Global Diversity of Ascidiae

Noa Shenkar\textsuperscript{1*}, Billie J. Swalla\textsuperscript{1,2}

\textsuperscript{1} Department of Biology, University of Washington, Seattle, Washington, United States of America, \textsuperscript{2} University of Washington Friday Harbor Labs, Friday Harbor, Washington, United States of America

Abstract: The class Ascidiae presents fundamental opportunities for research in the fields of development, evolution, ecology, natural products and more. This review provides a comprehensive overview of the current knowledge regarding the global biodiversity of the class Ascidiae, focusing in their taxonomy, main regions of biodiversity, and distribution patterns. Based on analysis of the literature and the species registered in the online World Register of Marine Species, we assembled a list of 2815 described species. The highest number of species and families is found in the order Aplousobranchia. Didemnidae and Styelidae families have the highest number of species with more than 500 within each group. Sixty percent of described species are colonial. Species richness is highest in tropical regions, where colonial species predominate. In higher latitudes solitary species gradually contribute more to the total species richness. We emphasize the strong association between species richness and sampling efforts, and discuss the risks of invasive species. Our inventory is certainly incomplete as the ascidian fauna in many areas around the world is relatively poorly known, and many new species continue to be discovered and described each year.

Introduction

Ascidians (Phylum Chordata, Class Ascidiae), or sea squirts, are the largest and most diverse class of the sub-phyllum Tunicata (also known as Urochordata). They comprise approximately 3000 described species found in all marine habitats from shallow water to the deep sea [1–3]. The group was initially difficult for zoologists to classify systematically, although ascidians were recognized as a distinct group as early as Aristotle [1]. The first clear description of an ascidian was made by Schlosser in 1756 in a letter entitled “An account of a curious, fleshy, coral-like colonial ascidian” [4]. The name “tunicate” (sub-phyllum Tunicata) was first coined by Lamarck [5] for ascidians, pyrosomes, and salps [6,7]. The name originates from the polysaccharide-containing tunic that envelops the animal and forms a somewhat flexible skeleton [1]. Milne Edwards [8] mistakenly included the Bryozoa in this group, and both, together with the Brachiopoda, were included in the Mollusca by Hancock [9]. Savigny [10] also recognized the Tunicata (ascidians, salps, dololids, and appendicularians) as a distinct group separate from the Mollusca [6]. Finally, the chordate nature of the ascidian tadpole larva was recognized by Kowalevsky [11], and they were reclassified with chordates [12]. The name Urochordata was not used until Balfour [13] created it as a replacement name for Tunicata, presumably to emphasize the chordate affinity. Indeed, recent phylogenetic studies place the tunicates as the sister group to the vertebrates [14–16], suggesting that they are our closest relatives among the invertebrates, which provides a fertile ground for evolutionary and developmental studies [17].

Following the original classification of Lahille [18], the class Ascidiae is now divided into three orders based on the structure of the adult branchial sac: Aplousobranchia (simple), Phlebobranchia (vascular) and Stolidobranchia (folded) (Fig. 1). This is the current classification used by most ascidian taxonomists that also corresponds to molecular phylogenetic analysis based on the 18S rDNA [7,19] as opposed to Perrier’s [20] division that was based upon the position of the gonads and other morphological considerations and comprised only two orders: Enterogona and Pleurogona. Ascidians belonging to the order Aplousobranchia are all colonial while the Phlebobranchia and Stolidobranchia include both colonial and solitary species [7].

Adult ascidians are sessile, inhabiting a wide variety of habitats such as soft sediments, coral reefs and rocky substrates. They successfully foul various artificial substrata such as jetties, ship hulls, floating docks and other man-made structures all over the world [21,22]. They remain sessile following larval settlement throughout their adult life, as they cannot avoid salinity or temperature changes, and thus larval behavior is critical [23,24]. Only a few species can survive in salinities below 20–25% [22,25], or above 44% [26], (Shenkar N, unpublished results). The tropical Ecteinascidia thurstoni has been recorded along the Suez Canal in habitats with salinity reaching 46% overgrowing metal pilings of jetties [26], while several species inhabit marine lakes in Indonesia with salinity of 28.5% [27]. In salinities below 22%o larval development is severely affected [24], as is the health of adult zooids [28]. Nonetheless, highly tolerant species such as Ciona intestinalis survive a wide range of salinities (12–40%), and can withstand short periods of lower salinities (<11%) [29,30]. In general, ascidians exhibit a wider tolerance to temperature range than salinities [22,31]. Antarctic species can tolerate temperature as low as -1.9°C [32], while others can survive seawater temperature higher than 35°C in the Arabian Gulf [33].

Both salinity and temperature are among the most important environmental variables influencing ascidian recruitment and reproduction [31,34–36]. Other factors that may affect spatial distribution and recruitment include light, substrate type, hydrodynamics, predation and competition...
Antimalarial compounds have been isolated from the solitary ascidians in both tropical and temperate waters. These compounds are now commonplace, with the potential and ability to thrive in eutrophic (nutrient-rich) environments. The solitary ascidian, Halocynthia roretzi, has long been a popular seafood in Japan and Korea, with a market value of $18 million in 2006 [46]. Recently, a unique infectious agent has been identified as the cause of mass mortality of these cultured ascidians [47].

Ascidians are a key ecological group because of their invasive potential and ability to thrive in eutrophic (nutrient-rich) environments. Introductions of non-indigenous ascidians into harbors in both tropical and temperate waters are now commonplace, with the rate of introductions increasing, sometimes creating severe damage to natural fauna by overgrowth [1,22,38-41] (reviewed in a special issue of Aquatic Invasions January 2009). For example, the solitary ascidians Styela clava and Ciona intestinalis have had an adverse effect on aquaculture along Canada’s east coast, mainly on mussel culture [42-44]. S. clava, when extremely abundant, may result in significantly decreased mussel growth and also cause severe problems in crop handling, resulting in increased production costs estimated at $1.5 million [45]. In contrast, several species of ascidians are cultured for food primarily in Japan, Korea and France. The solitary ascidian Halocynthia roretzi has long been a popular seafood in Japan and Korea, with a market value of $18 million in 2006 [46]. Recently, a unique infectious agent has been identified as the cause of mass mortality of these cultured ascidians [47].

Ascidians provide a fertile ground for studies in the field of natural products. Similar to sponges and bryozoans, many ascidians avoid predation or fouling by producing noxious secondary metabolites [48-52]. Because of these properties, numerous species of ascidians may thus be a potential source of new anti-cancer compounds [53,54]. Trabectedin (earlier known as ecteinascidin-743, commercial name Yondelis®), a marine-derived alkaloid isolated from extracts of Ecteinascidae turbinata, is now being used in treatment of soft-tissue sarcomas [55,56]. Antimalarial compounds have been isolated from the solitary ascidians Microcosmus kellii, Ascidia syphonensis and Phallusia nigra [57], and numerous other compounds with anti-cancer, anti-viral and anti-bacterial capabilities are in various clinical trial stages by the pharmaceutical industry. The management and use of these organisms as sources of natural products is dependent, however, on understanding their taxonomy, the integrative basis of biology.

Ascidians have a poor fossil record [58]. Although calcareous spicules of distinctive shapes are found in some species of the families Polycitoridae, Pyuridae, and especially the Didemnidae [59,60], their fossils are rarely reported by paleontologists [61]. This is possibly due to their susceptibility to dissolution, and small size; many are less than 0.1 mm [60]. Fossil didemnid ascidian spicules were encountered in rocks from various regions around the world, usually dating to the Late Pliocene-Early Pleistocene period [61,62]. Eight specimens of a solitary fossil tunicate have been discovered with a body size of 2-4 cm; they resemble the extant Clavelina genus and are presumably ~520 million years old [63].

Currently there are numerous web-based sources of taxonomic inventories (e.g., Encyclopedia of Life http://www.eol.org, Integrated Taxonomic Information System http://www.itis.gov), but only a few websites are dedicated to ascidians (e.g., The Dutch ascidians Home Page http://www.ascidians.com, Ascidian Home Page for United States http://deptswashington.edu/ascidian/), and they do not aim to provide an inventory list. Unfortunately, most web-based datasets often lack updates due to limitations in funding and expertise. The Asciacea World Database (http://www.marinespecies.org/ascidiae/), which is a part of the World Register of Marine Species (WoRMS), is unique; it contains a comprehensive list of ascidian species, including information on synonymy, taxonomic literature, and distribution [64]. This database is the result of a joint effort of several ascidian taxonomists who constantly update and revise the information. With the aid of this database and the large taxonomic literature, our aim is to provide a systematic review of the class Asciacea, describe the main regions of highest biodiversity, discuss the risks of invasive species, and summarize the current trends in ascidians global distribution patterns.

Methods

Biogeographic distribution

Ascidians specimens are held by museums and similar institutions all over the world. However, only a few institutions provide reliable on-line options to search their collections (e.g., Smithsonian Invertebrate Zoology Collections, The Santa Barbara Museum of Natural History, Yale Peabody Museum Catalog...
Figure 2. Systematic division of ascidian species. a) Number and percentage of species per order; b) number of species by family within each order. doi:10.1371/journal.pone.0020657.g002

Maps and geographic regions
Species distribution information was compiled based on the geographic regions of the Exclusive Economic Zone division v5 standard map provided by VLIZ Maritime Boundaries Geodatabase [65].

Species names and systematic validation
We followed the taxonomic classification and used the tabular keys of Monniot et al. 1991 [1] (revised by F. Monniot and G. Lambert 2008–2009, unpublished data). Annual check-
Figure 3. Discovery rate and author contribution. a) Cumulative number of valid ascidian species described between 1750–2010; b) Percentage and number of species described per taxonomic authority. Note: only taxonomic authorities with more than 100 species are mentioned by name. doi:10.1371/journal.pone.0020657.g003
Table 1. Documented locations of non-indigenous ascidians.

| Species | Introduced sites | Lifestyle | Order | Remarks | References |
|---------|------------------|-----------|-------|---------|------------|
| 1. Aplidium glabrum | Netherlands | C A NH | [69] |
| 2. Aplidium phortax | New Zealand | C A SH | [70] |
| 3. Aplidium acutangulum | Brazil | C A SH | [71] |
| 4. Ascidia arctica | Atlantic Panama | S P T, NH | [72] |
| 5. Ascidia cannelata | Mediterranean Sea | S P NH | [73-75] |
| 6. Ascidia sp. | California harbors | S P NH | [41,76] |
| 7. Ascidia sydneiensis | Atlantic Panama, Brazil, Guam, Hawaii, India South America | S P T | [39,40,77-82] |
| 8. Ascidia zara | California harbors | S P NH | [38,41,76] |
| 9. Ascidia aspera | Argentina, New England, New Zealand, South Africa, South Australia, Tasmania | S P | [82-87] |
| 10. Asterocarpa humilis | Chile, New Zealand | S S SH | [70,88] |
| 11. Botryllides leachi | South Australia and Tasmania | C S SH | [85,86] |
| 12. Botryllides perspicuum | California harbors | C S NH | [41,76] |
| 13. Botryllides sp. | New Zealand | C S SH | [70] |
| 14. Botryllides violaceus | Atlantic Canada, California harbors, England, Mediterranean Sea, Netherlands, New England, San Francisco Bay | C S NH | [41,69,73,75,76,83,89-95] |
| 15. Botryllus schlosseri | Atlantic Canada, California harbors, Incia, New England, San Francisco Bay, South Africa, South Australia and Tasmania, US West coast | C S | [38,41,73,75,82,83,85,86,89,90,92,93,96] |
| 16. Ciona intestinalis | Atlantic Canada, California harbors, Chile, China/Korea, New Zealand, South Africa, South Australia and Tasmania, Washington | S P | [38,41,73,75,82,83,85,89,90,92,93,96] |
| 17. Ciona savignyi | California harbors, Japana, New Zealand, Washington | S P | [38,41,76,86,100,101] |
| 18. Clavelina lepadiformis | NW Atlantic, South Africa | C A | [82,102] |
| 20. Chemidocarpa areolata (C. irene) | Brazil | S S T, SH | [80] |
| 21. Chemidocarpa humillis | South Africa | S S SH | [82] |
| 22. Chemidocarpa irene | Hawaii | S S T, NH | [103] |
| 23. Corella eumyota | England, Iberia Atlantic coast, New Zealand, NW France | S P | [70,91,104,105] |
| 24. Cystodytes philippinensis | Mediterranean Sea | C A NH | [73,75] |
| 25. Didemnum cineraceum | Atlantic Panama, Brazil | C A T | [72,80] |
| 26. Didemnum perlucidum | Brazil, Caribbean, Guam, Gulf of Mexico | C A T | [40,71,106,107] |
| 27. Didemnum sp. | Hawaii | C A T, NH | [81] |
| 28. Didemnum vexillum | England, New England, San Francisco Bay, Washington, widely distributed | C A | [83,90,98,108,109] |
| 29. Diplosoma esterianum | Brazil, Guam, Netherlands, New England, South Africa | C A T | [40,69,71,82,83] |
| 30. Distaplia bermudensis | Brazil, Florida, Mediterranean Sea | C A T | [71,73,75,110] |
| 31. Distaplia stylifera | Brazil | C A SH | [80] |
| 32. Ecteinascidia styeloides | Mediterranean Sea | C P NH | [73,75] |
| 33. Ecteinascidia thurstoni | Mediterranean Sea | C P NH | [74,75] |
| 34. Euclbito elongatum | New Zealand | C A SH | [111] |
| 35. Euosynystyla tincta | Incia | C S NH | [78] |
| 36. Herdmania momus | Hawaii, Mediterranean Sea | S S T, NH | [39,73-75] |
| 37. Herdmania pallida | Atlantic Panama, Hawaii | S S T, NH | [72] |
| 38. Lissoclinum fragile | Guam | C A T, NH | [40] |
| 39. Microcosmus exasperatus | Atlantic Panama, Guam, Hawaii, India, Mediterranean Sea | S S T, NH | [39,40,72,74,75,78] |
| 40. Microcosmus squamiger | California harbors, India, Mediterranean Sea, South Africa | S S T, NH | [38,41,73,75,78,82,112] |
| 41. Molgula citrina | Alaska | S S NH | [113] |
| 42. Molgula ficus | California harbors, Chile | S S | [88,114] |
lists are published on-line by the Catalogue of Life [66], and the Encyclopedia of Life [67]. Both databases are connected to the World Register of Marine Species (WoRMS) check list, to which the Ascidiacea World Database belongs [64]. Therefore, to avoid confusion, only the valid names and classification provided by the Ascidiacea World Database http://www.marinespecies.org/ascidiacea/ were used for systematic analysis of families, genera etc. Division of colonial versus solitary species was based on the Monniot et al. 1991 keys. Taxonomic contribution was analyzed according to the authority index in the World Ascidiacea Database, and only first authors were taken into consideration.

Records of non-indigenous ascidians

In order to compile a current list of non-indigenous ascidians, we gathered data not only from the available literature but also from different governmental reports which often provide the first record of an introduced species. In addition, valuable information was obtained from the proceedings of the International Invasive Sea Squirt conferences [J Exp Mar Biol Ecol 342 (1), 2007 and Aquat Inv 4 (1) 2009]. The list includes only species that are mentioned as introduced or non-indigenous. Species that are classified as “cryptogenic” (species that cannot be reliably demonstrated as being either introduced or native, 68) were not included.

Results

Systematic division of ascidian species

Our systematic list includes 2815 valid species of ascidians. The highest number of species and families is found in the order Aplousobranchia, with approximately 50% of the species (1480) in the class Ascidiacea (Fig. 2a). Based on the classification of the Ascidiacea World Database, there are currently 26 families in the class Ascidiacea, of which 13 belong to the Aplousobranchia, with the Didemminae having the highest number of species (578). The genera with the highest number of described species are Aplidium (529) and Didemnum (228), in the Aplousobranchia. However, the highest number of genera per family was found in the Styelidae (38), order Stolidobranchia, which also has the second highest number of species (535) (Fig. 2b). The majority of described species in the Ascidiacea are colonial (1730, 61.5%) (Table S1, supporting material).

| Species | Introduced sites | Lifestyle | Order | Remarks | References |
|---------|-----------------|-----------|-------|---------|------------|
| Malgula manhattensis | California harbors, China/Korea, Europe, NE Pacific, Netherlands, South Australia and Tasmania | S | S | | [38,41,69,76,86,94,115] |
| Perophora japonica | Atlantic Europe, England, Netherlands, Northern California | C | A | NH | [69,91,116,117] |
| Perophora multicirrata | Mediterranean Sea | C | A | | [73,75] |
| Phallusia nigra | Guam, Hawaii, India, Mediterranean Sea | S | P | T, NH | [39,40,73-75,78,81] |
| Polyandrocarpa anguinea | Brazil | C | S | T, SH | [80] |
| Polyandrocarpa sp. | Hawaii | C | S | T, NH | [39] |
| Polyandrocarpa zorritensis | California harbors, Gulf of Mexico, Mediterranean Sea | C | S | T, NH | [38,41,75,76,107] |
| Polycarpa aurita | Hawaii | S | S | T, NH | [103] |
| Polycarpa spongia | Brazil | S | S | SH | [71] |
| Polycarpa tunida | Brazil | S | S | T, SH | [80] |
| Polyclinum aurantium | Brazil | C | A | SH | [77] |
| Polyclinum constellatum | Guam, Brazil, Pacific Mexico | C | A | T | [40,71,118] |
| Pyura praepuntulis | Chile | S | S | | [88] |
| Pyura vittata | Atlantic Panama | S | S | T, NH | [72] |
| Rhodosoma turgidum | Mediterranean Sea, Florida | S | P | NH | [74,75] Lambert unpublished data |
| Styela canina | Atlantic Panama, Brazil, California harbors, Guam, India | S | S | T | [40,42,43,74,75,78,80,82] |
| Styela clava | Atlantic Canada, California harbors, China/Korea, Denmark, England, Germany, Mediterranean Sea, Netherlands, New England, New Zealand, San Francisco Bay, South Australia and Tasmania, England, Washington | S | S | | [38,41,70,73,75,76,85,86,90,91,95,98,99,119] |
| Styela plicata | Brazil, California harbors, China/Korea, Gulf of Mexico, South Africa, South Australia and Tasmania | S | S | T | [38,41,76,77,79,82,85,86,95,107,120] |
| Symplegma brakenhielmi | California harbors, Guam, Hawaii, Mediterranean Sea | C | S | T, NH | [38,40,73-75] |
| Symplegma reptans | California harbors, Hawaii | C | S | T, NH | [38,39,41,76] |
| Symplegma rubra | Brazil, Gulf of Mexico | C | S | T | [71,107] |
| Trididemnum cf. savignii | Mediterranean Sea | C | A | NH | [75] |

C- Colonial, S- Solitary, Order: A-Aplousobranchia, P-Phlebobranchia, S-Stolidobranchia, Remarks: T- Tropical, NH- Northern Hemisphere only, SH- Southern Hemisphere only, doi:10.1371/journal.pone.0020657.t001
Discovery rate and author contribution

The discovery rate of ascidian species from 1756 until 2010 is presented in Fig. 3a. The rate of discovery has accelerated since 1950, when the major taxonomists of this group, P. Kott, C. and F. Monniot, and R.H. Millar began publishing. Over 1600 species have been described by these experts including the numerous descriptions by C.P. Sluiter and W.A. Herdman from the late 19th century-beginning of the 20th century. Figure 3b summarizes the contribution of the major taxonomists to total ascidian species described. Only authors responsible for more than 100 descriptions are mentioned by name; Claude and Françoise Monniot were grouped together due to their numerous collaborative publications.

Non-indigenous ascidians

Review of the literature resulted in records of 64 non-indigenous species (Table 1). Thirty three species are colonial. Half of the introduced species (32) belong to the order Stolidobranchia, the rest divide between the other two orders. Almost half of the species (30) have records only from the northern hemisphere, 13 have records only from the southern hemisphere, and 21 have records from both sections.

Records of introduction of ascidians in tropical waters are mainly from Hawaii, Guam and Panama. Of the 64 documented global non-indigenous species, 27 species have records in tropical regions. However, only 14 have records that are restricted to tropical environments (Ascidia archaia, Ciona intestinalis, Ciona savignyi, Didemnum pustulatum, Didemnum sp. [Hawaii], Distaplia stylifera, Herdmania pulchra, Lissoclinum fragile, Polycarpa anguinea, Polyandrocarpa sp., Polycarpa aurata, Polycarpa tumida, Polyclunum constellatum, Pyura vittata). The majority of species (50) have introduction records from temperate environments, including both the northern and southern hemispheres. There are no records yet of non-indigenous ascidians from the arctic.

Ascidian global distribution

Fig. 4 provides a summary of species richness in different regions of the world. A complete list of sites and references is provided in Table 2. The highest number of ascidian species is found in the Indo-Pacific, with inventory numbers such as 317 species from New-Caledonia, 187 species from the Western Pacific ocean, 117 and 102 species from Guam and Gulf of Siam area (numbers represent the exact number of species mentioned in each citation). The ascidian fauna along the coasts of Australia and New Zealand was studied extensively; resulting in records of 717 species from Australia, 249 species from Tasmania, and 124 species from New Zealand. At higher latitudes, the Mediterranean and Japan each represent areas with high number of species with about 229 species from the Mediterranean and 163 species in Japan. Antarctica and South Africa also have extensive records of ascidian species of 107 and 168 species respectively. The North American coasts have been studied thoroughly with approximately 170 species along both the Atlantic and Pacific coasts.

Discussion

Even though the class Asciidiacea has been the object of much scientific interest in the last decade [170], there are extensive regions around the world where very little collecting of ascidians has been done, resulting in very low number of described ascidian species and general lack of data (e.g., South and Central America, Canada, Alaska, and many thousands of islands in the tropical west Pacific). The current study reveals a strong association between species richness and sampling efforts. In addition, there is a clear trend of arrival and spread of non-indigenous species that put the endemic fauna at risk. Both of these issues emphasize the need for additional research in the field of ascidian biodiversity and biogeography.

In geographical areas where taxonomists have long been active, we typically found high numbers of species. The majority of the
Table 2. Ascidian regional species richness.

| Area                                      | Number of species | Reference |
|-------------------------------------------|-------------------|-----------|
| Australia                                 | 717               | [121]     |
| New Caledonia                             | 317               | [122]     |
| Tasmania                                  | 249               | [123]     |
| Mediterranean Sea                         | 229               | [124]     |
| Western Pacific Ocean: Palau, The Philippines, Indonesia, and Papua New Guinea | 187               | [125]     |
| South Africa                              | 168               | [126]     |
| Western Mediterranean                     | 165               | [127]     |
| Japan                                     | 163               | [128]     |
| New Zealand                               | 124               | [129]     |
| Guam                                      | 117               | [130]     |
| Antarctica                                | 107               | [131]     |
| Indo West Pacific region                   | 102               | [132]     |
| French Polynesia                          | 92                | [133]     |
| India                                     | 88                | [134]     |
| South America                             | 87                | [135]     |
| Eastern Mediterranean                     | 86                | [136]     |
| Adriatic                                  | 85                | [137]     |
| North West Pacific (Kamchatka)            | 80                | [138-140] |
| Gulf of Mexico                            | 70                | [141]     |
| Gibraltar                                 | 66                | [142]     |
| Ibeia                                     | 64                | [143]     |
| Fiji                                      | 60                | [144]     |
| Pacific Northwest                         | 60                | [145]     |
| Panama                                    | 58                | [146]     |
| British                                   | 58                | [147]     |
| Timor and Arafura Sea                     | 57                | [148]     |
| Brazil                                    | 56                | [149]     |
| Africa north west coast                   | 55                | [150]     |
| Chile                                     | 55                | [151]     |
| Scandinavia                               | 48                | [152]     |
| Red Sea                                   | 47                | [153]     |
| Hawaii                                    | 45                | [154]     |
| California                                | 45                | [155]     |
| Belize                                    | 40                | [156]     |
| Jamaica                                   | 39                | [157]     |
| Cuba                                      | 39                | [158]     |
| Gulf of Saint Lawrence                    | 37                | [159]     |
| Florida                                   | 36                | [160]     |
| Hong Kong                                 | 31                | [161]     |
| Bermuda                                   | 31                | [162]     |
| West Indies                               | 31                | [163]     |
| Tanzania                                  | 31                | [164]     |
| Mozambique                                | 29                | [165]     |
| Circumpolar                               | 29                | [166]     |
| Venezuela                                 | 29                | [167]     |
| Massachusetts                             | 26                | [168]     |
| Gulf of Aqaba                             | 25                | [169]     |
| Azores Islands                            | 19                | [170]     |

Table 2. Cont.

| Area                                      | Number of species | Reference |
|-------------------------------------------|-------------------|-----------|
| Galapagos                                 | 16                | [171]     |
| Bering Sea                                | 16                | [172]     |
| Bahrain                                   | 15                | [173]     |
| Bay of Fundy                              | 12                | [174]     |
| Black Sea                                 | 10                | [175]     |

Data sorted by number of species. doi:10.1371/journal.pone.0020657.t002

The high diversity of some of the ascidian families is remarkable. With approximately 26 families in the class Ascidiae, the colonial Didemnidae family contains 20% of the described species, possibly due to highly diverse Didemnum genera, with more than 200 species. The Styelidae family is also highly diverse with 38 genera, and 535 described species, colonial and solitary. Colonial species characterize more than 60% of the described species. The high diversity of colonial ascidians is increasingly important since many contain very active secondary metabolites important to the pharmaceutical industry [178].

In general, it has been shown that in tropical environments colonial species dominate the substrate [179]. This is attributed to their asexual reproduction and indeterminate growth which provide them with a significant advantage for the exploitation of tropical habitats. Thus there are many more colonial ascidian species than solitary species in the tropics, representing about 80% of the species [125,126,131,143,180]. Although colonial ascidians are generally considered a minor benthic component on exposed surfaces of the natural coral reefs they can rapidly overgrow corals and outcompete them for space during periods of nutrient enrichment [181–183]. Since ascidians are able to filter even minute particulate matter [184,185], any rise in nutrient levels and organic material in coastal waters will have a direct influence on their abundance.

In temperate waters solitary ascidians comprise 52% of the American fauna [154], and 75% in European waters (but this includes abyssal forms, almost all of which are solitary), [125,186]. In the Antarctic, 58% of the species are solitary [32]. It is possible described ascidian species (more than 60%) are attributed to only seven taxonomic experts. This is demonstrated in the high species richness found in Australia [121], New Caledonia [122], Japan [126], the Caribbean Sea [171], and South Africa [172–174]. In contrast, along the coasts of South America, Indian Ocean, and Eastern Atlantic, there are vast areas with only scarce information regarding the occurrence of ascidians, and in some cases the only information comes from studies that may be out of date and not representative of the diversity these areas currently exhibit [154,175]. For instance, the ascidian fauna of the Western Mediterranean has been studied in great detail and has been recorded in number of publications with an estimate of 165 described species [127]. The nearby Red Sea, which supports one of the most diverse ecosystems in the world [176] is represented by only 47 described species [152]. Thus, this discrepancy appears to be a result of less research and fewer sampling efforts, rather than a decrease in ascidian diversity [177]. Our inventory of 2815 described species of ascidians is certainly incomplete, with an estimation that approximately 3000 species remain to be discovered and described (Appeltans et al. 2011 unpublished data). Applying molecular approaches may further assist in locating cryptic speciation of a single species.
that solitary ascidians in the Antarctic and the deep sea, many of which are stalked, have an advantage over encrusting colonial species since most of the benthos is composed of soft sediments, so their vertical growth lifts them above the sediment. This three dimensional structure may improve food capture and assimilation during periods of winter inactivity and sedimentation [107]. In addition, since in solitary ascidians fertilization and larval development usually occur in the water column (in contrast to colonial species which are brooders), it is possible that they have a higher potential for dispersal [74]. This may also be advantageous in the Antarctic in cases of anchor ice formation [188], and ice scouring [189] which have a key role in determining marine biodiversity in high latitudes, emphasizing the importance of larval dispersal processes.

Historical baselines for comparison to present day from museum collections and published literature are required in order to understand and respond to changes in global biodiversity [190]. The current study provides a list of 64 non-indigenous ascidians (NIAs) with published records of introduction. This number is likely to be an underestimate, due to difficulty in taxonomic identification of aplousobranch species in particular. In some cases it may be difficult to determine if a certain species record is of a new introduction, or of a previously undiscovered natural population [113]. Lambert [40] suggests two criteria for the designation of NIAs in Guam, following the general guidelines of Chapman and Carlton [68] for determining non-indigenous species: (1) restricted to artificial surfaces and (2) an extra Indo-West Pacific distribution. The first criterion may be especially important especially in tropical environments which may be more resistant to invasion due to their diverse communities [191,192]. In temperate and cold water environments there are records of rapid spread of NIAs on natural substrates such as Didemnum vexillum (Gulf of Maine) [109,193] and Microcosmus squamiger (Western Mediterranean) [112]. A molecular approach, therefore, may be more relevant in revealing the status of a certain species [194-196].

The majority of records of NIAs are from cold water environments, suggesting this environment may be more favorable to introductions of ascidians. Nonetheless, nearly half of the NIAs have geographical records from tropical environments. Under lab conditions, at high temperature, the growth rate of NIAs was higher compared to that of native species [197], and they were able to tolerate significantly higher temperatures [198]. Thus, there is growing evidence that global warming may facilitate a shift northward by non-native species, accelerating homogenization of the global biota [199]. Nevertheless, high rates of endemism can be found in tropical environments such as the Great Barrier Reef [121], New Caledonia and French Polynesia [122,131], and also in unique environments such as Southern New Zealand [123], and the Antarctic, with its isolated and homogeneous fauna [32].

The class Ascidacea presents vast opportunities for research in the fields of evolution and development, physiology, natural products, and marine bioinvasion. Yet, there are many areas around the world that are relatively poorly known, and in others the available data should be updated and revised. Many more species are yet to be discovered, contributing to our accumulating knowledge of this unique group.

Supporting Information

Table S1  Systematic division of ascidian species following the Ascidacea World Database [67].

Acknowledgments

We express our gratitude to G. Lambert for her advice and helpful suggestions and to C. Primo for her collaboration. N. Shenkar would like to thank the Israeli Taxonomy Initiative and the Dan-David foundation for financial support.

Author Contributions

Conceived and designed the experiments: NS BJS. Performed the experiments: NS. Analyzed the data: NS. Contributed reagents/materials/analysis tools: BJS. Wrote the paper: NS BJS.

References

1. Monniot C, Monniot F, Laboute P (1991) Coral Reef Ascidians of New Caledonia. Paris: ORSTOM.
2. Cameron CB, Garrett JR, Swalla BJ (2000) Evolution of the chordate body plan: new insights from phylogenetic analysis of deuterostome phylog. Proc Natl Acad Sci USA 97: 4403-4407.
3. Kost P (2005) New and little-known species of Didemnidae (Ascidiae, Tunicata) from Australia (Part 3). J Nat Hist 39: 2469-2497.
4. Forbes E, Harley SCT (1855) A history of British Mollusca and their shells. 4 vols. London: John Van Voorst.
5. Lamare JB (1816) Histoire naturelle des animaux sans vertèbres. Tome III. Tuniçiers. Paris: Detrerville. pp 80-130C.
6. Lambert CC (2005) Historical introduction, overview, and reproductive biology of the protochordates. Can J Zool 83: 1-77.
7. Zeng L, Swalla BJ (2005) Molecular phylogeny of the protochordates: chordate evolution. Can J Zool 83: 24-33.
8. Miller Edwards H (1843) Éléments de zoologie. Vol. 2. Animaux sans vertébrés. pp 313-316.
9. Hancock A (1850) On the anatomy of the fresh water Bryozoa. Ann Nat Hist 2: 175-204.
10. Savigny JC (1816) Mémoires sur les Animaux sans Vertébrés. Part 2. Paris: G Dutour.
11. Kowalevsky AO (1866) Entwicklungs geschichte der einfachen Ascidien. Mem Acad Sci St Petersb 10: 1-19.
12. Raff RA, Love AC (2004) Kowalevsky, comparative evolutionary embryology and the inerrnal linage of evo-devo. J Exp Zool (Mol Dev Evol) 302B: 19-34.
13. Balachoff BR (1881) A treatise of comparative embryology. London: Vol. 2.MacMillan.
14. Bourrat SJ, Jullienotir T, Lowe CJ, Freeman R, Antonowicz J, et al. (2006) Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenathurbida. Nature 444: 85-88.
15. Deluc B, Brinkmann H, Chourrout D, Philippe H (2006) Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 439: 965-968.
16. Swalla BJ, Smith AB (2008) Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. Phil Trans R Soc B 363: 1557-1568.
17. Nibhida H, Sasuda K (2001) Macho-1 encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. Nature 409: 724-729.
18. Lelie F (1886) Sur la classification des Tuniciers. CR Acad Sci Paris 102: 1573-1575.
19. Tsagkogeorga G, Turon X, Hopcroft RR, Tilik M, Feldstein T, et al. (2009) An updated 18S rRNA phylogeny of tunicates based on miton and secondary structure models. BMC Evol Biol 9: 187.
20. Perrier JO (1886) Note sur la classification des Tuniciers. CR Acad Sci Paris 126: 1758-1762.
21. Lambert G (2001) A global overview of ascidian introductions and their possible impact on the endemic fauna. In: Sasuda H, Yosuohi H and Lambert CC (ed.), The Biology of Ascidians. Tokyo, Springer-Verlag, pp 249-257.
22. Lambert G (2005) Ecology and natural history of the protochordates. Can J Zool 83: 34-56.
23. Svane I, Young CM (1989) The ecology and behavior of ascidian larvae. Oceanogr Mar Biol Annu Rev 27: 45-90.
24. Vasquez E, Young CM (1996) Responses of compound ascidian larva to haloclines. Mar Ecol Prog Ser 135: 175-190.
25. Sims LL (1984) Osmoregulatory capabilities of three macrozooplankton stolidolbranch ascidians, Styela clava Herdman, S. picta (Lenten) and S. montaguii (Dalh). J Exp Mar Biol Ecol 82: 117-129.
26. Gabi A (1990) Distribution of the sea squid Ecteinascidia thurstoni Herdman, 1880 (Ascidiae: Perophoridae) along Sri Canal and Egyptian Red Sea Coasts. Oceanologica 50: 239-253.
54. Scotto K W (2002) ET-743: more than an innovative mechanism of action.

53. McClintock JB, Amsler MO, Amsler CD, Southworth KJ, Petrie C, et al.

52. Pisut DP, Pawlik JR (2002) And-predatory chemical defenses of ascidians:

51. Davis AR (1998) Antifouling defense in a subtidal guild of temperate zone

50. Kumatagi A, Suto A, Ito H, Tanabe T, Takahashi K, et al. (2010) Mass

49. Nguyen TT, Taniguchi N, Nakajima M, Na-Nakorn U, Sukumasavin N,

48. Howes S, Herbinger CM, Darrell P, Vercaemter B (2007) Spatial and temporal

47. Bourque D, Davidson JG, MacNaughton JS, Arsenault G, LeBlanc AR, et al. (2007)

46. Sagular EK (2009) Fossil didemnid ascidian spicules in the Pliocene

45. Meador CC, Eldredge LG, Carlton JT (1999) Historical and recent

44. Casali PG, Sanfilippo R, D’Incalci M (2010) Trabectedin therapy for sarcomas.

43. Thompson R, MacNaughton JS (2004) An overview of the clubbed tunicate (,Styela

42. Robinson AR, MacNaughton JS (2003) A review of the recruitment and

41. Akber LA, Ovatt CA (2008) The solitary ascidian Hemicordella muscula: native (Red Sea) vs. non-indigenous (Mediterranean) populations. Biol Inv 10: 1431-1439.

40. Sherar N, Loya Y (2008) Antarctic ascidian populations in tropical waters. Pac Sci 56: 291-298.

39. Højlund C, Fortune R (2003) What is the view from the boat? Atlantic Canadian Tunicate Workshop Proceedings, Atlantic Veterinary College, PEI Canada, March 2003. 29 p.

38. Auer LA, Ovatt CA (2009) Factors influencing the recruitment and abundance of Didemnum in Narragansett Bay, RhodeIsland. ICES J Mar Sci 66: 765-769.

37. Dyer B (1967) Settlement of sessile animals on eelgrass slabs in two polls near Bergen. Sarsia 29: 137-180.

36. Thom P, Vansper ES (2009) Antarctic ascidians: an isolated and homogeneous fauna. Polar Res 28: 403-414.

35. Højlund C, Fortune R (2003) What is the view from the boat? Atlantic Canadian Tunicate Workshop Proceedings, Atlantic Veterinary College, PEI Canada, March 2003. 29 p.

34. Davis AR (1998) Antifouling defense in a subtidal guild of temperate zone

33. Auer LA, Ovatt CA (2009) Factors influencing the recruitment and abundance of Didemnum in Narragansett Bay, RhodeIsland. ICES J Mar Sci 66: 765-769.

32. Primo C, Vazquez E (2009) Antarctic ascidians: an isolated and homogeneous

31. Pisut DP, Pawlik JR (2002) And-predatory chemical defenses of ascidians:

30. Therriault TW, Herborg LM (2008) Predicting the potential distribution of the orange-blotched tunicate Chaetoderma intestinalis in Canadian waters: a risk assessment. ICES J Mar Sci 65: 785-794.

29. Dybern B (1967) Settlement of sessile animals on eelgrass slabs in two polls near Bergen. Sarsia 29: 137-180.

28. Dyer B (1967) Settlement of sessile animals on eelgrass slabs in two polls near Bergen. Sarsia 29: 137-180.

27. Moniati F (2009) Some ascidians from Indonesian marine lakes (Raja Ampat Islands, West Papua). Zootaxa 2106: 15-40.
Global Diversity of Ascidiae

Plos ONE | www.plosone.org

11 June 2011 | Volume 6 | Issue 6 | e20657

91. Arcenas F, Bishop JDD, Caruso JT, Dydynda PJ, Farnham WF, et al. (2006) Alien species and other notable records from a rapid assessment survey of marinas on the south coast of England. J Mar Biol Assoc UK 86: 1329–1337.

92. Carver CE, Mallet AL, Vercammen B (2006) Biological synopsis of the colonial tunicates, Botryllus schlosseri and Botrylloides violaceus. Canadian Museum Report of Fisheries and Aquatic Sciences. 2747 p.

93. Lejeune C, Bock DG, Thieriault TW, Maciaa HJ, Grisescu ME (2010) Comparative phyogeography of two colonial ascidians reveals contrasting invasion histories in North America. Biol Inv 13: 635–656.

94. Lambert G (2005) New records of ascidians from the NE Pacific: a new species of Trididemnum, range extension and redescriptions of Aglaphis pannosus (Ritter, 1899) including its larva, and several nonindigenous species. Zeotaxa 256: 65–67.

95. Gollasch S, Haydar D, Minchin D, Wolff WJ, Reise K (2009) Introduced aquatic species of the North Sea coasts and adjacent brackish waters. In: Biodiversity in Marine Ecosystems: Ecological, Management, and Geographic Perspectives. Rove G, Crooks J, eds. Berlin Springer-Verlag Heidelberg, pp 507–529.

96. Stone DS, Ben-Shlomo R, Rinkevich B, Weismann IL (2002) Genetic Variability of Botryllus schlosseri invasions to the East and West Coasts of the USA. Mar Ecol Prog Ser 245: 93–100.

97. Bursen BI (1959) Ascidians of New Zealand. Part IV. Ascidians in the vicinity of Christchurch. T Roy Soc NZ 78: 348–355.

98. Lambert G (2007) Washington state 2006 survey for invasive tunicates with records from previous surveys. Updated October 2010. Prepared for Washington Dept. of Fish and Wildlife. 8 p.

99. Sos KS, Looman D, Minchin D, Wolff WJ, Reise K (2009) First assessment of invasive marine species on Chinese and Korean Coasts. In: Biodiversity in Marine Ecosystems: Ecological, Management, and Geographic Perspectives, Rove G, Crooks J, eds. Berlin Springer-Verlag Heidelberg, pp 577–596.

100. Smith JK, Galah PL, Fidler AE (2010) First record of the solitary ascidian Ciona intestinalis Herdman, 1882 in the Southern Hemisphere. Aquat Inv 5: 363–368.

101. Zaygoninev D, Sanamyan DE, Khasenko SD (2007) On the Introduction of the Ascidian Ciona intestinalis Herdman, 1882 into the Great Bay, Sea of Japan. Russ J Mar Biol 33: 133–136.

102. Reinhardt JF, Stefanik LM, Hudson DM, Mangelio E, Glachy R, et al. (2010) First record of the non-native light bulb tunicate Clavelina lepadiformis Müller, 1776 in the northwest Atlantic. Aquat Inv 5: 165–190.

103. Godwin S, Harris L, Charette A, Moffitt R (2006) Marine invertebrate species associated with the biofouling of derrick fishing gear in the Papahanaumokuakea–Marine National Monument. A focus on marine non-native species transport. Kaneohe HI: Hawaii Institute for Marine Biology. 26 p.
155. Goodbody I (2003) The Ascidian fauna of Port Royal, Jamaica In Harbou and mangrove dwelling species. Bull Mar Sci 75: 457–476.

156. Hernández-Zanny AC, Carballo JL (2001) Distribution and abundance of ascidian assemblages in Caribbean reef zones of the Golfo de Batabano (Cuba). Coral Reefs 20: 139–162.

157. Brunei NJ (1953) Ascidians of the Bermudas. Biological Bulletin 106: 77–88.

158. Goodbody I (1984) Ascidians from Caribbean shallow water localities. Studies on the Fauna of Curacao and other Caribbean Islands 67: 62–76.

159. Millar RH (1956) Ascidians from Mozambique, East Africa. Ann Mag Nat Hist Nat Sci 9: 913–922.

160. Millar RH (1988) Ascidians collected during the International Indian Ocean Expedition, J Nat Hist 22: 825–848.

161. Roche RM, Guerra-Castro E, Lira C, Paul SM, Hernández J, et al. (2010) Inventory of ascidians (Tunicata, Ascidacea) from the National Park La Resinga, Isla Margarita, Venezuela. Biota Neotrop 10: 210–218.

162. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

163. Millar RH (1955) A collection of ascidians from South Africa. Ann Mag Nat Hist 86: 1–55.

164. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

165. Bone Q, Carre C, Chuang P (2003) Tunicate feeding filters, J Mar Biol Ass UK 83: 967–919.

166. Goodbody I (1984) Ascidians from Caribbean shallow water localities. Studies on the Fauna of Curacao and other Caribbean Islands 67: 62–76.

167. Millar RH (1955) A collection of ascidians from South Africa. Ann Mag Nat Hist 86: 1–55.

168. Berrill N J (1932) Ascidians of the Bermudas. Biological Bulletin 62: 77–88.

169. Brunei P, Bossé L, Lamarche G (1998) Catalogue of the Marine Invertebrates of the Euxine and Gulf of Saint Lawrence. Canadian Special Publication of Fisheries and Aquatic Sciences 126: 465.

170. Millar RH (1988) Ascidians collected during the International Indian Ocean Expedition, J Nat Hist 22: 825–848.

171. Millar RH (1955) A collection of ascidians from South Africa. Ann Mag Nat Hist 86: 1–55.

172. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

173. Millar RH (1956) Ascidians from Mozambique, East Africa. Ann Mag Nat Hist Nat Sci 9: 913–922.

174. Millar RH (1956) Ascidians from Mozambique, East Africa. Ann Mag Nat Hist Nat Sci 9: 913–922.

175. Millar RH (1955) A collection of ascidians from South Africa. Ann Mag Nat Hist 86: 1–55.

176. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

177. Millar RH (1956) Ascidians from Mozambique, East Africa. Ann Mag Nat Hist Nat Sci 9: 913–922.

178. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

179. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

180. Millar RH (1955) A collection of ascidians from South Africa. Ann Mag Nat Hist 86: 1–55.

181. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

182. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

183. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

184. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

185. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

186. Millar RH (1964) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

187. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

188. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

189. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

190. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

191. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

192. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

193. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

194. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

195. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

196. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.

197. Millar RH (1956) South African ascidians collected by Th. Mortensen, with some additional material. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening, bd., 127: 159–180.