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INVASION NOTE

The Southern Hemisphere ascidian *Asterocarpa humilis* is unrecognised but widely established in NW France and Great Britain

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Abstract Non-native ascidians can be a major feature of sessile communities, particularly in artificial habitats, but may be overlooked because of poor understanding of species' taxonomy and biogeographic status. The styelid unitary ascidian *Asterocarpa humilis*, up to now only reported in the Southern Hemisphere, has been found on the coast of NW France from St Malo to Quiberon, on the south coast of England from Falmouth to Brighton, and also in north Wales. The first documented occurrence was in 2005 in Brittany, but the species was found to be relatively widespread at a regional scale and common in many places during surveys in 2009, 2010 and 2011. It has possibly been present but overlooked for some time. The identification based on morphology was confirmed by comparison with specimens from New Zealand, within the species' presumed native range, by molecular barcoding based on mitochondrial (COI) and nuclear (18S) genes.

Keywords Non-native · Tunicata · *Cnemidocarpa* · Barcoding · NE Atlantic Ocean · Introduced species

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Introduction

Non-native tunicates are an important feature of sessile communities in coastal waters, particularly on artificial structures in harbours, shellfish farms etc. (Lambert 2007; Arenas et al. 2006; Marins et al. 2010) with noteworthy ecological and economic consequences (e.g. *Ciona intestinalis* Blum et al. 2007; Sephton et al. 2011). However, they can be overlooked due to misidentification or poor understanding of their historical biogeography, many being unrecognised as alien species or classified as cryptogenic (Carlton 2009; Haydar et al. 2011). Molecular barcoding can help by testing identifications based on morphology (McGlashan et al. 2008; Bucklin et al. 2011).

The genus *Asterocarpa* Brewin, 1946 (type species *Styela cerea* Sluiter, 1900) was until recently known

only from temperate waters in the Southern Hemisphere and is distinguished from other styelid genera mainly by the configuration of the gonads, which form a series of branched clusters, often in a somewhat radiating pattern lacking a major axis, with very short free gonoducts that are not directed towards the atrial opening (Brewin 1946; Kott 1985). These are the main features distinguishing *Asterocarpa* from the genus *Cnemidocarpa* Huntsman 1912, in which the gonads have a clear major axis and are generally unbranched, with gonoducts directed towards the atrial opening. Some authorities regard the separation of the genera on this basis to be unjustified, in which case the name *Cnemidocarpa* has priority.

Kott (1985) synonymised four nominal species with A*sterocarpa humilis* (Heller 1878): *Styela cerea* Sluiter, 1900, *Dendrodoa gregaria* Kesteven, 1909, *Tethyum asymmetron* Hartmeyer, 1912 and *Cnemidocarpa robinsoni* Hartmeyer, 1916. A fifth species, *Cnemidocarpa aucklandica* Bovien (1921), has also been synonymised with *A. humilis* (Millar 1982). This synonymy is followed here. On this basis *A. humilis*, which was originally described (as *Styela humilis*) from New Zealand, has subsequently been recorded from Tasmania, southern mainland Australia, and the remote Chilean Juan Fernandez archipelago (Van Name 1945; Millar 1982; Kott 1985; and references therein). In New Zealand, the name *Asterocarpa cerea* (Sluiter, 1900) has commonly been used for *A. humilis*, and a second, distinctive, blue species, *Asterocarpa coerulea* (Quoy and Gaimard 1834), also occurs (Morton and Miller 1973; Millar 1982). *Asterocarpa humilis* has also been reported from South Africa and continental Chile, but in both these regions it has been regarded as an introduced species, or at least as cryptogenic (Clarke and Castilla 2000; Monniot et al. 2001; Mead et al. 2011).

Here we report the first records of *Asterocarpa humilis* in the Northern Hemisphere, as an introduced species in NW Europe.

Methods

The records reported here mostly arose from monitoring the sessile biota of pontoons and other submerged surfaces in marinas and harbours, either through rapid assessment surveys, or during visits to tend settlement panels, or when collecting material for population-genetic studies of various species. In the UK, about 50 sites were surveyed on the south and east coasts of England between Falmouth and Hull in the last 2–3 years, and around 10 of these sites in SW England have been visited more frequently in connection with experimental work; this was all done from the surface (i.e. without diving). In France, 12 sites have been visited frequently since 2009 along the coast of Brittany from Lorient to St Malo as part of surveys, collecting visits and experimental work carried out both by diving and from the surface.

In addition to morphological examination, molecular barcoding was carried out. Representative samples from some of the European locations surveyed were sequenced (Table 1) as well as 2 specimens from New Zealand, within the presumed native range of the species. A preliminary search in GenBank found one sequence corresponding to a specimen classified as *Cnemidocarpa humilis* (GenBank No. FM244859; Tsagkogeorga et al. 2009); this sequence was for the 18S gene, which was therefore selected for further molecular analysis. In addition, because the nuclear 18S gene is a conservative marker, we also sequenced a fragment of the mitochondrial gene COI which is popular for molecular barcoding in marine metazoans (Bucklin et al. 2011). We designed specific primers for COI: Ah-COIF (Fwd) 5′-CTAATTCGTACTGAGCT TTC-3′ and Ah-COIR (Rev) 5′- GTTACTAATACC GTCCAACA-3′. These amplified a 467 bp fragment and after sequencing of the PCR products produced a 412 bp fragment to be analyzed. Details of the molecular methods are provided in Online Resource 1.

Results

Occurrence in Europe

The specimens yielding the DNA sequence of *Cnemidocarpa humilis* used in the general phylogenetic analysis of tunicates by Tsagkogeorga et al. (2009) had been collected with *Ciona intestinalis* in western Brittany (Brest or Camaret-sur-Mer), France, in May 2006, and were identified by X. Turon. Although not heralded as such, this was the first recognition of *A. humilis* in the Northern Hemisphere. Independently of this, the species was noted jointly by the Plymouth and Roscoff co-authors during collaborative surveys of marinas and harbours in Brittany and southern

Table 1 NW European localities at which *Asterocarpa humilis* has been recorded, with dates of first observation

Location	Survey			Molecular barcoding			
	Latitude (N)	Longitude (W)	Date obs.	Collection date	N	COI haplotype	18S haplotype
Brittany, NW France							
Bas-Sablons Marina, St-Malo	48° 38′ 21″	2° 1′ 37″	07/09/2011	07/09/2011	15	15 H1	15 H1
Perros-Guirec Marina	48° 48′ 17″	3° 26′ 30″	18/08/2010				
Trebeurden Marina	48° 46′ 11″	3° 35′ 11″	18/08/2010	09/12/2010	7	7 H1	7 H1
Astan, Morlaix area, CTD profiler offshore	48° 44′ 55″	3° 57′ 40″	15/09/2011	15/09/2011	3	3 H1	3 H1
Roscoff Harbour (for ferry/fishing vessels)	48° 43′ 4″	3° 57′ 58″	02/03/2010	09/08/2011	1	1 H1	1 H1
Penzé estuary, natural habitat, Figuier to Cheminee	48° 40′ 28″	3° 56′ 09″	29/04/2011				
Oyster Farm, Penzé estuary	48° 40′ 60″	3° 56′ 28″	12/08/2010	12/08/2010	2	2 H1	2 H1
Aber Wrac'h Marina	48° 35′ 55″	4° 33′ 39″	08/02/2010	12/08/2011	13	13 H1	12 H1
Château Marina, Brest	48° 22′ 45″	4° 29′ 24″	17/08/2010				
Moulin Blanc Marina, Brest	48° 23′ 34″	4° 25′ 51″	07/12/2009				
Camaret-sur-Mer Marina	48° 16′ 48″	4° 35′ 44″	01/09/2005	29/11/2010	10	9 H1	10 H1
Crozon Morgat Marina	48° 13′ 26″	4° 29′ 42″	19/08/2010				
Quiberon Marina	47° 29′ 18″	3° 6′ 8″	01/03/2011				
North Wales							
Holyhead Marina	53° 19′ 14″	4° 38′ 36″	23/11/2011				
Southern England							
Falmouth Marina	50° 9′ 48″	5° 5′ 4″	28/03/2011	11/08/2011	12	11 H1	12 H1
Port Pendennis Marina (outer), Falmouth	50° 9′ 8″	5° 3′ 41″	14/07/2010				
Mussel farm, Fal estuary	50° 12′ 53″	5° 1′ 39″	29/06/2010				
Queen Anne's Battery Marina, Plymouth	50° 21′ 53″	4° 7′ 49″	21/04/2011	24/08/2011	6	6 H1	6 H1
Plymouth Yacht Haven (marina)	50° 21′ 30″	4° 7′ 18″	01/09/2011				
Kingsbridge–Salcombe Estuary	c.50° 15′ 00″	c. 3° 45′ 30″	23/10/2009				
Brixham Marina	50° 23′ 57″	3° 30′ 24″	09/06/2011	25/08/2011	12	11 H1	11 H1
Torquay Marina	50° 27′ 37″	3° 31′ 38″	21/03/2010				
Weymouth Harbour	50° 36′ 25″	2° 27′ 8″	09/10/2009				
Brighton Marina	50° 48′ 33″	0° 5′ 53″	16/06/2011				
New Zealand							
Picton				12/2010	2	1 H2	2 H1

For molecular analyses, dates of collection, number of specimens analysed (N), haplotype (H1, H2) and number of specimens possessing haplotype

England in 2005–2011, and eventually identified by them as *A. humilis*. Retrospective examination of preserved specimens and photographs indicated the earliest documented presence of *A. humilis* to be in Brittany in September 2005, at Camaret-sur-Mer, while the first documented records in Great Britain were in October 2009.

To date, the species has been found on the coast of Brittany in 13 locations (distributed between 9 bays or estuaries) from St Malo in the north to Quiberon in the

south (Table 1). Most of the sites are marinas or harbours but in the Bay of Morlaix area the species was also found (1) on artificial structures in an oyster farm and nearby natural habitat (Penzé estuary in Table 1) where it occured with native oysters (*Ostrea edulis*), scallops (*Pecten maximus*) and the invasive slipper limpet (*Crepidula fornicata*) and (2) fouling a continuous SBE CTD profiler operating off-shore (Astan in Table 1; the nearby natural rocky habitats were not surveyed). *Asterocarpa humilis* was not found in two other bays, namely Lorient (Port Kernével Marina and the city centre marina, both surveyed on 26/05/2011) and St-Quay-Portrieux (marina surveyed on 07/09/2011).

In England, the species has been found in 10 locations on the south coast between Falmouth and Brighton (a distance of c. 350 km), although not during visits to 17 marinas in the Solent region (Lymington to Southsea, central south coast) in autumn 2009. It was not recorded on the east coast of England, i.e. from the Thames estuary to Hull in autumn 2009, but was present in north Wales, in Holyhead Marina, when visited in November 2011.

Morphological identification of European specimens

Illustrations and further details of the internal anatomy of European specimens, and consideration of the generic assignment of *A. humilis*, are provided in Online Resources 1 and 2.

European specimens agree well with the redescriptions of Southern Hemisphere *Asterocarpa humilis* by Van Name (1945) (Juan Fernandez, as *Cnemodocarpa robinsoni*), Brewin (1946) and Millar (1982) (both New Zealand, as *A. cerea*), Kott (1985) (Australia), Clarke and Castilla (2000) (Chile) and Monniot et al. (2001) (South Africa, as *C. humilis*). In NW Europe, individuals are up to 40 mm in length, and may occur in clumps attached to one another (Fig. 1e, f). Exposed tunic of living specimens is orange–red, often with small warty protuberances or papillae on the upper surfaces and especially on the siphons (Fig. 1d–f). Tunic attached to the substrate or another individual is paler (often silvery-white) and thinner. The open siphons show four prominent cream or white stripes plus secondary white, radial markings on a reddish background, giving a pattern reminiscent of the face of a compass (Fig. 1a, b). The main stripes remain discernible in partially closed siphons as pale bars (Fig. 1d), but are not evident when the siphons are closed tightly, such as in specimens out of water (Fig. 1e, f). The siphon markings are lost upon preservation in ethanol.

Internally, the arrangement of the gonads distinguishes *A. humilis* from all other styelids present in European seas. Several small gonads are arrayed just to the right of the ventral midline (endostyle) and coalesce into a smaller number of branched clusters that may ultimately form a single complex strip parallel to and partially overlying the endostyle (Online Resource 2b–d), as noted by Brewin (1946) and Monniot et al. (2001); very short, transparent gonoducts project from each cluster, with their openings remote from the atrial siphon and facing in a variety of directions (Online Resource 2d, left inset). On the left side, only one or two gonad clumps occur; these lie anteriorly, close to the ventral midline near the top of the gut loop (Online Resource 2a and d right inset, arrowed). In general the branching of the gonadal clumps in our material is much less clearly radial than in the illustration by Brewin (1946).

Molecular barcoding

Both the nuclear and mitochondrial genes analyzed, when compared to specimens from the native range, unambiguously assigned the sampled European specimens to *Asterocarpa humilis* (Table 1). Briefly, exactly the same sequence was obtained (GenBank n°JX312280) from the 18S nuclear gene for every specimen analyzed from New Zealand, Brittany and Great Britain (n = 81). There was an almost perfect match (99 % of 1502 bp) between this sequence and the one available in GenBank (i.e. GenBank n° FM244859); the differences were 4 one-base-pair indels and 4 substitutions. For the COI gene, no sequence was available in GenBank. The smallest fragment obtained was for the New Zealand specimen (394 bp). Using this fragment to compare with sequences available in the GenBank database, the highest similarity was obtained for *Polycarpa pomaria* (80 % identity only), another styelid species. Interestingly, no polymorphism was observed among the French and UK specimens (COI haplotype H1 in Table 1; n = 78; GenBank n° JX312278) whereas the single New Zealand specimen successfully sequenced was characterized by another haplotype (H2 in

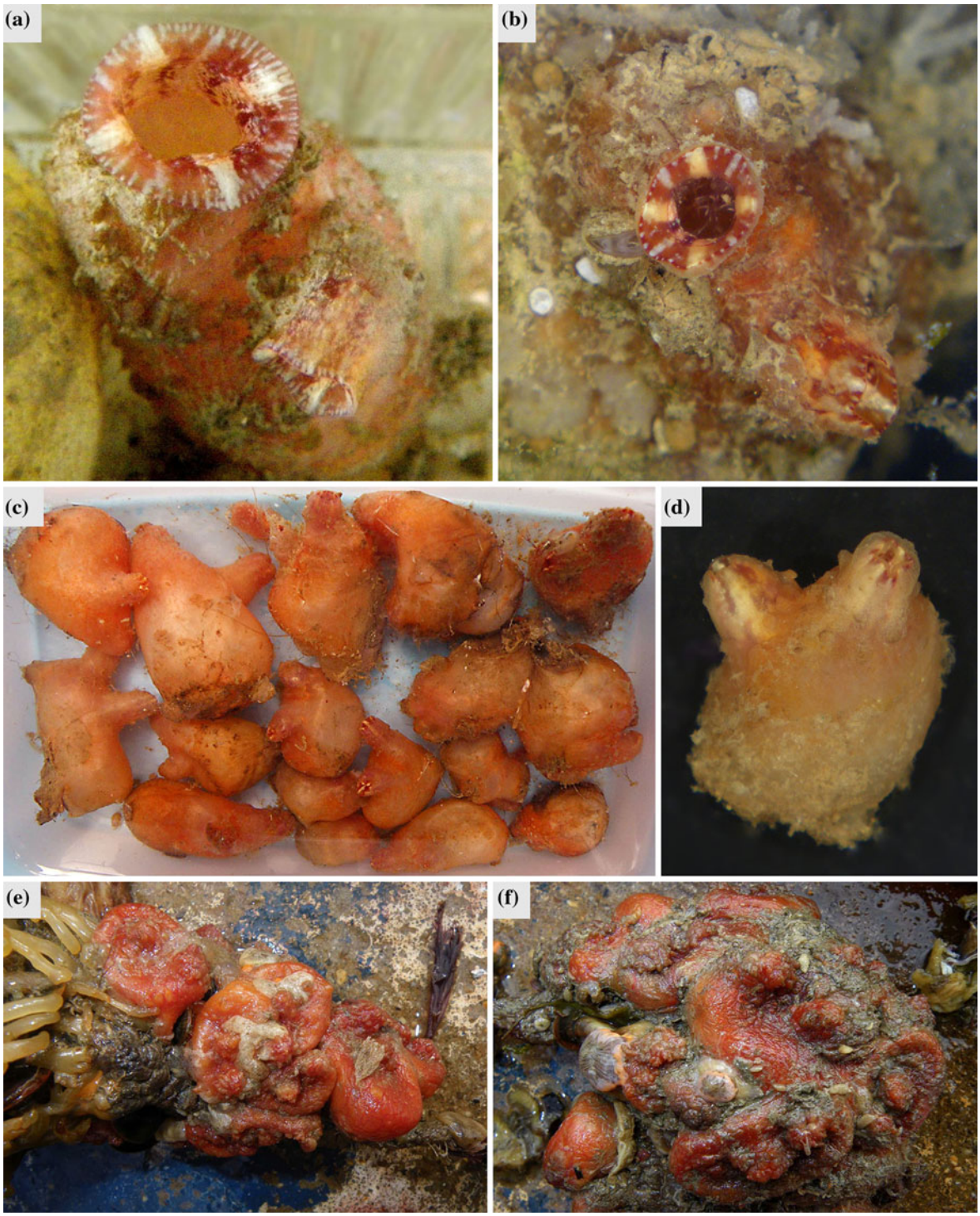

Fig. 1 *Asterocarpa humilis* **a** and **b** live specimens (c. 25 mm length) showing pattern of open siphons (**a** Brest, France; **b** Plymouth, England) **c** live specimens mechanically removed from harvested mussels being prepared for sale at aquaculture site (Fal estuary, England), siphons wholly or partially closed, (**d**) live specimen (c. 10 mm length, Plymouth) showing striped appearance of partially closed siphons (**e**) and (**f**) clumps of individuals strongly contracted following removal from the water (Salcombe, England) (Images are in colour in online version)

Table 1; GenBank n° JX312279), differing by 4 base pairs.

Brooding

Brewin (1946), Millar (1982) and Clarke and Castilla (2000) all reported finding larvae in the atrial cavity of *A. humilis*. Brooding in the atrial cavity was confirmed in two specimens collected on 23/11/2011 in Holyhead (N. Wales, UK), which contained hundreds of hatched larvae (Online Resource 2i). These larvae are 1.0–1.1 mm in length, slightly shorter than indicated by Brewin (1946), Millar (1982) and Clarke and Castilla (2000). They are white, and the sensory vesicle has a single circular black spot, stated by Millar (1982) to be the ocellus.

Discussion

The records reported here are to our knowledge the first for *Asterocarpa humilis* in the Northern Hemisphere, but the species is already widespread in NW France and Great Britain, and is common in at least some of the known localities, indicating the potential to be a major presence in future. The species' known occurrences suggest that it is not a recent arrival. However, it was not recorded during surveys of 12 marinas/harbours on the south coast of England in September 2004 (Arenas et al. 2006), including 5 sites at which it has subsequently been found. The survey team in 2004 included Gretchen and Charles Lambert, ascidian experts already very familiar with *A. humilis* from visits to the Southern Hemisphere. Similarly, *A. humilis* was not noted in marinas in NW France during surveys in summer 2005 by a team including the Lamberts, again visiting sites which now host the species. The only record of *A. humilis* from the time of the 2005 survey, made retrospectively from photographs, was amongst material at the Station Biologique de Roscoff which had been collected by the divers of the Station staff at an additional site, Camaret-sur-Mer. The species was present in February 2010 in Aber Wrac'h Marina, Brittany, which was not built until 2007. At Queen Anne's Battery Marina in Plymouth, UK, a site included in the 2004 survey and regularly visited by the Plymouth co-authors for over a decade, *A. humilis* was not detected until April 2011. This all suggests that *A. humilis* has become more widespread, or at least much more numerous where it does occur, compared with a few years ago. It is of course possible that earlier records will now come to light based on photographic evidence or examination of preserved material.

The geographical limits of *A. humilis* in Europe remain to be properly investigated. The great majority of the current records are from artificial habitats (marinas, harbours or aquaculture facilities) but this pattern may simply reflect a strong bias towards this kind of habitat in our survey work. Indeed, since the species has been recognised, divers have observed specimens on some natural estuarine and off-shore substrates in Brittany (in the Penzé estuary and Astan sites in Table 1). Again, the presence of *A. humilis* in natural habitats remains to be properly explored, but holds important implications for the potential effects of this introduced species.

Within its putative native range, *Asterocarpa humilis* has been recorded as a fouling species on commercial ships (Coutts and Dodgshun 2007) and aquaculture equipment (Hodson et al. 2000). Introduction to Europe via commercial ports as a fouling organism on 'niche' areas on the hulls of merchant ships is thus a possibility, but importation via commercial shellfish movements is a very strong alternative. The species has been reported from scallop aquaculture ropes in Chile (Clarke and Castilla 2000), and is common in a rope-culture mussel farm in SW England and in an oyster farm in the Penzé estuary, Brittany.

Brewin (1946) reported the production of larvae by *Asterocarpa humilis* from the middle of September to the end of April in Otago Harbour, New Zealand. The occurrence of brooded larvae in late November in Holyhead is relatively slightly later in the (Boreal) autumn than reported by Brewin in New Zealand.

Most unitary ('solitary', non-budding) ascidians spawn both eggs and sperm through the atrial siphon for external fertilization and development. *Asterocarpa humilis* is an exception which retains its eggs and broods its young in the atrial cavity. It shares this distinction with another unitary ascidian that is native and widespread in the Southern Hemisphere but has successfully established as a non-native species in NW Europe, the phlebobranch *Corella eumyota* (Lambert 2004; Arenas et al. 2006). In both species, the gonoducts are very short and eggs and sperm are discharged into the atrial cavity remote from the atrial

opening. In *C. eumyota*, this arrangement is associated with a degree of self-fertilization, accompanied by the alternative capacity for cross-fertilization to yield a mixed mating system (Lambert et al. 1995; Lambert 2004; Dupont et al. 2007). The anatomy of the reproductive organs in *A. humilis* suggests a similar capacity for selfing. It is possible that the ability to self facultatively and to retain progeny to a late developmental stage may have played significant roles in enabling the colonisation of distant habitats by these two species. The genetic data obtained here were for species identification only and thus not intended for population genetics or mating system analyses. It is however noteworthy that the COI gene widely used to trace introduction pathways and analyze genetic diversity in introduced populations was found to be monomorphic across every specimen of *A. humilis* sampled from UK and Brittany. Selfing, which reduces effective population size, may have exacerbated any loss of genetic diversity caused by primary founding events. This hypothesis should be further explored, but this will necessitate further sampling and the development of polymorphic genetic markers.

Molecular barcoding is likely to be an efficient tool to identify introduced species in the light of taxonomic complexity and the loss of taxonomic expertise (Bucklin et al. 2011). Such molecular tools can help to detect mis-identification (e.g. McGlashan et al. 2008) or confirm correct morphological identification (this study). However for barcoding to be efficient and accurate, a large reference molecular database is needed. Tunicate diversity is unfortunately relatively poorly represented in such databases. Efforts should be dedicated to support barcoding initiatives in this complex subphylum, especially in light of (1) their commonness as invasive, cryptogenic and cosmopolitan species, (2) phylogenetic and taxonomic complexity and (3) the frequent paucity of simple, external distinguishing characteristics for their identification in the field, likely to result in new arrivals being overlooked until they have become established and widespread, as exemplified by *Asterocarpa humilis* among other species.

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References

Arenas F, Bishop JDD, Carlton JT, Dyrynda PJ, Farnham WF, Gonzalez DJ, Jacobs MW, Lambert C, Lambert G, Nielsen SE, Pederson JA, Porter JS, Ward S, Wood CA (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

Blum JC, Chang AL, Liljesthröm M, Schenk ME, Steinberg MK, Ruiz GM (2007) The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. J Exp Mar Biol Ecol 342:5–14

Brewin BI (1946) Ascidians in the vicinity of the Portobello marine biological station, Otago harbour. Trans R Soc NZ 76:87–131

Bucklin A, Steinke D, Blanco-Bercial L (2011) DNA Barcoding of marine Metazoa. Annu Rev Mar Sci 3:471–508

Carlton JT (2009) Deep invasion ecology and the assembly of communities in historical time. In: Rilov G, Crook JA (eds) Biological invasions in marine ecosystems. Springer, Berlin, pp 13–56

Clarke M, Castilla JC (2000) Dos nuevos registros de ascidias (Tunicata: ascidiacea) para la costa continental de Chile. Rev Chil Hist Nat 73:503–510

Coutts ADM, Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: a protected mechanism for marine bioinvasions. Mar Poll Bull 54:875–886

Dupont L, Viard F, David P, Bishop JDD (2007) Combined effects of bottlenecks and selfing in populations of *Corella eumyota*, a recently introduced sea squirt in the English channel. Divers Distrib 13:808–817

Haydar D, Hoarau G, Olsen JL, Stam WT, Wolff WJ (2011) Introduced or glacial relict? Phylogeography of the cryptogenic tunicate *Molgula manhattensis* (Ascidiacea, Pleurogona). Divers Distrib 17:68–80

Hodson SL, Burke CM, Bissett AP (2000) Biofouling of fish-cage netting: the efficacy of a silicone coating and the effect of netting colour. Aquaculture 184:277–290

Kott P (1985) The Australian Ascidiacea. Part 1, Phlebobranchia and Stolidobranchia. Mem Qld Mus 23:1–438

Lambert G (2004) The south temperate and Antarctic ascidian *Corella eumyota* reported in two harbours in north-western France. J Mar Biol Assoc UK 84:239–241

Lambert G (2007) Invasive sea squirts: a growing global problem. J Exp Mar Biol Ecol 342:3–4

Lambert GC, Lambert IM, Lambert G (1995) Brooding strategies in solitary ascidians: *Corella* species from north and south temperate waters. Can J Zool 73:1666–1671

Marins FO, Novaes RLM, Rocha RM, Junqueira AOR (2010) Non indigenous ascidians in port and natural environments in a tropical Brazilian bay. Zoologia 27:213–221

McGlashan DJ, Ponniah M, Cassey P, Viard F (2008) Clarifying marine invasions with molecular markers: an illustration based on mtDNA from mistaken calyptraeid identifications. Biol Invasions 10:51–57

Mead A, Carlton JT, Griffiths CL, Rius M (2011) Revealing the scale of marine bioinvasions in developing regions: a South African re-assessment. Biol Invasions 13:1991–2008

Millar RH (1982) The marine fauna of New Zealand: Ascidiacea. Mem NZ Oceanogr Inst 85:1–117

Monniot C, Monniot F, Griffiths CL, Schleyer M (2001) South African ascidians. Ann S Afr Mus 108:1–141

Morton J, Miller M (1973) The New Zealand sea shore, 2nd edn. Collins, London and Auckland, pp 1–653

Sephton D, Benedikte V, Nicolas J, Keays J (2011) Monitoring for invasive tunicates in Nova Scotia, Canada (2006–2009). Aquat Invasions 6:391–403

Tsagkogeorga G, Turon X, Hopcroft RR, Tilak MK, Feldstein T, Shenkar N, Loya Y, Huchon D, Douzery EJP, Delsuc F (2009) An updated 18S rRNA phylogeny of tunicates based on mixture and secondary structure models. BMC Evol Biol 9:187

Van Name WG (1945) The North and South American ascidians. Bull Amer Mus Nat Hist 84:1–476 and plates 1–31