

Evolutionary history of the dynamic horseshoe crab

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The American Horseshoe Crab *Limulus polyphemus* is an important ecological component of the United States Atlantic coastline, particularly in areas such as Delaware Bay, where large numbers congregate on beaches to spawn. These spawning events are crucial sources of food for shorebirds including migrating Red Knots *Calidris canutus*, which feed upon the eggs deposited within the sand. Red Knot numbers have steadily declined over the last few decades in direct response to decreases in the numbers of breeding horseshoe crabs, which are harvested by the biomedical industry and for bait by eel and conch fisheries. Red Knot conservation is intrinsically tied to horseshoe crab conservation, and harvesting restrictions have been in place since the early 2000s, although with apparently limited effect. Horseshoe crabs have a fossil record stretching back hundreds of millions of years, and their apparent morphological stasis over this time has fed their reputation as ecological generalists that are able to survive any environmental change. Despite their reputation for morphological and ecological conservatism, horseshoe crabs have undergone major marine to freshwater transitions at least two times in the past. The four extant horseshoe crab species (none of which have a fossil record) represent just a portion of the geographic and ecological diversity of a lineage which is not particularly immune to extinction. This review explores the evolutionary history of horseshoe crabs, from their origins in Ordovician seas, through the height of their diversity in Carboniferous coal swamps, to their restricted modern distribution. The economic, ecological, and scientific importance of horseshoe crabs is reviewed, and conservation concerns surrounding both American and Asian species are discussed.

Keywords

American Horseshoe Crab
Limulus polyphemus
evolution
paleontology
living fossil
Xiphosura
conservation

INTRODUCTION

Few modern organisms evoke a sense of the primeval as does the horseshoe crab. Commonly seen shuffling awkwardly onto land to reproduce, yet surprisingly graceful in their usual aquatic habitat, horseshoe crabs are striking in both their outward dissimilarity from any other extant arthropod group (Fig. 1) and their sheer size. Large size among arthropods is often evoked as a relic of the distant past, from an age when giant arthropods ruled the Carboniferous. With a maximum length of just over half a meter, horseshoe crabs certainly appear to be remnants of grander times. Despite their antiquated look, horseshoe crabs can still yield scientific surprises; for example, the fact that they fluoresce under ultraviolet light was only documented in 2017 (Rubin *et al.* 2017).

Perhaps in part because of their archaic appearance, horseshoe crabs have a checkered taxonomic history. As indicated by their common misnomer, horseshoe ‘crabs’ were once considered to be relatives of crustaceans (Owen 1873), largely due to their aquatic habitat. Detailed comparative anatomical studies of the internal morphology of the horseshoe crab *Limulus* and scorpions led Lankester (1881) to propose a close relationship between horseshoe crabs and arachnids. Despite some initial disagreement (Packard 1882), the realization that horseshoe crabs are chelicerates set the foundations for modern interpretations of arthropod relationships.

The historical ambiguities of horseshoe crab taxonomy extend down to the species level. The number of valid extant species was under debate as recently as the late

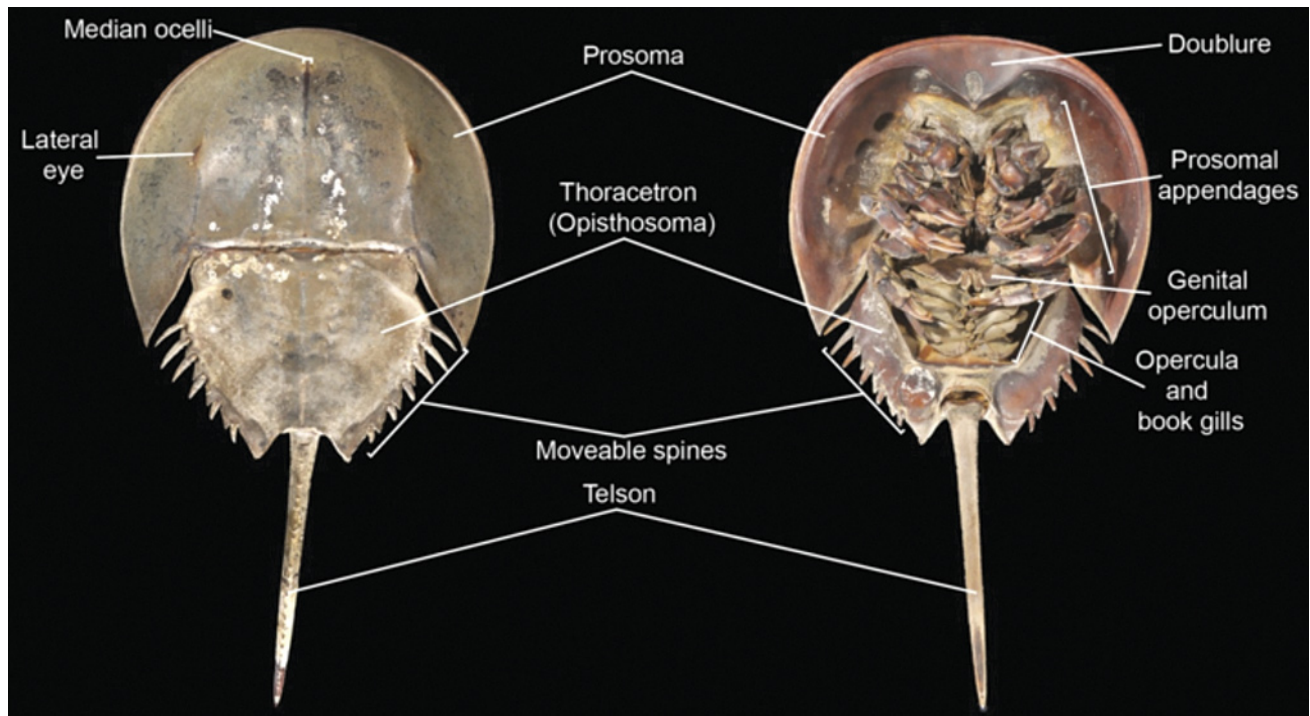


Fig. 1. Morphological terminology of horseshoe crabs as demonstrated by *Limulus polyphemus* (YPM IZ 070174).

1950s, confounded by over-splitting of the Asian horseshoe crab species (Waterman 1958). Four extant species are now recognized (Fig. 2): *Limulus polyphemus* (Linnaeus 1758), from the Atlantic coast of North America and the Gulf of Mexico; *Carcinoscorpius rotundicauda* (Latreille 1802) and *Tachypleus gigas* (Müller 1785), from the coast of Indonesia and the Bay of Bengal; and *Tachypleus tridentatus* (Leach 1819), from the South and East China Seas.

HORSESHOE CRABS AS A SOCIOECONOMIC, ECOLOGICAL, AND SCIENTIFIC RESOURCE

Despite occurring along the coastlines of different continents in different oceans (with a distance of some 12,000 km between the closest Asian and American populations), horseshoe crabs have been utilized as a resource by humans in similar ways across their range. Indigenous peoples in both America and Asia utilized the horseshoe crab populations as a source of food, consuming the eggs as well as boiling and eating the meat of the legs (Speck & Dexter 1948, Christianus & Saad 2009), and horseshoe crab eggs are still considered a delicacy in parts of Asia today (Nelson *et al.* 2015, Shin *et al.* 2009). The Wampanoag, who once lived in what is present-day Massachusetts, used the horseshoe crab carapace to make instruments, needles, awls, spears and charms (Speck & Dexter 1948), while in Malaysia and Thailand the head shield has been used as a decorative ornament (Christianus & Saad 2009). Horseshoe crabs were also used as fertilizer for crops (Morison 1972, Christianus & Saad 2009), a technique subsequently adopted by European colonists in North America (Kreamer & Michels 2009).

The exploitation of horseshoe crabs changed drastically with the onset of the 20th Century. Horseshoe crabs were harvested for fertilizer on an industrial scale (Berkson & Shuster 1999, Kreamer & Michels 2009), a practice that ceased in North America with the spread of cheap, artificial fertilizer (Walls *et al.* 2002) but still continues in Asia (Nelson *et al.* 2015, Pati *et al.* 2017). Horseshoe crabs are also now the primary source of bait for eel and conch fisheries in America and Asia (Bianchini *et al.* 1981, Berkson & Shuster 1999). However, the most radical change to the relationship between humans and horseshoe crabs came with the advent of *Limulus* Amoebocyte Lysate (LAL; Novitsky 1984, 2009, Kumar *et al.* 2015) and its Asian-species equivalent, *Tachypleus* Amoebocyte Lysate (TAL; Hodes *et al.* 1987, Akbar John *et al.* 2012, Bal *et al.* 2016). LAL and TAL are derived from horseshoe crab blood and are important in the biomedical industry as a test for the detection and quantification for bacterial endotoxins (Das *et al.* 2015). As a result, horseshoe crabs are now regularly harvested for their blood (Rudloe 1983, Rutecki *et al.* 2004), with harvests in the US regulated as capture-release programs (Botton & Ropes 1987a).

Horseshoe crabs are an important resource for a number of species aside from humans. Most famously, the seasonal spawning of horseshoe crabs along the east coast of North America provides a crucial stop-off point for migrating Red Knots *Calidris canutus*, which feed on the freshly-laid *Limulus* eggs (Castro & Myers 1993, Karpanty *et al.* 2006). Numerous other shorebirds also feed upon the eggs, as do sand shrimp and a variety of fish (Shuster 1982, Walls *et al.* 2002). After hatching, the larvae and juveniles are preyed upon by amphipods and green, blue,

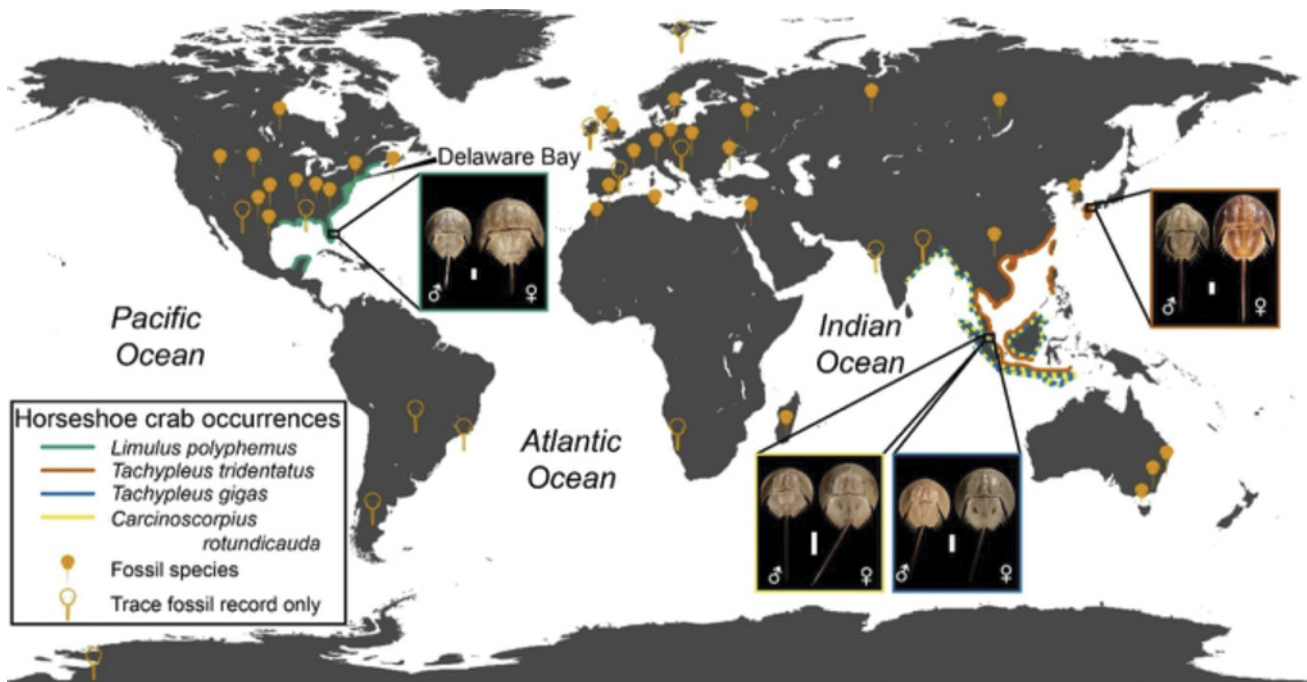


Fig. 2. Geographic distribution of modern and fossil horseshoe crabs. Fossil occurrences are derived from Dunlop *et al.* (2018) for body fossils, with additional trace fossil occurrences from Patel & Shringarpure (1992), da Rosa *et al.* (1994), Pickford (1995), Wignall & Best (2000), Mikuláš & Mertlík (2002), Buta *et al.* (2005), Lermen (2006), Chakraborty & Battacharya (2012), Hasiotis *et al.* (2012), Fernández & Pazos (2013), Naurstad (2014), Lerner & Lucas (2015), Alberti *et al.* (2017), and Mujal *et al.* (2018). *Limulus polyphemus* is represented by YPM IZ 055605 (male) and YPM IZ 070174 (female), *Carcinoscorpius rotundicauda* by YPM IZ 055595 (male) and YPM IZ 055574 (female), *Tachypleus gigas* by YPM IZ 055578 (male) and YPM IZ 055570 (female), and *Tachypleus tridentatus* by YPM IZ 055581 (male) and YPM IZ 055576 (female). Scale bars = 50 mm.

fiddler, and spider crabs alongside a range of fish (Botton 2009). Few species predate adult horseshoe crabs, but Leopard Sharks *Triakis semifasciata*, Loggerhead Turtles *Caretta caretta*, and American Alligators *Alligator mississippiensis* are some of their few direct predators, and are capable of crushing the thick external carapace (Keinath *et al.* 1987, Reid & Bonde 1990, Ehlinger *et al.* 2003, Seney & Musick 2007, Botton 2009). Long-tailed Macaques *Macaca fascicularis* and domestic pigs *Sus domesticus* have been observed predated the Asian horseshoe crab species (Ang 2016, Pati & Dash 2016). American Herring Gulls *Larus smithsonianus* and Great Black-backed Gulls *L. marinus* feed upon overturned horseshoe crabs stranded on beaches during spawning events (Botton & Loveland 1993), tearing off the opisthosomal opercula and feeding upon the muscle tissue within the thoracetrone, a predation style shared with House Crows *Corvus splendens* that feed on the Asian species (Debnath & Choudhury 1988). Finally, conch species of the genus *Melongena* predate horseshoe crabs by drilling through their carapace with the radula and consuming the flesh inside (Hathaway & Woodburne 1961).

Although they have a variety of societal and biomedical uses, horseshoe crabs are also of great scientific interest due to their potential to contribute toward our understanding of a variety of evolutionary processes. The horse-

shoe crab fossil record extends some 480 million years into the past but is generally sparse, with rarely more than three or four species occurring at the same time (Størmer 1952), although decay experiments have suggested that the relative changes in diversity through time are largely accurate (Klompemaker *et al.* 2017). There have been a number of studies on horseshoe crab phylogeny over the years (Anderson & Selden 1997, Lamsdell 2013, 2016, Lamsdell & McKenzie 2015), as well as analyses of the visual system (Fahrenbach 1981, Battelle 2006, Harzsch *et al.* 2006), development (Scholl 1977, Sekiguchi *et al.* 1982, 1988, Farley 2010, 2012), and decay and preservation (Babcock & Chang 1997, Babcock *et al.* 2000, Klompemaker *et al.* 2017). Much of the scientific interest in the horseshoe crab fossil record, however, has focused on a single evolutionary phenomenon: namely, the concept of evolutionary stasis and living fossils (e.g. Renwick 1968, Barthel 1974, Fisher 1984, 1990, Chatterji & Abidi 1993, Kin & Błazejowski 2014).

LIVING FOSSILS?

The term 'living fossil' originates from Darwin (1859), who applied it to the Platypus *Ornithorhynchus* and South American Lungfish *Lepidosiren*, based on their supposed positions as surviving representatives of once widespread lineages now restricted to confined geographic areas.

These restricted species were in turn considered to be subjected to less severe competition, resulting in them having rather anomalous morphologies in comparison to other modern taxa. Numerous taxa have since been assigned the 'living fossil' epithet, including a variety of flora (including the ferns *Angiopteris*, *Dipteris*, and *Matonia*, gymnosperms *Cycas*, *Ginkgo*, *Sequoia*, and quillworts such as *Stylites*), vertebrates (including the coelacanths *Latimeria*, Sixgill Shark *Hexanchus*, gars *Lepisosteus*, 'primordial' frogs *Leiopelma*, Earless Monitor Lizard *Lanthanotus*, Tuatara *Sphenodon*, kiwis *Apteryx*, opossums *Didelphis*, treeshrews *Tupaia*, Fossa *Cryptoprocta*, tapirs *Tapirus*, and Sumatran Rhinoceros *Dicerorhinus*), and invertebrates (including brachiopods *Lingula*, mollusks *Entemnotrochus*, *Nautilus*, *Neopilina*, and *Nucula*, echinoderms *Metacrinus* and *Platasterias*, onychophorans *Peripatopsis*, and arthropods *Anaspides*, *Hutchinsoniella*, *Limulus*, *Liphistius*, and *Pycnogonum*) (Gordon & Jablonski 1979).

Despite its widespread use in scientific and public literature, there is little consensus over the definition of what makes an organism a living fossil. Darwin (1859) explicitly tied the concept to membership of a previously common and widespread fossil lineage, the possession of a restricted geographic range, and unusual morphology. However, alternative definitions have frequently focused predominantly on either temporal duration or the morphological similarity to extinct taxa. As such, living fossils have been defined as either: 1) a living species that has persisted over a long interval of geologic time; 2) a living species that is morphologically and physiologically similar to a fossil species as seen over long intervals of geologic time; 3) a living species that has a preponderance of 'primitive' morphological traits; 4) a living species that has persisted over a long period of geologic time, is morphologically and physiologically similar to a fossil species as seen over long intervals of geologic time, or has a preponderance of 'primitive' morphological traits, and has a relict distribution; 5) a living species once thought to be extinct; or 6) an extant clade of low taxonomic diversity whose species have either persisted over a long interval of geologic time, are morphologically similar to fossil species that have persisted over long periods of geologic time, or have 'primitive' morphological traits (Schopf 1984). A recent review (Werth & Shear 2014) noted that living fossils share a number of characteristics, either alone or in combination: retention of an ancestral morphology, apparent stasis over geologic time, resemblance to ancient fossil forms, restricted relict distribution, and low taxonomic diversity. A reader may, however, be inclined to believe that these first three stipulations are recapitulations of the same condition, and 160 years after Darwin first coined the term, the definition of a living fossil is as mutable as it was at inception, relying upon some condition of similarity with fossil taxa, a limited geographic or environmental distribution, and a low number of species. Irrespective of the exact definition, horseshoe crabs are considered archetypal examples of a living fossil: *Limulus* and its extinct counterpart, *Mesolimulus*, grace the cover

of the first print hardcover of Richard Fortey's (2011) book *Survivors*, which explores a variety of species representing the apparently unchanged remnants of ancient lineages.

The 'living fossil' terminology is much maligned in biology and paleontology, and has been criticized as being inaccurate and misleading, as it is frequently influenced heavily by the morphological complexity of the organism in question (Schopf *et al.* 1975). While the term has been defended as describing a genuine phenomenon (Eldredge 1976), there have been a number of attempts to introduce new terminology, each focusing on a different aspect of the 'essence' of what makes a living fossil. Horseshoe crabs featured predominantly in these reformulations. The preeminent alternative to living fossils for much of the 20th century was the concept of bradytely, derived from Simpson's (1944) discourse on tempo and mode in evolution and championed for horseshoe crabs by Fisher (1984, 1990). Bradytelic lineages are clades that exhibit apparent slow rates of evolution (Fisher 1990) as evidenced by little morphological variation between species. One clade of horseshoe crabs – the Limulina, which includes the species alive today – was determined to exhibit bradytely (Fisher 1984). The concept of bradytely has also been criticized, however, as bradytelic lineages often exhibit the same degrees of genetic variability as clades that do not exhibit bradytely (Schopf 1984). A more recent conceptual alternative has been proposed: stabilomorphism (Kin & Błazejowski 2014). Stabilomorphs are defined as organisms that exhibit relative morphological stability in time and spatial distribution, having a taxonomic status not exceeding the genus level, and have survived one or more mass extinction events. The concept essentially is that stabilomorphs are so well-adapted that they do not express phenotypic variants in response to environmental changes. The theoretical feasibility of the concept notwithstanding, one advantage of stabilomorphism is that it can apply to extinct taxa and so is a more general concept without being tied to the contingent factor of the organisms needing to exist in our discrete time period. It does, however, tie the definition to two other arbitrary conditions: invoking the genus level as the taxonomic level of focus (any taxonomic rank above the level of species being subjective), and the requirement to have survived at least one mass extinction (thereby once again making the definition contingent on a random point in time).

Ultimately, quantitative studies of living fossils, bradytelic lineages, or stabilomorphs have found little support for the supposed archaic nature of taxa traditionally considered to show extreme morphological stasis. The other living fossil 'poster children', the coelacanths *Latimeria* and nautiloids *Nautilus*, have been stripped of their titles as it was revealed that coelacanths exhibit neither morphological stasis nor low molecular substitution rates (Casane & Laurenti 2013) and *Nautilus* is in the process of a diversification event (Wray *et al.* 1995). Another classic example, living monoplacophoran mollusks, were shown to have radiated fairly recently (Kano *et al.* 2012). Most critically,

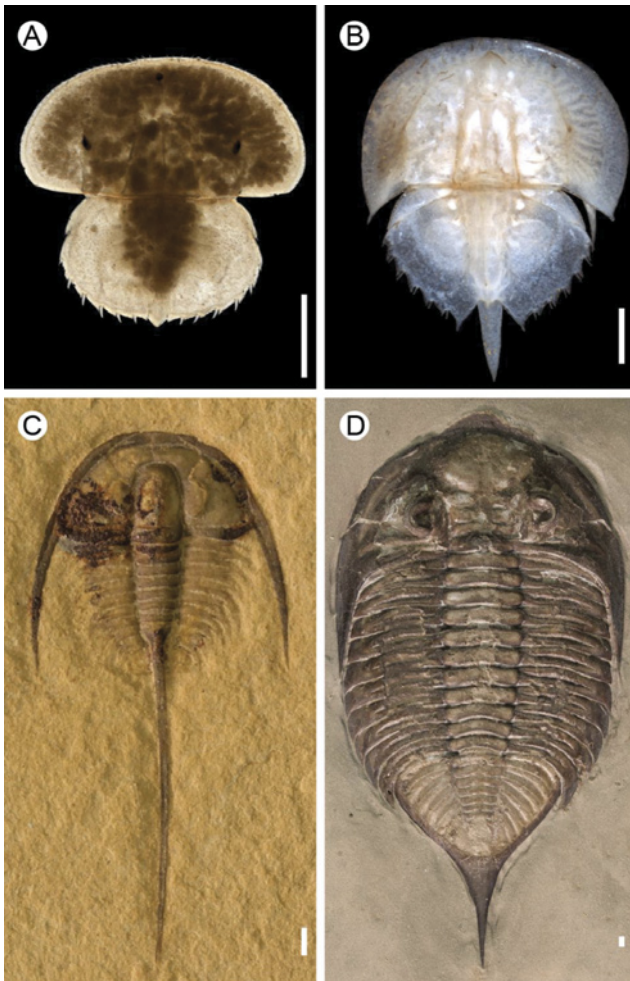


Fig. 3. Comparisons between larval and juvenile horseshoe crabs and trilobites. **a.** ‘Trilobite’ larva of *Limulus polyphemus* (YPM IZ 076994). **b.** Stage 5 instar of *Limulus polyphemus* (YPM IZ 098247). **c.** *Cedarina schachtii* (YPM IP 531072), one of the most ‘horseshoe crab’-like trilobites. The similarity is not, however, striking. Note that the apparent long tail spine is actually a dorsal spine coming off one of the body segments. **d.** *Dalmanites limulurus* (YPM IP 428856), a more typical-looking trilobite. Scale bars = 1 mm.

restudy of the branchiopod crustacean *Triops cancriformis*, one of the few living species with a fossil record ostensibly extending into the Mesozoic, determined that modern and fossil populations exhibit distinct ontogenetic trajectories with clearly distinguishable adult morphotypes (Wagner *et al.* 2017). As a result, the fossil specimens represent a separate species (Lagebro *et al.* 2015), and *Triops cancriformis* does not in fact have a known fossil record. The one exception to this trend is *Sphenodon punctatus*, the Tuatara; proposing a slow rate of lineage evolution and a morphology positioned close to the centroid of clade morphospace as a testable definition for a living fossil, a study of Rhynchocephalia found that *Sphenodon* does indeed fit the title (Herrera-Flores *et al.* 2017).

Similarly, many of the ‘living fossil’ characteristics applied to horseshoe crabs do not hold up under scrutiny. None of the modern species possess a fossil record. The so-called ‘trilobite larva’ do not exhibit any real similarity to trilobite morphology (Fig. 3), and despite claims that horseshoe crabs are the closest living relative to trilobites (Shuster 1982), this is not borne out by any phylogenetic analysis (Lamsdell 2013, Lamsdell *et al.* 2013, Legg *et al.* 2013, Stein *et al.* 2013, Aria *et al.* 2015). Furthermore, analysis of the horseshoe crab genome indicates the lineage underwent a whole-genome duplication event prior to the divergence of the extant species (Kenny *et al.* 2016), resulting in redundant genes that can accrue mutations and provide the genetic raw material for evolutionary innovation (Ohno 1970). Yet, horseshoe crabs are still regularly evoked as living fossils. There is some danger in this line of thought, especially as articulated in the stabilomorph concept, as living fossils are often considered to have persisted through mass extinctions and changing environments for millions of years without any change to their morphology or ecology. By implicit assumption, there is a public perception horseshoe crabs will therefore be able to weather any future changes, an opinion not shared by the scientists that study them (Mishra 2009, Shin *et al.* 2009, Smith *et al.* 2009, Beekey & Mattei 2015, Botton *et al.* 2015, Pati *et al.* 2017). As we shall see, the fossil record demonstrates that, while horseshoe crabs have maintained a distinct and recognizable morphology for long periods of geologic time, they have occupied a variety of ecologies over their evolutionary history and also experienced their fair share of extinction.

THE DYNAMIC HORSESHOE CRAB: PHYLOGENY AND EVOLUTIONARY HISTORY

Horseshoe crabs are first known from the Ordovician, approximately 480 million years (MY) ago (Fig. 4). The oldest described species is the diminutive *Lunataspis aurora*, known from 445 MY-old marine rocks in Canada (Rudkin *et al.* 2008). The oldest known horseshoe crab, however, is an undescribed species from Morocco (Van Roy *et al.* 2010) that lived 477 MY ago at a high, southern polar latitude (Van Roy *et al.* 2015). These unassuming creatures set the template for horseshoe crabs through the remainder of the Paleozoic (which terminates at the end-Permian mass extinction, 252 MY ago); the segments of the body were fused into a thoracetron but the vestiges of their segment margins remained visible, and the telson was elongated and approximately equal in length to the rest of the body (Fig. 4a, b). Their appearance in the fossil record is sudden, and this, combined with the distinctiveness of their morphology compared to their closest relatives, suggests either rapid rates of evolutionary change associated with their origin, a cryptic fossil record extending into the Cambrian, or a combination of these scenarios. They are also the only true horseshoe crabs known for the next 100 MY.

This sizeable gap in the fossil record went unrecognized until the discovery of *Lunataspis*. Previously, a plethora of

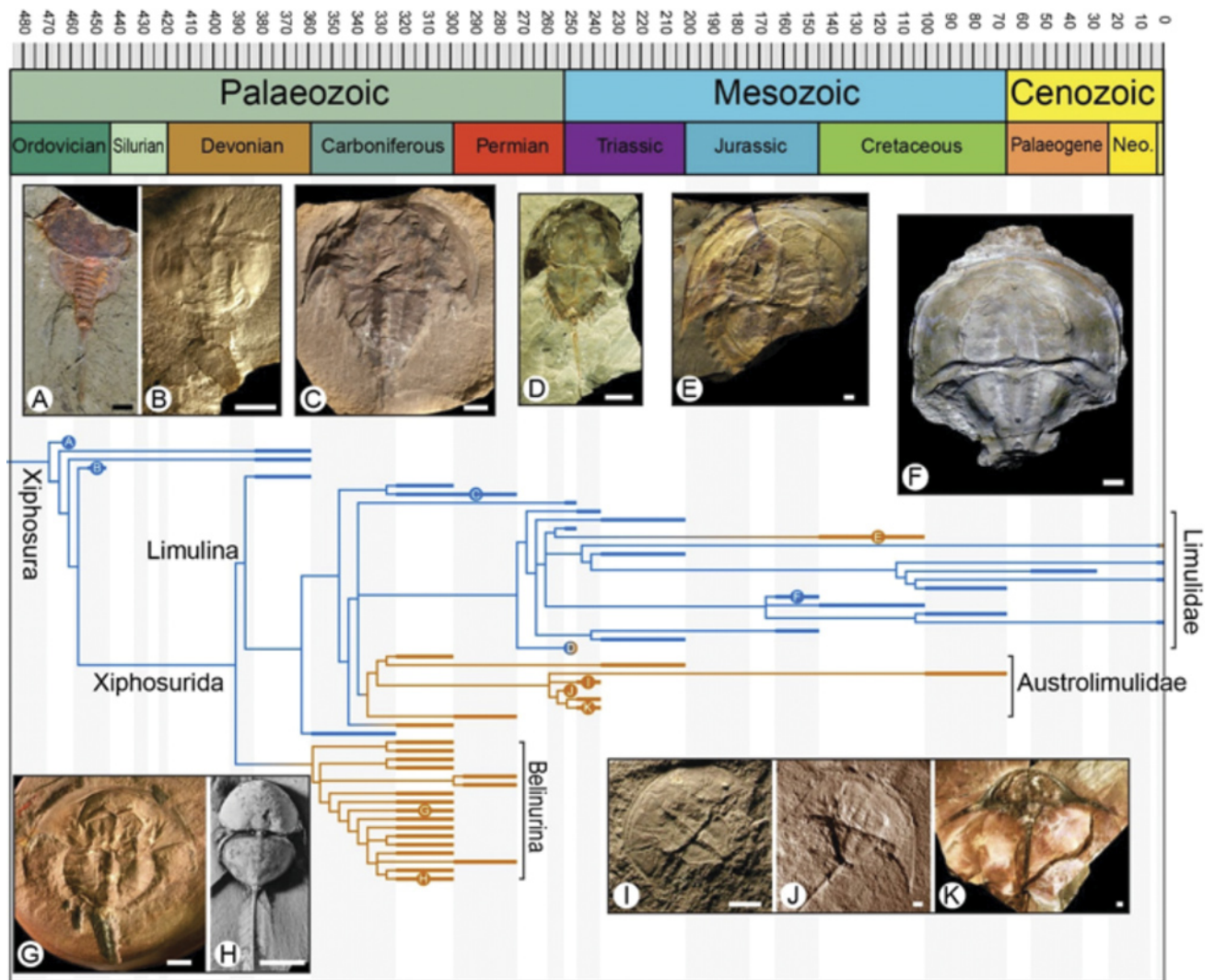


Fig. 4. Horseshoe crab evolutionary history. Phylogeny (derived from Lamsdell 2016) with taxon ranges shown by thickened bars. Branches are color coded by ecological occupation: marine environments are shown in blue, while freshwater environments are orange. The position of figured taxa are denoted by letters. **a.** Unnamed xiphosurid, Morocco (YPM IP 531838). **b.** *Lunataspis aurora*, Canada (MM I-3989). **c.** *Paleolimulus signatus*, United States (FMNH PE56851). **d.** *Limulitella bronni*, France (Grauvogel-Gall collection). **e.** *Victalimulus mcqueeni*, Australia (NMVM P22410-3). **f.** '*Limulus*' *darwini*, Poland (ZPAL X.1/O-B/XA2). **g.** *Euproops danae*, United States (YPM IP 1816909). **h.** *Alanops magnificus*, France (MNHN SOT 88526). **i.** *Dubbolimulus peetae*, Australia (NMVM P315229). **j.** *Vaderlimulus tricki*, United States (UCM 140.25). **k.** *Austrolimulus fletcheri*, Australia (AM F.38274). Scale bars = 5 mm.

chelicerate arthropods, called synziphosurines, had been considered to represent early horseshoe crabs (Eldredge 1974, Bergström 1975). While not overly common, numerous species are known from the Paleozoic; these animals had freely articulating body segments that had not been fused into a thoracetrone, and so were considered to be the ancestral stock from which modern horseshoe crabs (xiphosurids) evolved towards the end of the Paleozoic (Anderson & Selden 1997). *Lunataspis* upset the paleontological order of events, as the new fossils pre-dated all of the supposedly ancestral synziphosurines; instead, these species must themselves have been descendants of offshoots from an even more ancient ancestral stock. Subsequent discoveries of synziphosurines with biramous limbs (Briggs *et al.* 2012) – retaining the upper branch of the arthropod

limb, which has been lost in other chelicerates (Dunlop & Lamsdell 2017) – or displaying a variety of eurypterid or arachnid characteristics (Selden *et al.* 2015, Lamsdell *et al.* 2015) along with ensuing phylogenetic analyses led to the removal of synziphosurines from horseshoe crabs, as it was revealed that synziphosurines are in fact a disparate group of chelicerate arthropods that include stem euche-licerates and closer relatives to arachnids and eurypterids (Lamsdell 2013, 2016). There are therefore no horseshoe crabs currently known for the entirety of the Silurian (444–419 MY ago), although logically the lineage must have been present.

When horseshoe crabs next appear, towards the end of the Devonian (around 365 MY ago), they had already

attained a global distribution. Although only three species are known, they are found in North America (Babcock *et al.* 1995), Australia (Pickett 1993), and Russia (Chernyshev 1933), which at the time formed parts of three distinct paleocontinents. This widespread distribution is maintained for much of the rest of their evolutionary history: horseshoe crab fossils are known from every continent, including Antarctica (Fig. 2). While no detailed analysis has been done, this prehistoric distribution likely explains the disparate geographic occupation of modern horseshoe crabs as a relict of this past worldwide occupation.

The horseshoe crab fossil record becomes much more abundant during the Carboniferous with the radiation of the Belinurina, the most speciose clade of horseshoe crabs known. This high diversity is somewhat inflated by over-splitting, wherein a number of characteristics that change through ontogeny have been used to define species (Haug *et al.* 2012, Haug & Rötzer 2018), and through their occurrence in coal swamps, a sheltered environment more conducive to the preservation of unmineralized cuticle, although by all accounts the heightened diversity of belinurines remains a genuine phenomenon. The cause of this proliferation may be linked to their habitat: coal swamps are freshwater environments, and the belinurines are the first of several horseshoe crab lineages to invade the non-marine realm (see Fig. 4). It has been suggested that the change in niche occupation resulted in a subsequent change in population structure, with increasing likelihood of allopatric speciation through geographic isolation (Lamsdell 2016), a similar process to that observed today in modern fish (DeWoody & Avise 2000).

The switch from a marine to freshwater habitat requires extreme physiological changes in salinity tolerance (Little 1990). Adults of extant *Limulus* and *Tachypleus* can tolerate a wide range of salinities, but cannot survive in fresh water for extended periods of time (Towle & Henry 2003, Sekiguchi & Shuster 2009). The larvae, however, are capable of tolerating a wider range of salinities for extended periods of time (Shuster 1982, Botton *et al.* 2010), and it has been shown that they have a greater tolerance for salinities lower than 35‰ than they do for hypersaline environments (Ehlinger & Tankersley 2007). Belinurines, and other lineages of non-marine horseshoe crabs, may have made the transition out of the marine realm by retaining aspects of their larval physiology. Interestingly, belinurines exhibit a general paedomorphic evolutionary trend and retain increasingly juvenile morphologies into adulthood (compare Fig. 4g, h with Fig. 3a, b). However, developmental studies have shown that larval horseshoe crabs in low salinities exhibit slower rates of development (Jegla & Costlow 1982, Ehlinger & Tankersley 2004); as a consistently low salinity environment could therefore conceivably result in individuals reaching sexual maturity before otherwise completing their morphological development, it is impossible to discern whether the paedomorphic trend in belinurines facilitated their transition to freshwater environments or was a result of it.

Despite the rapid diversification undergone by belinurines in freshwater environments, their dependence on coal swamps ultimately proved to be their undoing when rainforest ecosystems collapsed towards the end of the Carboniferous (DiMichele *et al.* 2006, 2009). Belinurines underwent a major extinction, with only a few species surviving into the Permian before they, too, went extinct. Marine horseshoe crabs were unaffected by this dramatic terrestrial environmental change: *Paleolimulus signatus* (Fig. 4c) has a long geologic range extending from the mid Carboniferous well into the Permian (Beecher 1904, Dunbar 1923, Raymond 1944) and is equally abundant before and after the loss of the rainforests. Early representatives of austrolimulids, a distinctive group of horseshoe crabs originally recognized from the Mesozoic of Australia, also weathered the environmental upheaval from marginal marine environments.

Horseshoe crabs appear to recover remarkably quickly from the end-Permian mass extinction, when up to 96% of all marine species went extinct (Erwin 1990). Two clades diversified early in the Triassic, the austrolimulids and the limulids. Austrolimulids were by this point inhabiting fully freshwater streams and rivers (Lamsdell 2016). They achieved a degree of success that, while not matching the diversity of the belinurines, indicates that they were also able to thrive outside of marine salinities across the globe: austrolimulids have been described from Europe (Hauschke & Wilde 1987), North America (Lerner *et al.* 2017) and, as their name suggests, Australia (Riek 1955, Pickett 1984). These horseshoe crabs do not show an obvious paedomorphic trend as is apparent in belinurines; however, austrolimulids do exhibit extremely aberrant morphologies that clearly set them apart from other horseshoe crabs (Fig. 4i–k), with an extremely broad prosomal carapace and elongated and laterally-splayed genal spines in later species. Austrolimulids underwent a major drop in diversity towards the end of the Triassic (201 MY ago), with only a single post-Triassic species, *Casterolimulus kletti* (Holland *et al.* 1975), known from the Cretaceous (94 MY ago). This long gap may be indicative of either a cryptic fossil record, or that *Casterolimulus* may be taxonomically misplaced: a species of limulid, *Victalimulus mcqueeni* (Riek & Gill 1971), is also known from the Cretaceous, again from a freshwater environment. It is possible that *Casterolimulus* should be more closely aligned to *Victalimulus*. However, this still leaves a similar temporal gap between *Victalimulus* and its current closest relative (some 100 MY), and so it is clear that the Jurassic horseshoe crab record is incomplete irrespective of the exact taxonomic affinities of *Casterolimulus*.

While austrolimulids were diversifying in freshwater environments in the early Mesozoic, limulids were radiating in the marine realm. A number of these early limulids are known from an abundance of individuals: over 200 specimens of *Limulitella bronni* (Fig. 4d) are preserved in marginal marine settings from the Triassic of France (Selden & Nudds 2012). Multiple specimens of *Mesolimulus walchi* are also known from the Jurassic of Solnhofen in

Germany. These horseshoe crabs can exhibit exceptional preservation of muscle tissue (Briggs *et al.* 2005) and are considered to have been fossilized after being swept into anoxic lagoonal conditions by storm events (Barthel *et al.* 1990). Famously, some of these *Mesolimulus* specimens are preserved dead at the end of their trackways (Seilacher 2007); these trackways can extend for several meters, even showing the impact mark of the animal hitting the lagoon floor and its erratic movements as it succumbs to anoxia (Lomax & Racay 2012). Trackways, such as the Solnhofen 'death marches', provide important evidence regarding the behavior of extinct horseshoe crabs (ichnology, the study of organismal traces in the fossil record, is the only way of directly studying ancient behavior; Plotnick 2012). Horseshoe crab trackways are easily identifiable in the fossil record as they closely resemble the trackways of modern species (Fig. 5). Trackways are generally more common than body fossils, as a single animal can leave many tracks over its lifetime, and tracks are the only record of horseshoe crabs from a number of continents (Fig. 2). Trackways also provide the first evidence of mass spawning, which is perhaps the defining behavioral characteristic of modern horseshoe crabs and the main reason for the ecological importance of Delaware Bay (Shuster & Botton 1985). Extensive trackways from the Middle Triassic (243 MY ago) of Germany reveal that horseshoe crabs were congregating and spawning on beaches during high tides much as their modern relatives do (Diedrich 2011). Fascinatingly, these horseshoe crabs were preyed upon by large quadrupedal archosaurs, distant relatives of the birds that rely on horseshoe crab eggs as a food resource in the modern Delaware Bay ecosystem.

It was around the Middle Triassic that species potentially assignable to extant genera began to appear. The oldest of these is *Tachypleus gadeai*, from the Triassic of Spain, although this has also been assigned to its own genus *Heterolimulus* (Via Boada & De Villalta 1966). A species assigned to *Limulus* (Fig. 4F) has also been reported from the Jurassic (148 MY ago) of Poland (Kin & Błazejowski 2014). Phylogenetic analyses have, however, failed to unequivocally resolve the species within the genus *Limulus* (Lamsdell & McKenzie 2015, Lamsdell 2016) and additional horseshoe crab specimens assignable to the extinct genus *Crenatolimulus* are known from the same locality (Błazejowski 2015). It is likely that the combined material represents a single species that should be assigned to *Crenatolimulus* (Tashman 2014). The first probable occurrences of *Limulus* and *Tachypleus* are from the Cretaceous (~105 MY ago), the most convincing of which is *Tachypleus syriacus* (Fig. 6). This species, known from marine strata of Lebanon, preserves clear prosomal sexual dimorphism with males exhibiting a pronounced anterior scalloped margin, which is otherwise only known from the extant *Tachypleus tridentatus*. As this morphological development is absent in *Tachypleus gigas*, phylogenetic analysis resolves *Tachypleus syriacus* within the *Tachypleus* crown group (Lamsdell & McKenzie 2015).

While there is broad agreement that the modern Asian species form a clade (Tachypleinae), early molecular

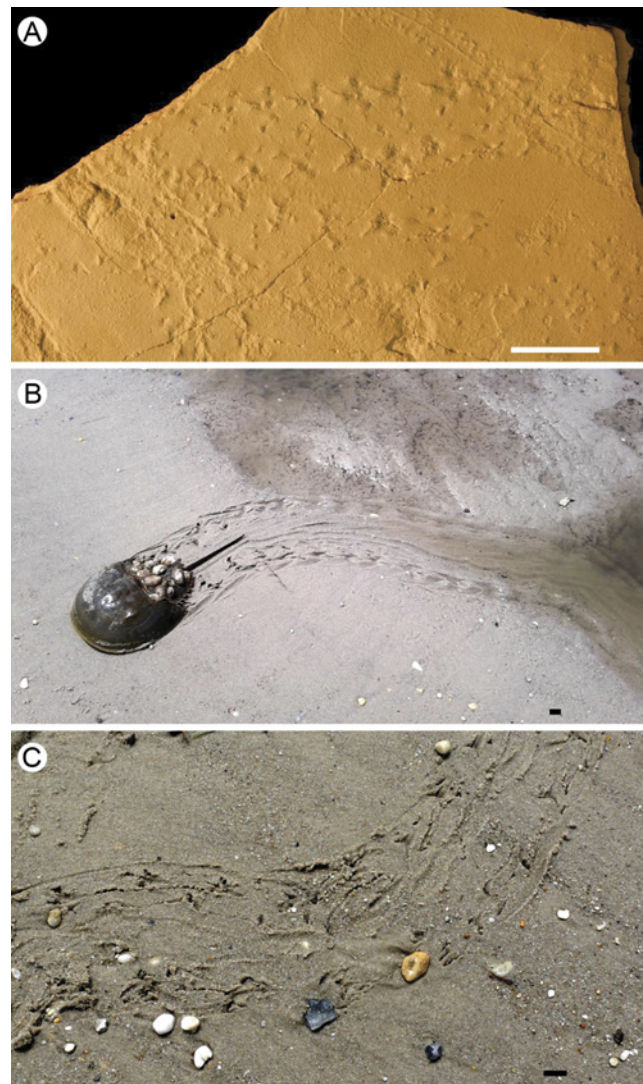


Fig. 5. Horseshoe crab trackways. **a.** Fossil limulid tracks (YPM IP 3954). **b.** Modern trackway from Pickering Beach, Delaware, with *Limulus* trackmaker. **c.** Details of modern trackway from Pickering Beach, Delaware in more consolidated sand. Scale bars = 20 mm.

analyses suggested that *Tachypleus* was paraphyletic in relation to *Carcinoscorpius* (Shishikura *et al.* 1982, Xia 2000, Kamaruzzaman *et al.* 2011), while morphological data retrieves *Tachypleus* monophyly (Lamsdell & McKenzie 2015, Lamsdell 2016). However, recent molecular analyses converge on the morphological topology (Obst *et al.* 2012, Periasamy *et al.* 2017), and there now appears to be general support for a monophyletic *Tachypleus*. The one caveat to this is that it leaves *Carcinoscorpius* with a 250 MY gap in its fossil record. *Carcinoscorpius* is one of two limulid species known to persist for extended periods in non-marine salinities, the other being *Victalimulus* from the Cretaceous of Australia (Fig. 4e). It is possible that *Carcinoscorpius* is actually nested within *Tachypleus*, and that morphological changes as *Carcinoscorpius* has adopted a more marginal marine lifestyle obfuscates this relationship. Perhaps more likely, a number of fossil horseshoe crab

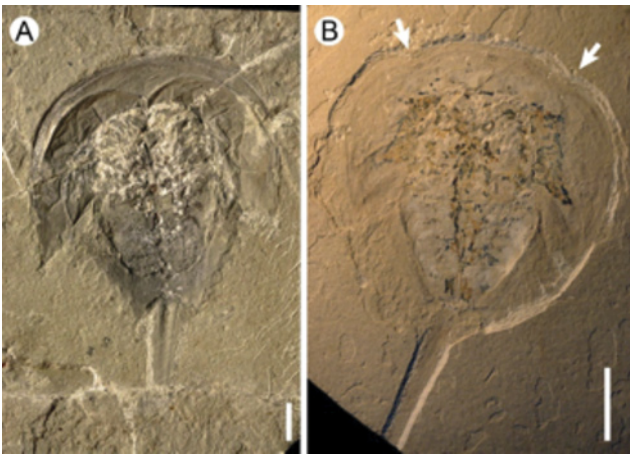


Fig. 6. Sexual dimorphism in *Tachypleus syriacus*, from the Cretaceous of Lebanon. **a.** Female (MSNM i27468). **b.** Male, with anterior scalloping of the prosomal carapace indicated by white arrows (MSNM i9352). Scale bars = 20 mm.

species (such as *Tachypleus gadeai*) may be closer related to *Carcinoscorpius* than currently thought.

Modern horseshoe crabs are ecological generalists that can survive in a wide range of salinities and temperatures (Sekiguchi & Shuster 2009) although it is worth remembering that, outside of spawning, the majority of adults spend their lives on the continental shelf in fully marine conditions (Botton & Ropes 1987b). It is therefore noteworthy that the majority of past horseshoe crab diversity and morphological disparity has been associated with entirely non-marine, freshwater environments. It has been shown that modern horseshoe crab populations are genetically discrete (King *et al.* 2005), each with some variation on their exact physiological tolerances (Sekiguchi & Shuster 2009). It has been suggested that this pool of genetic variability will ensure that at least one population has the potential to survive any environmental changes and secure the evolutionary future of horseshoe crabs into the next geologic epoch (Sekiguchi & Shuster 2009). However, to borrow a concept from the writing of Stephen Jay Gould (1989), any observer of horseshoe crabs during the Carboniferous would be justified in predicting that the future of the lineage lay among the large number of freshwater coal swamp species and not among the handful of species inhabiting the seas at the time. Similarly, a traveler to the Triassic would be hard pressed to predict whether it would be the marine limulids or freshwater austrolimulids that persisted through to our present day. This is to say that, while the genetic diversity between populations may increase the chances of the long-term persistence of the lineage, it should not be taken for granted that the remaining horseshoe crab species will successfully adapt to future environmental changes. Furthermore, extirpation of local populations could drastically influence the overall genetic diversity of a species and severely impact the long-term chances of species survival.

HORSESHOE CRABS: PAST, PRESENT, FUTURE?

Concerns surrounding horseshoe crab conservation have generally focused on the health of populations within the United States and its impact on Red Knots (Karpanty *et al.* 2006), as it has been shown that a decline in volume of horseshoe crab eggs leads to a decline in bird survivorship (Baker *et al.* 2004). As discussed earlier, horseshoe crabs have been an economic resource for decades, and the industrialization of this exploitation has put immense pressure on their North American populations (Faurby *et al.* 2010). Estimates of the harvest of horseshoe crabs from Pleasant Bay, Massachusetts in 2001 indicated that harvesting resulted in mortality of 1–2% of the population (Rutecki *et al.* 2004), while also noting that the population at Pleasant Bay was one of the least exploited populations along the east coast. Modern harvests are split between taking bait for eel and conch fisheries (Bianchini *et al.* 1981, Berkson & Shuster 1999) and the biomedical industry (Rudloe 1983). Harvesting quotas are now regulated (Atlantic States Marine Fisheries Commission 1998, 2006) and biomedical companies operate capture-release programs (Botton & Ropes 1987a). Despite these restrictions, the annual horseshoe crab harvest has been consistently higher since the 1980s than when records began in 1970 (Niles *et al.* 2009, Millard *et al.* 2015). It is worth noting that, with the extreme exploitation for fertilizer in the early 1900s (Berkson & Shuster 1999, Kreamer & Michels 2009), our observed 1970s baseline for horseshoe crab numbers is most likely derived from an already depleted population.

The harvest restrictions have also resulted in an increase in illegal catching in North America and importation of Asian species to supply the bait market (Botton *et al.* 2015). Horseshoe crabs in Asia experience many of the same human-driven pressures as their American counterparts, as well as additional threats from human infringements on their spawning grounds (Nelson *et al.* 2016, Pati *et al.* 2017). Several conservation programs aiming to preserve the Asian species are underway (Tsuchiya 2009, Kwan *et al.* 2017), although they are generally limited in scope and many countries have no conservation efforts at all (Mishra 2009).

Aside from the direct human influence on horseshoe crab populations, human impact on global climate may also be a threat to their long-term survival. Sea level is predicted to rise over the next 100 years, driven by global temperature increases (Meehl *et al.* 2005, Rahmstorf 2007, Vermeer & Rahmstorf 2009), which will result in a natural landward progression of the beach-marsh systems that horseshoe crabs rely upon as their breeding grounds (Loveland & Botton 2015). However, human engineered stabilization of coastal environments through groins, barriers and bulkheads will halt this landward progression and result in the total loss of breeding ground habitat (Botton *et al.* 1988, Botton 2001, Berkson *et al.* 2009, Hsieh & Chen 2009). Another possibility that does not appear to have been considered is that rising temperature may directly impact horseshoe crab reproduction. Sea

surface temperatures are reaching record highs (Blunden & Arndt 2016), and developmental studies have demonstrated that horseshoe crab embryos develop more rapidly in warmer temperatures (Jegla & Costlow 1982, Carmichael & Brush 2012). If rising temperatures were to result in earlier larval emergence times, it is unclear what impact this would have on horseshoe crab populations, especially as horseshoe crab larvae do not disperse far from their hatching beaches (Botton *et al.* 2010) and undergo major shifts in diet as they develop into juveniles and eventually adults (Gaines *et al.* 2002). Perturbations in embryonic development could conceivably result in a disconnect between emergence time and food source availability, and if this were to happen, horseshoe crab populations would rapidly collapse.

Horseshoe crabs have a rich evolutionary history extending for hundreds of millions of years. During this time they have repeatedly invaded freshwater environments, developed bizarre morphologies, and occupied every continent on Earth. Our modern species, with their much more limited geographic distributions, represent only a fraction of the lineage's past ecological and morphological diversity. As our understanding of the horseshoe crab fossil record has evolved, it has become apparent that the most successful lineages were those that moved out of their usual marine habitat, although all of these ecological invasions ultimately ended in failure. Horseshoe crabs do not appear to be especially immune to extinction, and genetic studies have shown that while the modern species are indeed ecological generalists, they are so in the sense that their populations each have somewhat distinct physiological tolerances, rather than in any one individual being able to survive transplantation into a new environment. Everything we learn about these fantastic creatures demonstrates that we should not remain unconcerned about the survival of the four remaining species, especially as they face concerted pressures from human activities unlike any the lineage would have experienced over the course of their evolution. International discussion and collaboration is the way forward if we want to develop effective conservation measures (Botton 2001, Berkson *et al.* 2009, Hsieh & Chen 2009, Botton *et al.* 2015); however, social and political interest beyond that of researchers and conservationists is going to have to be leveraged if we want to avoid losing our horseshoe crab species to the geologic past.

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