral within-population genetic diversity does not necessarily reflect genetic variation relevant to ecological success (Roman and Darling 2007). This is particularly likely when selective forces outweigh the effect of genetic drift, in which case genetic variation of neutral molecular markers will not be indicative of diversity at quantitative trait loci related to fitness (Reed and Frankham 2001; McKay and Latta 2002; Roman and Darling 2007). As such, levels of polymorphism estimated using neutral markers may be less informative in the importance of genetic diversity per se to the success of ascidian invasions. Long-term studies incorporating both high-throughput sequencing and ecologically relevant quantitative trait polymorphisms are required to know whether and why genetically depauperate introduced populations are more likely to become extinct or, similarly, whether and why genetically polymorphic ones are predisposed to spread and become invasive (e.g., Pérez-Portela et al. 2012; Reem et al. 2013). Although these studies have been conducted on model species including *Arabidopsis thaliana* (Crawford and Whitney 2010) and *Eurytemora affinis* (Lee et al. 2011), studies on ascidians await investigation.

Intraspecific genetic diversity between populations

In addition to documenting within-population levels of genetic diversity, researchers have investigated how genetic variation is partitioned between populations. When

Table 2 Examples of recent studies of comparing genetic diversity of ascidian species populations

Species	Main research question/hypothesis	Molecular markers	Ranges included	Patterns recovered	References
Botrylloides violaceus	Vectors and pathways of post-establishment spread	SSRs, mtDNA	I	Variable genetic diversity, contrasting colonization patterns across the introduced range	Bock et al. (2011)
Botrylloides violaceus *Botryllus shlosseri*	Genetic structure and invasion history	mtDNA	N, I	Variable genetic diversity, contrasting patterns of invasion for the two species	Lejeusne et al. (2011)
Botryllus schlosseri	Range limits across a species phylogeny	SSRs, mtDNA, rDNA	N, I	Variable genetic diversity, cryptic species in a model ascidian, with contrasting ranges	Bock et al. (2012)
Botryllus schlosseri	Vectors and pathways of post-establishment spread	SSRs, mtDNA	I	Variable genetic diversity, differential contribution of vectors to post-establishment spread	Lacoursiere-Roussel et al. (2012)
Botryllus schlosseri	Invasion source of populations in California	SSRs	I	Either European or Asian populations are the most likely source for Californian populations	Stoner et al. (2002)
Botryllus schlosseri	Genetic structure of harbor and openshore populations	mtDNA	N, I	Strong genetic structure, long-distance colonization, occasional gene flow-through ship transport	López-Legentil et al. (2006)
Ciona intestinalis	Degree of population connectivity at various spatial scales	SSRs, mtDNA, rDNA	N, I	High population connectivity at local and continental scales, cryptic species in a model ascidian	Zhan et al. (2010)
Ciona intestinalis	Present-day genetic patterns in the introduced range	SSRs, mtDNA	I	Variable genetic diversity, complex genetic patterns, multiple evolutionary processes may be responsible for such complex patterns	Zhan et al. (2012)
Corella eumyota	Genetic diversity in native and introduced populations	SSRs	N, I	Reduced genetic diversity, high selfing rate inferred	Dupont et al. (2007)
Diplosoma listerianum	Present-day and historical genetic patterns	mtDNA	N, I	Variable genetic diversity, cryptic species, complex patterns of population genetic connectivity	Pérez-Portela et al. (2013)
Clavelina lepadiformis	Genetic relationships between populations in different regions	mtDNA	N	Low genetic divergence and high gene flow between regions	Turon et al. (2003)
Herdmania momus	How genetic variation is partitioned between sides of the Suez Canal	mtDNA	N, I	Two well-differentiated colonization histories across the eastern Mediterranean	Rius and Shenkar (2012)
Microcosmus squamiger	Invasion history and pathways of introduction	mtDNA, SSRs	N, I	High genetic diversity, non-independent colonizations, invasion histories well resolved using genetic analyses	Rius et al. (2008, 2012)
Molgula manhattensis	Natural versus human-mediated dispersal	mtDNA	N, I	Low diversity in Europe due to a recent expansion, *M. manhattensis* remains cryogenic in Europe.	Haydar et al. (2011)
Perophora japonica	Temporal genetic patterns and spatial genetic structure	mtDNA	I	Loss of genetic diversity, genetic structure was consistent with the historical records	Pérez-Portela et al. (2012)

Table 2 continued

Species	Main research question/hypothesis	Molecular markers	Ranges included	Patterns recovered	References
Styela clava	Vectors and pathways of post-establishment spread	SSRs	I	High genetic diversity, source-sink post-colonization population dynamics	Dupont et al. (2009)
Styela clava	Vectors and pathways of post-establishment spread	SSRs, mtDNA	I	Moderate genetic diversity, differential contribution of pre-border and post-border vectors	Goldstien et al. (2010)
Styela clava	Invasion history and pathways of introduction	mtDNA	N, I	Moderate genetic diversity, multiple introduction in some populations	Goldstien et al. (2011)
Styela clava	Vectors and pathways of post-establishment spread	SSRs	I	Multi-vector post-establishment spread, multiple introduction in some populations	Darling et al. (2012)
Styela plicata	Present-day and historical genetic patterns	mtDNA, nuDNA	N, I	Variable genetic diversity, stochastic introduction events	Pineda et al. (2011)

"SSR" denotes nuclear microsatellites, "mtDNA" refers to mitochondrial DNA sequences, "nuDNA" refers to non-ribosomal nuclear DNA sequences, "rDNA" denotes nuclear ribosomal DNA sequences, "I" indicates populations sampled in the invaded range, while "N" indicates populations sampled in the native range

incorporating native range sampling, a main goal has been to clarify the history of ascidian invasions and to identify the most probable sources and pathways of introductions and post-establishment spread (e.g., Lejeusne et al. 2011; Pineda et al. 2011; Rius et al. 2012). This is an issue of primary importance, since any interpretation regarding contemporary genetic patterns of invasions stands on firmer footing when information on the initial introduction source is available. Unfortunately, the majority of studies did not find convincing genetic evidence to clarify native ranges of invasive ascidians, mainly owing to genetic homogenization derived from frequent human-mediated introductions and recurrent introductions from introduced ranges to native ranges. Moreover, evolutionary trajectories in established populations may well differ depending on the type of dispersal vector(s) involved (Wilson et al. 2009).

While greatly benefiting from the integration of historical taxonomic records with new analytical approaches such as approximate Bayesian computation (ABC; e.g., Rius et al. 2012), the study of ascidian invasion histories has been greatly complicated by two obstacles. The first obstacle is that most ascidians have long and intricate invasion histories. Frequent association with vessel- and aquaculture-based transplantation, two crucial vectors that continuously shuffle genetic variation between populations, can obscure pathways of invasion beyond the point where confident inferences of invasion source may be drawn (e.g., Pineda et al. 2011). The second obstacle is that a number of highly invasive ascidians are native to regions that have been relatively inaccessible to researchers until recently and, thus, have been severely undersampled (e.g., the Indo-Pacific; Shenkar and Swalla 2011). This problem is vividly illustrated by *S. plicata*, a widely introduced ascidian native to the northwest Pacific Ocean for which even the "type" specimen used to describe this species was obtained from the hull of a ship in Philadelphia (Pineda et al. 2011). Since confident inferences of invasion histories and vectors rely upon comprehensive coverage of native ranges, increased efforts targeting, for example, sampling and clear invasion history are needed (Muirhead et al. 2008).

Studies of ascidian invasion routes have also considered only populations from outside a taxon's native range, when aiming to understand the dynamics of colonization after initial establishment. In this context, patterns of population genetic connectivity have been used to formulate hypotheses regarding the contribution of environmental variables to colonization dynamics (e.g., David et al. 2010), the importance of sexual versus asexual propagules of dispersal (e.g., Bock et al. 2011), and the relative roles of alternative pathways and vectors of post-establishment spread (e.g., Dupont et al. 2009; Goldstien et al. 2010; Bock et al. 2011). Unfortunately, only a few studies have attempted to verify hypotheses formulated from genetic data with independent measures of population connectivity obtained in the field. We are aware of only two such studies: one using direct information on the intensity and directionality of boat traffic during the spread of *S. clava* in the northeastern Pacific (Darling et al. 2012) and the other using interviews with recreational boat owners as an measure of vector activity during the spread of *B. schlosseri* in the northwestern Atlantic (Lacoursiere-Roussel et al. 2012). Both studies leveraged vector information to provide a more complete picture of post-establishment spread. Future studies are required to integrate multiple data sources such as shipping data, field survey data and genetic data to test hypotheses regarding population connectivity at different geographical scales.

Interspecific genetic diversity

Although intraspecific patterns of genetic polymorphism have been tremendously revealing, perhaps one of the most surprising results to emerge during the past decade from studies of ascidian invasion genetics is at the interspecific level of biological organization. Notably, genetic surveys have made clear that cryptic speciation is very common in invasive ascidians (e.g., Caputi et al. 2007; Pérez-Portela et al. 2009, 2013; Zhan et al. 2010; Bock et al. 2012). Interestingly, in at least three of these cryptic species complexes, namely *C. intestinalis* (Zhan et al. 2010), *B. schlosseri* (Bock et al. 2012), and *Diplosoma listerianum* (Pérez-Portela et al. 2013), one or a limited number of sister taxa have been inferred as being invasive, while the rest appear to be highly geographically restricted. These patterns raise the intriguing possibility that differential invasive potential has evolved in parallel in each of these lineages. Given that species from two of these species complexes (*B. schlosseri* and *C. intestinalis*) are genetic model systems that have had their genomes sequenced (Dehal et al. 2002; Voskoboynik et al. 2013), it now seems appropriate and possible to leverage high-throughput sequencing technologies to investigate the underpinnings of ascidian invasiveness at the genome level (see recent reviews by Chown et al. 2015; Rius et al. 2015a). One promising approach would be to use whole-genome re-sequencing or reduced representation libraries to perform population genomic scans for "outlier loci." Such studies could provide valuable information on the number and location of targets of selection during the evolution of invasiveness. In addition, provided that "invasive" traits are identified for the *B. schlosseri* and *C. intestinalis* systems, mapping approaches, consisting of biparental mapping and/or genome-wide association mapping, can be used to clarify their genetic architecture. In addition, the advent and fast development of high-throughput sequencing has made it possible to perform surveys at the genome level for non-model species. Over a longer term, this information could be applied to understand the timing of genomic changes that prompted parallel evolution of invasiveness in both model and non-model ascidians.

In summary, in addition to the power of genetic methods on resolving longstanding questions such as invasion sources and pathways (see review by Geller et al. 2010), invasion genetics can help deeply understand one of the major challenges in biological invasions, that is, evolutionary and ecological processes in natural populations. Indeed, studies of invasive ascidians have mirrored many advances (Table 2) and are expected to continuously contribute to our understanding of the causes and consequences of biological invasions. In particular, the two model ascidians, *C. intestinalis* and *B. schlosseri*, represent ideal models for ecological and evolutionary studies in the wild. Their relative small genomes (~160 MB for *C. intestinalis* and ~600 MB for *B. schlosseri*) make them attractive species to study genetic and evolutionary components of invasion success using natural populations at a whole-genome level. Genome-level studies can largely help decipher evolutionary potentials of invasive species in the invaded habitats and, more widely, understand the microevolutionary causes and consequences of natural populations in response to changing environments.

Environmental changes

Many ecological factors including hydrodynamics (Holloway and Connell 2002), substrate condition (Shenkar et al. 2008), predation (Pisut and Pawlik 2002; Rius et al. 2014a), and competition (Castilla et al. 2004; Rius et al. 2014a) can potentially influence the spatial distribution of ascidians. Environmental changes associated with human activities (such as coastal construction, overfishing and aquaculture) and global climate changes (such as global warming and El Niño events) have directly and/or indirectly affected such ecological factors, thus facilitating spread and colonization of invasive ascidians (Lambert 2007).

Human activities have strongly disturbed coastal environments. Increased sediment runoff caused by coastal construction and sewage outflow associated with rapid shoreline development and/or urbanization can enhance food supply to suspension feeders including ascidians (Lambert 2007; Simkanin et al. 2012; Airoldi et al. 2015). In addition, alternation of natural communities caused by various human activities can largely reduce species richness and biodiversity, potentially increasing community vulnerability to invasion (Stachowicz et al. 2002). Rapid development of aquaculture in recent decades provides man-made structures (ropes, nets, cages, etc.) and even live animals (shells of oysters, mussels, lobsters, etc.) for colonization by ascidians (McKindsey et al. 2007). Man-made substrates are often the initial site of settlement for ascidians owing to availability of space and simple or nonexistent fouling communities (Lambert 2007). Global climate changes including surface warming and/or El Niño conditions have affected temperate waters in many regions where invasive ascidians have colonized (Lambert 2007; Rius et al. 2014b). For example, *Styela canopus* has successfully established along the coast of Southern California during an El Niño event in the 1990s (Lambert and Lambert 1998, 2003). A recent empirical study suggests that altered seawater temperature regimes associated with climate change foster the spread and abundance of invasive ascidians across multiple spatial scales (Rius et al. 2014b).

In summary, the rapid and widespread colonization by ascidians along many coastal areas globally allows us to investigate the contribution of environmental change to invasion success. Repetitive invasion patterns such as invasions by the same species in different types of habitats or invasions by different species to the same habitats may help us understand the generality of environmental changes as a driver of ascidian invasions.

Current policy, rapid response, and control of spread

Current policy

Given that many factors may contribute to the invasion success, a huge challenge exists to develop effective control and prevention strategies for management of ascidian invasions. Most countries currently lack science-based policies to prevent human-mediated introductions of ascidians, though some have made progress (see a summary in Table 3). Even though much effort has been made to understand the causative factors responsible for invasion success, as mentioned above, current policies and management solutions largely target vectors, especially those associated with human activities. The policy information we summarize below might not be complete mainly owing to language and/or information barriers.

Ballast water management, generally conducted as ballast water exchange, is the most common management requirement and is mandatory for commercial shipping entering the USA, Canada, Australia, and New Zealand (United States Coast Guard (USCG) 1993; International Maritime Organization (IMO) 2004; Government of Canada 2006; Locke et al. 2009; Locke and Hanson 2009a; New Zealand Government 2010; Australian Government 2011). In addition to ballast water exchange, clean hulls are required for all ships entering New Zealand's waters (New Zealand Government 2010). Canada and Australia currently follow voluntary guidelines for hull husbandry (International Maritime Organization (IMO) 2011; Australian Government 2013), but there are either loose or no regulations for other major vectors such as fishery and aquaculture industries (Locke et al. 2009). Aquaculture managers in Prince Edward Island, Canada, had to obtain permits to transfer mussels between farming zones to prevent the spread of invasive ascidians during the periods of hard infestation of aquaculture farms (Locke et al. 2009; Locke and Hanson 2009a). Similarly, the Netherlands temporarily halted the import of mussels for aquaculture from infested countries such as Ireland during infestation of the Zeeland area, as well as within country movement from infested to uninfested areas (Gittenberger 2009). Currently, these applied policies appear effective to prevent new introductions and spread (Gittenberger 2009; Locke et al. 2009; Locke and Hanson 2009a; Department of Fisheries, Aquaculture and Rural Development 2012, 2014) and should be adopted by countries that currently lack policies for regulating human-mediated movement of ascidians.

Table 3 List of current policies to prevent human-mediated movement of ascidians

Management	Policy's target	Country	Mandatory/voluntarily	References
Ballast water exchange	Prevention of movement of all aquatic species	Australia	Mandatory	Australian Government (2011)
		Canada	Mandatory	Government of Canada (2006)
		New Zealand	Mandatory	New Zealand Government (2010)
		The USA	Mandatory	United States Coast Guard (USCG) (1993)
Ships hulls husbandry	Prevention of movement of all aquatic species	Australia	Voluntarily	Australian Government (2013)
		Canada	Voluntarily	International Maritime Organization (IMO) (2011)
		New Zealand	Mandatory	New Zealand Government (2010)
Restrictions of movement of aquaculture species	Prevention of movement of ascidian species	Canada—only Prince Edward Island	Mandatory—but temporary	Locke et al. (2009) and Locke and Hanson (2009a)
		The Netherlands	Mandatory—but temporary	Gittenberger (2009)

Early detection, rapid response, and eradication

Rapid response may incorporate pre-introduction, early detection, and post-incursion elements (McEnnulty et al. 2001; Wotton and Hewitt 2004; Locke and Hanson 2009b). Development of appropriate protocols and action plans before the introduction of new species enhances rapid response by insuring: (1) availability of tools with which to respond; (2) legal authority approval to conduct rapid response actions; and (3) availability of resources to carry out the response (Locke and Hanson 2009a). Managers and government officials can create a "watch list" for particular species in regions with high invasion risk (Ricciardi and Rasmussen 1998; Locke 2009). The "watch list" represents the first step in predicting which species may be introduced to an area and may be combined with environmental suitability assessments to further refine establishment risk upon potential introductions (Ricciardi and Rasmussen 1998; Locke 2009). For example, applying both shipping (i.e., introduction) and climate zone filters (i.e., environmental suitability), Locke (2009) constructed a "watch list" with 17 out of a possible 57 species with invasion histories worldwide that might be introduced to and successfully survive in waters of Atlantic Canada.

The created "watch list" should be further used for screening of areas under concern. Based on the vectors described above, areas under concern include: (1) those in close proximity to shellfish aquaculture sites and processing facilities; (2) commercial ports; (3) marinas and/or yacht clubs; and (4) commercial fishing harbors (Sephton et al. 2011). Inspection of these areas should be conducted regularly, for example by examining floating docks and aquaculture equipment (Grey 2009), or by deploying monitoring collectors to assess for presence of species of concern (Sephton et al. 2011). Given such surveys are labor-intensive and time-consuming, environmental DNA (eDNA) in combination with high-throughput sequencing or microarrays represents a promising screening strategy for "dangerous" ascidians. More conveniently, such a strategy can be used to detect target species by sampling water from the areas of concern without ever detecting living animals (Lodge et al. 2012; Thomsen et al. 2012; Egan et al. 2013). Moreover, these genetic techniques are very powerful at detecting species at low population abundance (Zhan et al. 2013) and may be used to powerfully screen for all invasive ascidian species simultaneously as long as technical issues are well addressed (e.g., Zhan et al. 2014a, b; Zhan and MacIsaac 2015). In addition, to promote community-based monitoring, ascidian identification cards and posters should be produced and distributed to the general public, aquaculture managers, fish harvesters, processing plant managers, and boaters (Sephton et al. 2011). Web sites and toll-free telephone numbers to report sighting should also be available (Locke and Hanson 2009a).

Detection of any species either using a "watch list" or genetic methods should be followed by conducting well-prepared action plans in advance (Locke and Hanson 2009a). An action plan should include: (1) notification of aquaculture managers, fish harvesters, processing plant managers, and boaters of newly introduced species; (2) determination of distribution of introduced species; (3) containment of the invaded area; (4) assessment of the likely efficacy of eradication attempts; and (5) monitoring of the success of eradication attempts (Locke and Hanson 2009a). If a species is detected that is not present on the "watch list", a preliminary risk assessment should be undertaken to determine whether it should be added to a "watch list" and whether rapid response actions are required (Locke and Hanson 2009a). Given previous experiences of ascidian invasions in Prince Edward Island, all unrecognized ascidians or ascidian-like organisms are now treated as a species of concern by the Prince Edward Island Aquatic Invasive Species Steering Committee (Locke and Hanson 2009a). Eradication should be performed immediately and before the development of individuals capable of dispersing (Locke et al. 2009). Eradication may be: (1) physical/mechanical removal of individuals by hand/machinery, by exposure to heat, or by protracted drying of aquaculture structures, docks, and buoys (Clancey and Hinton 2003; Locke and Hanson 2009a; Muñoz and McDonald 2014); and/or (2) chemical exposure of aquaculture structures, docks, and buoys to sodium hypochlorite, salt brine, hydrated lime, or freshwater and acetic acid (Carver et al. 2003; Locke et al. 2009; Muñoz and McDonald 2014). Post-eradication should be followed by monitoring for surviving individuals (Locke and Hanson 2009a). The Aquatic Invasive Species Steering Committee in Prince Edward Island agreed that the whole province would be "clean" after 2 years without detection of a particular ascidian species (Locke and Hanson 2009a). While some areas, such as the Foxley River, have been declared clean, many others remain infected by ascidian species (Department of Fisheries, Aquaculture and Rural Development 2012, 2014).

Control of spread

The order of actions in rapid response is: (1) prevention of introductions; (2) eradication of introduced populations; (3) control of spread; (4) reduction in populations to tolerable level; and (5) do nothing. If a species is already well established and eradication is not possible, reducing spread and population density to an economically and ecologically tolerable level are good options (Locke and Hanson 2009a).

In addition, the movement of aquaculture equipment from infested to uninfested areas should be strictly restricted, screening systems should be placed on the outflow of all aquaculture sites and processing facilities, and all equipment should be cleaned before movement to uninfested areas (Locke et al. 2009). Ideally, separate equipment is used in infested and uninfested waters. Cleaning of hulls of recreational boats and commercial fishery vessels is also necessary (Bernier et al. 2009; Locke et al. 2009).

In summary, successful quarantine measures and control of human activity-associated vectors in several countries largely affected by invasive ascidians such as Canada provide good examples on science-based policies and management solutions to other countries. All obtained results based on invasive ascidians, as well as many ongoing projects, provide models to evaluate the effectiveness and efficiency of applied policies and management solutions on how to prevent colonization and spread of both invasive ascidians and other similarly notorious invasive species.

Future perspectives

Even though the study of ascidian invasions has become a productive and insightful area of marine invasion biology, many fundamental questions remain to be answered and effective technologies and management strategies are largely required to possibly stop future invasions. Below we propose future research perspectives to fill many research gaps in marine invasion biology using invasive ascidians as models.

Cause of introductions and widespread

Given that human activities such as shipping and aquaculture are increasing globally, both the ecological and economic impacts caused by the introduction and spread of ascidians are expected to increase at local, regional, and global scales. Although efforts have been made to dissect how and why biological invasions occurred over the past several decades, causative factors for successful invasions are not well understood for many invaders. Future studies should also be directed to explore the roles of and interactions among introduction vectors, biological characteristics, and environmental/ecological changes. Blackburn et al.'s (2011) model for biological invasions provides a good platform to organize a series of comprehensive studies to understand how these factors, either alone or by interactions, underlie the invasion success. Such studies are expected to not only contribute to control and prevention of future introductions and spread, but also answer numerous fundamental questions in evolutionary biology and ecology, such as dynamics of rapid local adaption, tolerance to environmental factors/pollution, and interactions between members in communities.

Invasion genetics

With the recent development of sequencing technologies (Helyar et al. 2011), which enable the simultaneous discovery and genotyping of thousands of genome-wide genetic variants, invasion genetics is transitioning from the genetic to the genomic scales. As a result, it is now possible to perform analyses that only a few years ago were out of reach. These include conducting genomic scans for "outlier loci" that may be involved in possible rapid local adaptation during biological invasions, or identifying specific chromosomal regions that may have been transferred between genotypes via intraspecific or interspecific admixture (Chown et al. 2015; Rius et al. 2015a). To fully exploit these technological advances, however, genetic data should also be paired with sound experimental approaches to identify and quantify quantitative variation in traits that confer increased virulence for specific genotypes of invasive ascidians. These additions will allow us to bridge the invasive genotype–phenotype gap and to better understand the evolution of invasiveness (see review by Chown et al. 2015). In addition to further genetic investigation, mounting evidence suggests that heritable variation in ecologically induced traits could be derived from a suite of epigenetic mechanisms, even in the absence of genetic variation (see perspective by Bossdorf et al. 2008). Consequently, a combination of multiple approaches, especially those derived from recently developed fields such as genomics, epigenomics, transcriptomics, and proteomics, should be systematically employed to investigate the structure of invasive populations as well as the interrelations between genetic, epigenetic, phenotypic variation, and ecological interactions. Since model invasive ascidians such as *C. intestinalis* species complex, *C. savignyi* and *B. schlosseri* species complex have relatively small genomes, these species provide an effective and time-/cost-saving models to perform genome-wide surveys.

Management solutions

Governments throughout the world should prevent future spread of invasive ascidians by developing and implementing policies and prevention measures. Management efforts to reduce the risk of new introductions provide realistic opportunities to mitigate the ecological and economic uncertainty imposed by invasive ascidians, and they are more effective and less costly than eradication or curtailment of spread of established populations (Leung et al. 2002; Lodge et al. 2006; Hulme et al. 2008; Reaser et al. 2008). Besides prevention of new introductions, screening of areas of concerns regularly will be necessary to detect individuals that successfully evade prevention measures (Sephton et al. 2011). In addition to effective policy and management solutions, developing practical antifouling technologies that can be used for commercial shipping, recreational boats, and aquaculture could reduce the scale of both primary and secondary introductions (e.g., Cahil et al. 2012). Finally, future development and application of robust detection tools such as microarrays and high-throughput sequencing based on environmental DNA may greatly enhance the power of early detection of new infestations (Lodge et al. 2012; Thomsen et al. 2012; Egan et al. 2013; Zhan et al. 2013).

Conclusions

Even though the major causes for invasiveness and invasion success often remain unknown, studies on invasive ascidians have successfully identified many factors favoring their invasions. These factors include the availability of multiple vectors, biological and genetic characteristics, and environmental changes. Various ongoing studies and proposed future surveys on invasive ascidians, especially those based on model species complexes including *C. intestinalis* and *B. schlosseri*, can recover key ecological and evolutionary processes responsible for successful invasions. The use of these model species facilitates answers to both fundamental questions such as factors underlying invasion success and applied topics such as policies and management solutions. In addition, the comparison between invasive and noninvasive species with different biological characteristics and life history traits may help elucidate factors underlying invasiveness and favoring invasion success. Based upon a better understanding of invasion patterns and dynamics, more effective policies and management strategies may be developed to predict and possibly prevent future invasions. In conclusion, invasive ascidians provide promising models to test hypotheses in marine invasion biology, to evaluate the generality of results obtained in species with different biological/genetic characteristics to explain causes and consequences of invasion success, and to evaluate the efficiency of science-based policies and management strategies.

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