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Molecular evidence for three separate cryptic introductions of the red seaweed *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in South Africa

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The red seaweed genus *Asparagopsis* Montagne (Bonnemaisoniales) contains two widely introduced species that are considered notorious seaweed invaders worldwide, *Asparagopsis armata* and *A. taxiformis*, both characterised by heteromorphic, diplo-haplontic life histories. To uncover cryptic introductions of *Asparagopsis* along the South African coastline and identify 'Falkenbergia' isolates (i.e. tetrasporophytic life-history phase morphologically identical between species), the mitochondrial *cox2–3* spacer was sequenced from gametophytes of *Asparagopsis taxiformis* from Scottburgh, KwaZulu-Natal, on the East Coast, Knysna Lagoon on the South Coast and from tetrasporophytes, otherwise unidentifiable to species level, collected from False Bay near Cape Town on the South-West Coast and Tsitsikamma on the South Coast. Only tetrasporophytes of the temperate *Asparagopsis armata* were encountered from the Cape Peninsula (Cape Town) probably as far east as to Port St Johns, Eastern Cape province. This is considered an introduced species, and was first collected at Kommetjie (Cape Peninsula) in 1935. Gametophytes of the warm-temperate to tropical *A. taxiformis* were first collected at Reunion Rocks near Durban in 1984; the KwaZulu-Natal material studied here belongs to an Atlantic Mediterranean cryptic lineage. This taxon is an ecological dominant in some intertidal and shallow subtidal areas in northern KwaZulu-Natal, and is thus considered 'introduced' and 'invasive'. In contrast, *A. taxiformis* gametophytes, collected in Knysna Lagoon in 2008, clustered with individuals of Indo-Pacific lineage 2. The latter is considered a major invasive lineage in the western Mediterranean, but at present is categorised as introduced in South Africa. This study provides molecular evidence of three independent, cryptic introductions in South Africa, one of them probably very recent, and this is discussed with respect to potential vectors responsible for transport.

Keywords: Bonnemaisoniaceae, cryptogenic, introduced, invasive, seaweed

Introduction

Species of the genus *Asparagopsis* and their importance as introduced seaweeds

The red seaweed genus *Asparagopsis* includes two invasive species: the warm-temperate to tropical *Asparagopsis taxiformis* (Delile) Trevisan, originally described from Alexandria, Egypt (Mediterranean Sea), and the cold to warm-temperate *Asparagopsis armata* (Harvey), with syntype localities in Western Australia and Tasmania (Silva et al. 1996; see Figure 1 in Andreakis et al. 2007a for global species distribution). A distinctive heteromorphic, diplo-haplontic life history characterises the genus: haploid gametophytes of both species (i.e. the '*Asparagopsis*' phase) are found as upright seaweeds, generally c. 8–10 cm high (up to 30 cm in *Asparagopsis taxiformis*; Womersley 1996) with an obvious main axis and plumose branching; on the other hand, diploid tetrasporophytes (i.e. the '*Falkenbergia*' phase) are made

up of branched filaments with a narrow axial cell and three periaxial cells, forming pinkish-red tufts or clumps often entangled with other algae, or free-floating, sometimes described as pink 'pompoms' (Stegenga et al. 1997, De Clerck et al. 2005a). Gametophytes of *A. taxiformis* are morphologically distinct from those of *A. armata* in that they lack lateral basal branches with retrorse spines (harpoon-like branches) present in *A. armata* (for a detailed description of both species in Australia, see Womersley 1996). No macroscopic morphological differences have been reported between tetrasporophytes of *A. armata* and *A. taxiformis*. These were previously identified as the separate species *Falkenbergia rufolanosa* (Harvey) Schmitz and *F. hillebrandii* (Bornet) Falkenberg respectively, and were often considered responsible for taxonomic confusion (Feldmann and Feldmann 1942).

Asparagopsis taxiformis and *A. armata* are considered 'among the most widespread and well-documented seaweed invaders in the world' (Williams and Smith 2007). In particular, *A. taxiformis* has been previously described as one of nine invasive species ('invasive, i.e. playing a conspicuous role in the recipient ecosystems, taking the place of keystone taxa, and/or being economically harmful') out of 85 introduced seaweeds into the Mediterranean basin (Boudouresque and Verlaque 2002). This species has a recent rapid range expansion, especially in the South Aegean Sea (Tsiamis et al. 2010). Similarly, *A. armata* was one of only 10 seaweed species considered to be introduced into the North Sea by 1999 (Maggs and Stegenga 1999). In a study of 113 seaweeds introduced into Europe, Nyberg and Wallentinus (2005) placed *A. armata* in fourth position in a list of the 'top five risk species'.

Molecular phylogenies inferred from nuclear, mitochondrial and plastid sequence markers (Andreakis et al. 2004, 2007a) showed that *A. armata* is a genetically homogeneous species that lacks global geographic structuring, giving evidence for multiple recent introductions. This species is thought to be endemic to cold-temperate waters of Australia and New Zealand, and was introduced into Europe in the 1920s (Ní Chualáin et al. 2004). In contrast, *A. taxiformis* has been found to consist of four morphologically cryptic yet genetically divergent mitochondrial lineages, characterised by distinct geographical distributions worldwide (Andreakis et al. 2007a). Three of these lineages (1, 2 and 4) occur widely in the Indo-Pacific region; lineage 3 has a specifically Atlantic (Brazil, Puerto Rico, Canary Islands) and eastern Mediterranean distribution. Of these lineages, Indo-Pacific lineage 2 exhibits a polyploid status — an invasive character — and has recently expanded its distribution range in southern Portugal and the Mediterranean Sea, where high genotypic variation encountered within populations suggests either multiple founder events or an initial founder constituted by a large group of genetically diverse individuals (Andreakis et al. 2009).

Published records of *Asparagopsis* in South Africa

Kylin (1938) is the first published South African record of the tetrasporophyte of *Asparagopsis*, recording *Falkenbergia rufolanosa* from False Bay (St James) and Port Elizabeth (Reef Bay). This author was describing material sent to him by TA Stephenson, collected in the ecological survey of the South African intertidal in the years 1931–1940 (Stephenson 1948, Bolton 1999). Stephenson (1948), in his list of South African seaweeds, gives the same two collecting sites, describing the species as 'common'. *Falkenbergia rufolanosa* was consequently listed in a catalogue of South African seaweeds by Seagrief (1984), and illustrated in a field guide to the seaweeds of the eastern Cape coast (Seagrief 1988). The tetrasporophyte was later recorded in species lists from Bird Island, Eastern Cape (Anderson and Stegenga 1989) and De Hoop Nature Reserve, Western Cape (Bolton and Stegenga 1990). Stegenga et al. (1997) documented the distribution of '*Falkenbergia rufolanosa*' as being 'from Platbank (Cape Peninsula) eastward, more common in the Eastern Cape'. Stegenga et al. (1997, Fig. 85: 3) also include a drawing of a fertile tetrasporophyte, but these authors did not find any record of gametophytes.

The first published record of the gametophyte of any *Asparagopsis* species was the description of *A. taxiformis* from KwaZulu-Natal by Norris (1992). The authors noted collections from Reunion Rocks, Hulley Point and Kosi Mouth. In their *Guide to the Seaweeds of KwaZulu-Natal*, De Clerck et al. (2005a) state that *A. taxiformis* is 'common in the shallow subtidal where the species often forms extensive mats ... collected once near Scottburgh in central KwaZulu-Natal, but common north of Cape Vidal'.

There are no published records of the gametophyte of *Asparagopsis armata* in South Africa.

Seaweed species introduced to South Africa

Despite intensive collecting since the 1980s (Stegenga et al. 1997, Bolton 1999, Bolton et al. 2001, Anderson and Bolton 2005), there have been much fewer documented cases of introduced seaweeds into South Africa than on many similarly sized marine coastlines around the world. Indeed, De Clerck et al. (2002) described their finding in a Cape Town public aquarium of *Schimmelmanna elegans* Baardseth, presumably introduced from the only previously identified locality on the South Atlantic island of Tristan da Cunha, as 'South Africa's first introduced seaweed?' This species was recorded (by JJB and RJA) for the first time in South Africa outside Cape Town Harbour, growing as a healthy population in a wave-exposed gully at the Cape of Good Hope, on 18 November 2009. It is thus now more clearly in the 'introduced' category and apparently spreading. De Clerck et al. (2002) also suggest a possible introduced status for two filamentous red algae, *Antithamnionella spirographidis* (Schiffner) E.M.Wollaston and *Antithamnionella ternifolia* (J.D.Hooker & Harvey) Lyle. In listing marine introduced seaweed species in South Africa, Robinson et al. (2005) include only these three red algae, describing *Schimmelmanna elegans* as introduced and the two *Antithamnionella* species as 'cryptogenic'. In addition, in a molecular study of subspecific variation in the green alga *Codium fragile* (Suringar) Hariot, using living and historical herbarium specimens, Provan et al. (2008) demonstrated the presence of four separate haplotypes of the species in South Africa. Three of these were designated South Africa haplotype 1 (confined to South Africa), South African haplotype 2 (also found at Cape Horn, South America) and New Zealand haplotype 2 (found in New Zealand, South Africa and the Falkland Islands). Most interestingly, the highly invasive haplotype of the species, designated by Provan et al. (2008) as *Codium fragile* subsp. *tomentosoides* haplotype, was demonstrated to occur in South Africa, and the first recorded specimen of this invasive haplotype was the type specimen of *Codium fragile* subsp. *capense* PC Silva. This was sequenced from a herbarium specimen in the Natural History Museum, London (NHM43), and was collected in Melkbosch on the West Coast by GF Papenfuss in 1937.

Aims of the study

Because four mitochondrial lineages of similar morphologies are encountered within *A. taxiformis* and *Falkenbergia* phases are practically identical, molecular phylogenetic analysis becomes of primary importance to discriminate among lineages and between *Falkenbergia* isolates. In this study, gametophytes and tetrasporophytes of *A. armata* and

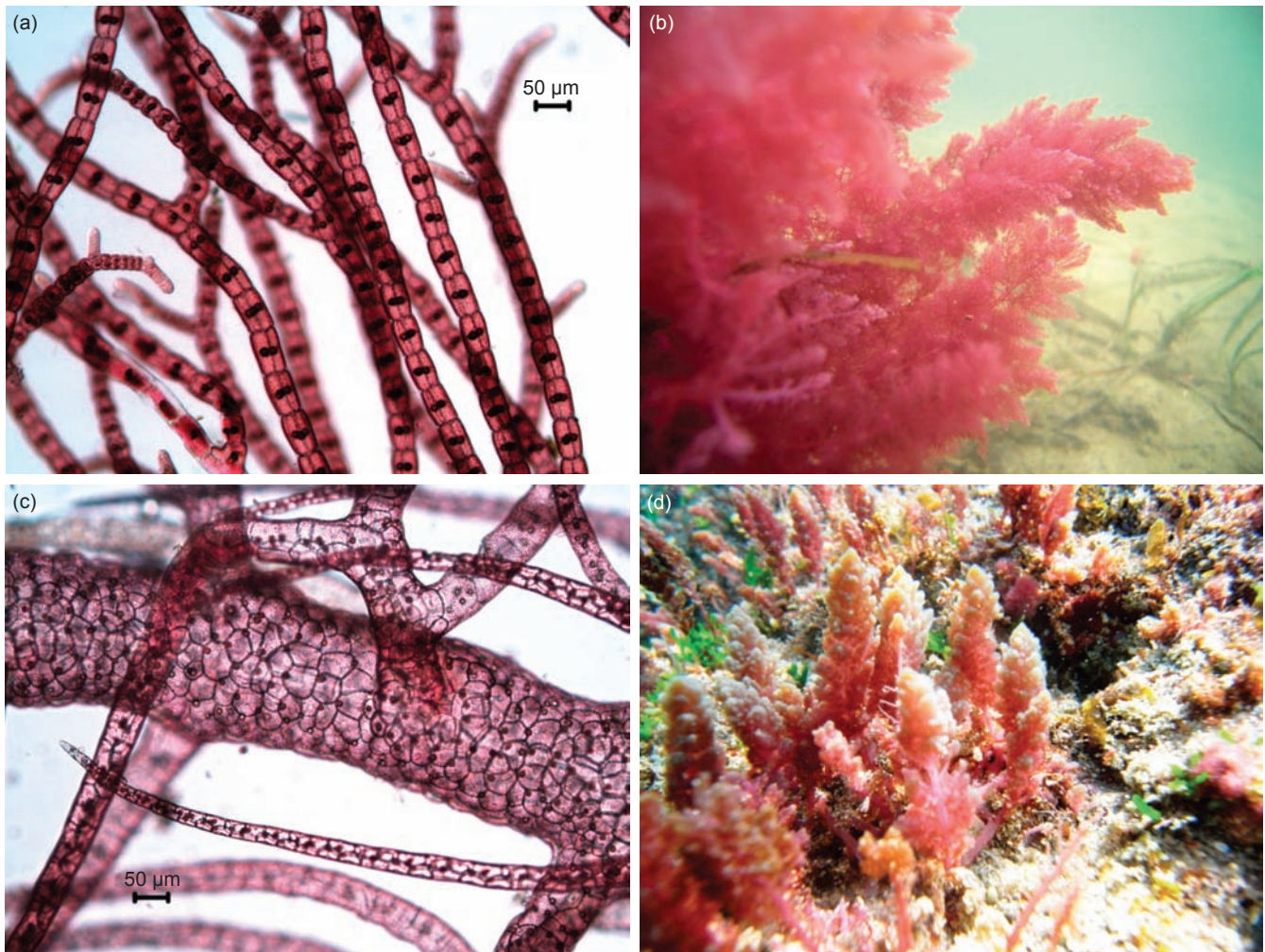


Figure 1: South African *Asparagopsis*: (a) tetrasporophyte of *A. armata* ('*Falkenbergia*'); (b) and (c) *A. taxiformis* in Leisure Isle Harbour, Knysna Lagoon, habit and microscopic detail of Indo-Pacific Mediterranean lineage 2; and (d) *A. taxiformis* (Atlantic Mediterranean lineage 3) in the shallow subtidal at Sodwana Bay, northern KwaZulu-Natal

A. taxiformis were collected from different biogeographical regions of South Africa (South-West, South and East coasts *sensu* Bolton and Anderson 1997, Bolton and Stegenga 2002, Bolton et al. 2004) (Figure 1). Sequences were obtained from the mitochondrial cytochrome oxidase subunit 2–subunit 3 spacer (*cox2–3* spacer; Zuccarello et al. 1999), a suitable marker for cryptic lineage assignment and species identification within the genus *Asparagopsis* (Sherwood 2008). Our aim was to identify lineages of *Asparagopsis* species occurring in South Africa. Episodes of multiple cryptic introductions of *Asparagopsis* in the study area and the possible timings of these introductions are discussed on the basis of evidence from sequencing, herbarium collections and the literature.

Material and methods

South African collections

Herbarium records of *Asparagopsis* species (including '*Falkenbergia*') were investigated in all the major seaweed herbaria in South Africa as well as the GENT herbarium

(Belgium), in which a large recent collection of particularly East Coast material is available (Bolton 1999, Bolton et al. 2001, De Clerck et al. 2005b). A single specimen of *A. taxiformis* gametophyte collected during 2004 from French Polynesia has been also included in the analysis.

DNA extraction, sequencing and phylogenetic analysis

Sample preservation and DNA extraction were carried out as in Andreakis et al. (2004, 2007b). The fast-evolving mitochondrial *cox2–3* spacer was PCR amplified as described in Zuccarello et al. (1999). The resulting PCR products were purified using the QIAEX II Gel Extraction kit 500 (Qiagen GmbH, Hilden, Germany) and directly sequenced on a Beckman Ceq 2000, using a dye-terminator cycle sequencing kit (Beckman) following manufacturer's instructions. Sequences were assembled using the DNASTAR computer package (Lasergene) supplied with the Beckman sequencer. The specimens used in the analysis are listed in Table 1. *Asparagopsis* spp. tetrasporophytes and *A. taxiformis* gametophytes were identified at the species

Table 1: List of *Asparagopsis taxiformis* and *A. armata* specimens used in this study. 'G' and 'T' denote gametophyte and tetrasporophyte life stages respectively

Sample number	Sample site	Haplotype acc. number	Life stage
<i>Asparagopsis armata</i>			
124	South Marseille, France	AY589520	G
130	South Marseille, France		G
E761	Sydney, Australia		G
E762	Sydney, Australia		G
233	Praia Castelo, Albufeira, Portugal		G
228	Praia Castelo, Albufeira, Portugal	DQ228878	G
500	Buffels Bay, Cape Town, South Africa	JF820069	T
6	Tsitsikamma, South Africa	JF820070	T
<i>Asparagopsis taxiformis</i>			
35	Kaalawai, Oahu, Hawaii, USA	DQ228892	G
51	Kaalawai, Oahu, Hawaii, USA		G
55	Kaalawai, Oahu, Hawaii, USA		G
70	Kaalawai, Oahu, Hawaii, USA		G
260	Kawaihae, North Kohala, Hawaii, USA	AY589525	G
261	Lelewi State Beach, Hawaii, USA		G
422	Cabo Rojo, Lighthouse, Puerto Rico	DQ228886	T
421	Luna Reef, La Parguera, Puerto Rico		G
110	Tenerife, Canary Island, Spain	AY589524	G
80	Tenerife, Canary Island, Spain		G
259	Ilha Anchieta, Sao Paolo, Brazil	DQ228891	G
258	Praia Fortaleza, Sao Paolo, Brazil		G
490	Scottburgh, South Africa	JF820071	G
496	Barbara, Lebanon	DQ228888	G
368	Isla Naos, Balboa, Panama	DQ228884	T
997	Hawaii, USA	DQ228885	T
367	Isla Naos, Balboa, Panama		T
483	Dikwella village, Sri Lanka	DQ228883	G
419	French Polynesia	JF820072	G
372	Isla Perico, Balboa, Panama	DQ228893	T
24	Naples, Italy	AY589525	G
10	Naples, Italy		G
31	Ischia, Italy		G
32	Ischia, Italy		G
263	Capri, Italy	AY589530	G
264	Capri, Italy		G
340	Trapani, Italy	AY589532	G
348	Trapani, Italy	AY589533	G
KNY/D/4	Knysna Lagoon, South Africa	JF820073	G
KNY/D/5	Knysna Lagoon, South Africa	JF820074	G

and lineage levels respectively by aligning the aforementioned sequences against a reduced panel of *cox2*–3 spacer sequences previously obtained from *A. armata* and *A. taxiformis* cryptic lineages collected globally (Andreakis et al. 2004, 2007a). The ClustalW (Thompson et al. 1994) algorithm was used for the alignment as implemented in Bioedit v. 4.8.5 (Hall 1999); finally the alignment was refined by eye. Hierarchical likelihood ratio tests were performed using Modeltest Version 3.06 (Posada and Crandall 1998) to find the best-fitting parameters for maximum likelihood (ML) analysis given the alignment and ML phylogenies were inferred in PAUP* 4.0b10 (Windows version; Swofford 2002). Computations were performed using heuristic searches and 10 random sequence additions to find the highest likelihood tree. Bootstrap support for individual clades (Felsenstein 1985) was calculated on 1 000 replicates using the same

options and constraints as used in the tree-inferences, but with identical sequences represented only once.

Results

Collections of *Asparagopsis* in South Africa

The first record of *Asparagopsis* in South Africa was a specimen of *Falkenbergia* sp. collected by GF Papenfuss at Kommetjie on the west coast of the Cape Peninsula on 13 October 1935 (SABIF 4662), currently in the NAT herbarium in Pietermaritzburg. This is also the westernmost record thus far. There are quite a number of records of the tetrasporophyte eastwards in the Western and Eastern Cape provinces. The easternmost record of the *Falkenbergia* sporophyte with a South Coast distribution (*sensu* Bolton and Anderson 1997) is a specimen collected by MA Pocock and GF Papenfuss at Port St Johns, Transkei, on 31 July 1938 (NAT, SABIF 4661). There is a large gap in distribution between the sporophyte collected from Port St Johns and the next collection to the east at Rocky Bay, Park Rynie, KwaZulu-Natal. The first record of *Falkenbergia* at Rocky Bay was collected by RN Pienaar on 23 April 1968 (SABIF 4648 in NAT), and it has subsequently been collected in 1983 and 1986 at the same site. There are few records of the sporophyte from KwaZulu-Natal, with no records in herbaria between Rocky Bay and Cape Vidal.

The first record of a gametophyte of *Asparagopsis* in South Africa, as reported in Norris (1992), was a specimen of *A. taxiformis* collected at Reunion Rocks, just south of Durban, on 16 April 1984 by RN Pienaar, RE Norris, ME Aken, SR Meyer and G Lambert (SABIF 696, NAT 2057). Subsequent finds have been farther south at Scottburgh (this paper), and a number of specimens from Cape Vidal to Kosi Bay mouth in NAT and GENT. Subsequently, a population of *A. taxiformis* was discovered luxuriantly in the boat harbour at Leisure Isle, Knysna Lagoon, on the South African south coast some 1 000 km coastal distance from Scottburgh on 4 July 2008 by H Stegenga (National Herbarium of the Netherlands, Leiden), RJA and JJB. The species was extremely abundant in a dense seaweed vegetation in the harbour, which was dominated by the green seaweed *Codium tenue* (Kützting) Kützting.

Phylogenetic analysis

Maximum likelihood analysis, constrained with the best model given the data (HKY + G) and optimal model-tested parameters (nucleotide frequencies: A = 0.3405, C = 0.1226, G = 0.1190, T = 0.4179; ti/tv ratio = 1.5183; proportion of variable sites assumed to follow a gamma distribution with shape parameter α = 0.3956) resulted in a single ML topology ($-\ln L$ = 1 217.3337; Figure 2). Two tetrasporophytes from Cape Town (Buffels Bay, False Bay) and Tsitsikamma were encountered within the *A. armata* clade and they were identical to the most frequent mitochondrial haplotype of the species distributed globally. *Asparagopsis* gametophytes from Knysna Lagoon, however, clustered together with the most frequent haplotype of the Indo-Pacific lineage 2, and the gametophyte specimen collected in Scottburgh grouped with the Atlantic Mediterranean lineage 3. Finally, specimen 419, collected in French Polynesia, clustered with individuals of the Pacific lineage 1.

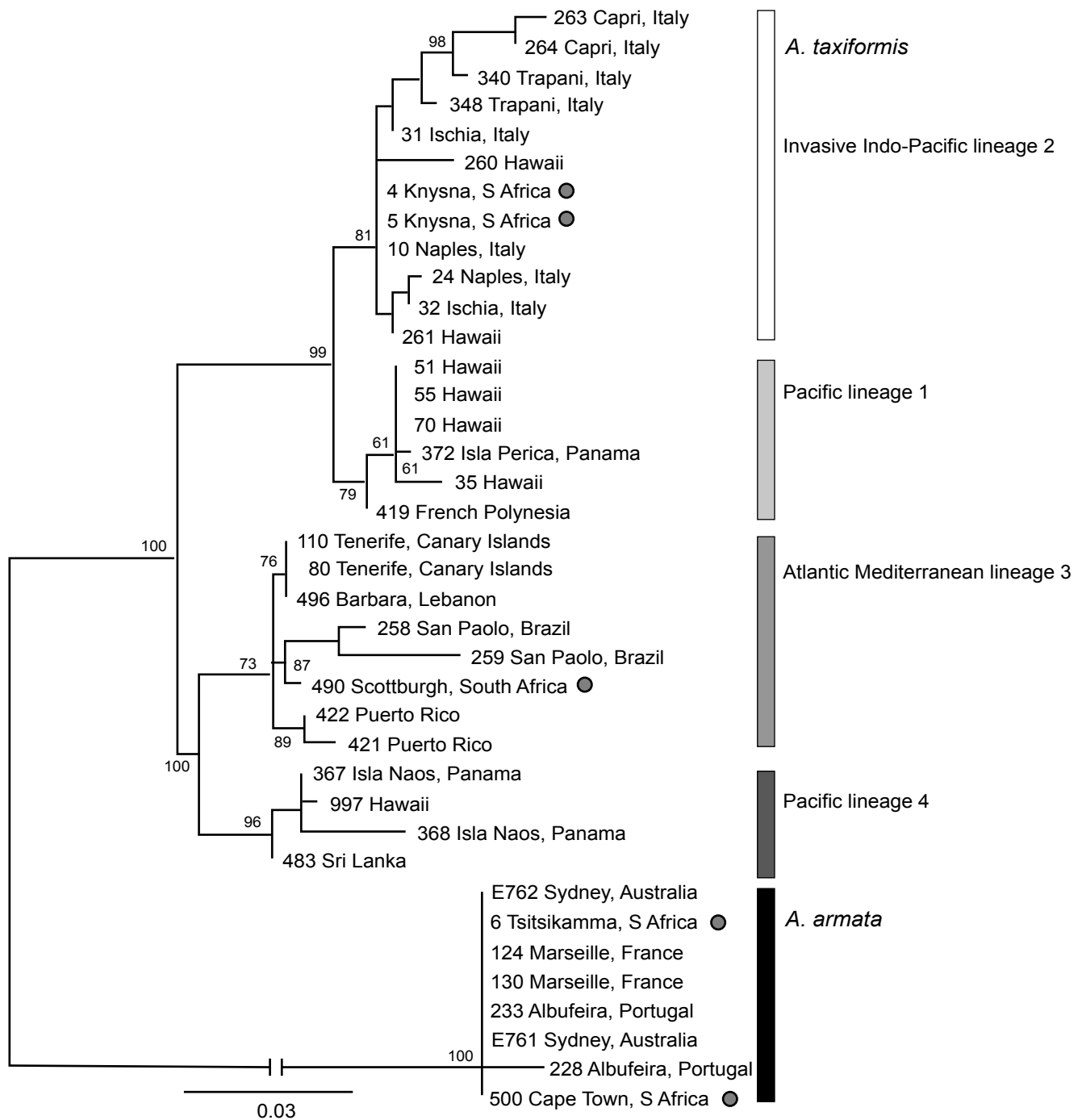


Figure 2: Midpoint rooted maximum likelihood phylogenetic reconstruction of *Asparagopsis* spp. based on *cox2-3* spacer sequences. Numbers on nodes indicate bootstrap support; grey circles indicate sequences from South African isolates produced in this study

Discussion

The molecular phylogeny provides evidence of two genetically distinct mitochondrial lineages of *A. taxiformis* in South Africa, considered to be morphologically cryptic species (Andreakis et al. 2007a). One is of Atlantic Mediterranean origin (lineage 3 in Andreakis et al. 2007a) and now dominates intertidal and shallow subtidal areas in northern KwaZulu-Natal; it is thus here considered introduced and

invasive. The other, collected in Knysna Lagoon in 2008, is of Indo-Pacific origin and is now regarded as a major invasive lineage throughout the Mediterranean Sea (invasive lineage 2 in Andreakis et al. 2007a). At present, we consider lineage 2 as a recently introduced taxon in South Africa. Morphologically cryptic *Falkenbergia* isolates collected from Tsitsikamma and False Bay (Cape Town) were identified as the tetrasporophyte phase of *A. armata*. This is a genetically homogeneous species, regarded to be invasive

in the western Mediterranean Sea and western European coasts; it is found from Platbank (False Bay) probably as far east as to Port St Johns, Eastern Cape (Figure 3). As this species survives only as the sporophyte, and as there is much evidence from the literature that the species has been introduced widely to many regions around the world, *A. armata* is considered an introduction to the South African flora, although first collected in 1935. The single gametophyte specimen of *A. taxiformis* that was collected in French Polynesia during 2004 was successfully assigned to the Pacific lineage 1, thus extending the geographical distribution range of that lineage to this location (described in Andreakis et al. 2007a).

Falkenbergia isolates belonging to *A. armata* from Tsitsikamma were found in sympatry with gametophytes of *A. taxiformis*, yet the two mitochondrial lineages of *A. taxiformis* reported here are separated at a geographical scale (c. 1 000 km). For comparison, three mitochondrial lineages of *A. taxiformis* have been reported from the Hawaiian archipelago with one of them, lineage 2, being considered native; the other two, lineages 1 and 4, are considered recently introduced with the latter as recently as 1991 (Sherwood 2008).

Collections of *A. taxiformis* from KwaZulu-Natal were successfully assigned to the Atlantic Mediterranean lineage 3, previously reported from the Canary Islands, Brazil, Puerto Rico, Yucatan, Florida and the eastern Mediterranean Sea (Andreakis et al. 2007a). These populations were first recorded in 1984, and lineage 3 in this case represents a recent introduction, as we consider it very unlikely that *Asparagopsis* gametophytes were overlooked by previous collectors on the KwaZulu-Natal coastline. In addition, Atlantic Mediterranean lineage 3 of *A. taxiformis* is here considered 'invasive' (*sensu* Boudouresque and Verlaque 2002) across its South African distribution range. This is because lineage 3 has been previously described as a common local component and capable of forming extensive mats north of Cape Vidal (De Clerck et al. 2005a). This lineage has been observed by the present authors to be an ecologically dominant species in the intertidal and shallow subtidal areas at Sodwana Bay, northern KwaZulu-Natal.

Isolates of *A. taxiformis* recently collected in Knysna Lagoon are genetically distinct from the KwaZulu-Natal isolates, and identical to the most frequent Indo-Pacific haplotype of lineage 2. This lineage is found in Eastern Australia, Hawaii, California, Vietnam and New Caledonia and was introduced throughout the Mediterranean basin. Lineage 2 represents a very recent introduction, because *A. taxiformis* gametophytes were not observed on two previous collecting expeditions in Leisure Isle Harbour, Knysna Lagoon, by the present authors in the 1990s. There are good grounds to suspect that oyster transport for commercial aquaculture was the vector responsible for the introduction of lineage 2 into the Knysna system. For example, seven species of alien marine animal growing on the Knysna oyster farms have been recently reported by Haupt et al. (2010).

Cryptic versus cryptogenic species in South Africa

Marine species are generally designated as introduced when there is some specific evidence of an introduction,

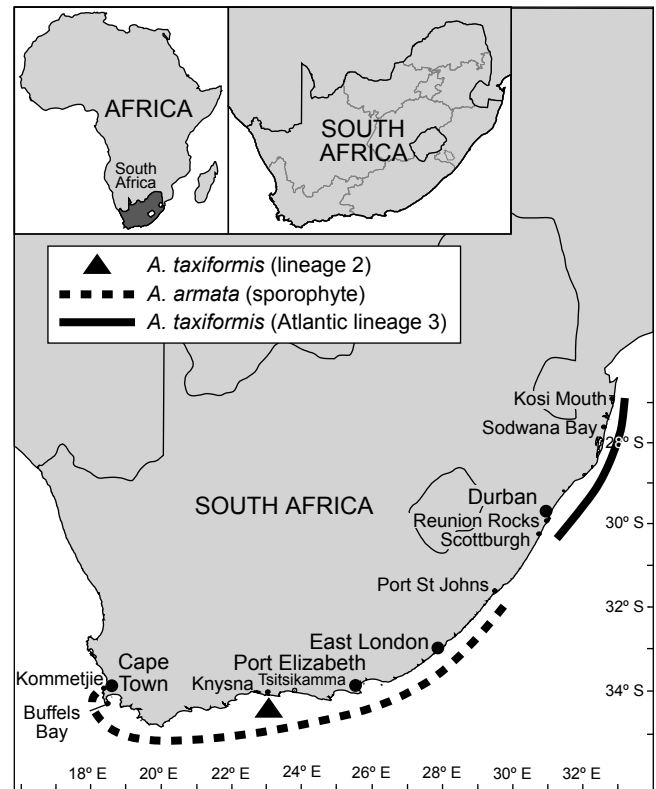


Figure 3: Geographical distribution of species and haplotypes of *Asparagopsis* in South Africa. Triangle = invasive Indo-Pacific lineage 2 of *A. taxiformis*; solid line = Atlantic Mediterranean lineage 3 of *A. taxiformis*; dashed line = tetrasporophyte of *A. armata*

particularly good evidence that a species was not present previously. Carlton (1996) described species as 'cryptogenic' when they are 'species which are not demonstrably native or introduced'. Robinson et al. (2005) refined this term, using the designation 'cryptogenic' for potentially introduced South African marine species that have a global range or cross biogeographic boundaries, show life-history characteristics which facilitate dispersal by humans, and for which there is evidence of relevant human vectors. The designation of seaweed species as cryptogenic is problematic without data from molecular sequencing, as in the current study, to confirm that taxa from different regions are indeed the same genetic entity. As South African examples, species that are recorded to have worldwide distributions include *Grateloupia filicina* (J.V.Lamouroux) C.Agardh (De Clerck et al. 2005c described South African material as a new endemic species, *G. capensis* O.De Clerck) and *Centroceras clavulatum* (C. Agardh) Montagne (Won et al. 2009 showed that true *C. clavulatum* does not occur in South Africa, but demonstrated the presence in South Africa of four previously undescribed species of *Centroceras*). The listing of cryptogenic species of seaweeds purely on the basis that the name has been used worldwide is clearly invalid (e.g. Hewitt et al. 2004).

Asparagopsis species as successful colonisers

The discovery of *Asparagopsis* species has always been

reported as a recent introduction event of demonstrated invasive character (Altamirano et al. 2008, Andreakis et al. 2007a, 2009). This is because both *A. taxiformis* and *A. armata* exhibit a number of features that make them successful invaders. These include vegetative propagation of both the *Asparagopsis* and the *Falkenbergia* phases, sexual and asexual reproduction systems, as well as a well-developed suite of halogenated secondary metabolites that results in them being consumed by few predators (Boudouresque and Verlaque 2002, Paul et al. 2006a, 2006b, Williams and Smith 2007). In addition, a high level of nuclear microsatellite variation has been reported in Mediterranean populations belonging to the invasive lineage 2 (Andreakis et al. 2009), and differences in ploidy levels due to endo-polyploidisation or endo-reduplication have been previously suggested for that lineage (Andreakis et al. 2007b). Differences in ploidy level, the DNA content and in the number of nuclei in cells reported in *Asparagopsis*, as in other red algae (Kapraun 2005, Andreakis et al. 2007b, 2009), may account for high numbers of genetic variants. Some of these, positively selected, may be responsible for the remarkable expansion of *Asparagopsis* outside its known distribution range. Furthermore, *Falkenbergia* phases are particularly well suited to human-mediated transport and capable of surviving for long periods as free-floating or loosely attached filaments transported on ships, in ballast waters, or on animals and other materials in aquaculture (Carlton 1985, Boudouresque and Verlaque 2002). In South Africa, no records of *A. armata* gametophytes have been reported so far, despite the fact that these would be much more obvious than the tetrasporophyte. The long-term survival (since 1935) of solely vegetative sporophytes of *A. armata* in South Africa indicates the ability of the *Falkenbergia* phase to persist outside the ecophysiological tolerance limits of the respective gametophytic phase.

Asparagopsis distribution in South Africa

There is therefore good evidence that the tetrasporophyte of *A. armata* has been present on warm-temperate coasts of South Africa at least since 1935. Although it is sometimes abundant (particularly as floating pompoms seen at times in False Bay near Cape Town), it is generally insignificant, and before the readily available description in Stegenga et al. (1997), would have needed a good seaweed taxonomist (e.g. Kylin, Papenfuss, Pocock) to identify it. The distribution is still patchy, with a number of detailed collection trips along the South Coast by JJB and RJA with H Stegenga in spring over the past two decades having failed to record it. Fertile sporophytes are described as 'rare' by Stegenga et al. (1997). They are rare enough for the gametophyte never to have been observed. The rarity of fertile sporophytes is very likely linked to the requirement for short daylength for production of tetrasporangia (Oza 1976, Lüning 1981). *Asparagopsis armata* probably occurs from Kommetjie to at least Port St Johns (Figure 3). The gap in records of any *Asparagopsis* material between Port St Johns and Park Rynie suggests that material north-east of the latter site is likely to be *A. taxiformis*, although this is not definite as the two species occur together in some parts of the world. *Asparagopsis armata* thus appears to be a South Coast species, in that Kommetjie is the western limit of the

biogeographic region designated by Stephenson (1948) as the 'Western Overlap', and many South Coast species have their easternmost record immediately south of the KwaZulu-Natal border (Stephenson 1948, Bolton and Anderson 1997, Bolton and Stegenga 2002).

Conclusions

Because of inadequate morphological resolution, cryptic introduction events of non-native *Asparagopsis* lineages can be revealed only by molecular techniques and may represent a serious and underestimated threat to local marine biota (Boudouresque and Verlaque 2002, Klein and Verlaque 2009). The number of potential benefits mentioned in the literature for this genus is, however, remarkable. These include considerable antimicrobial activity useful in the cosmetics industry (Salvador et al. 2007) and animal aquaculture (Bansemir et al. 2006, Manilal et al. 2009), anti-HIV activity (Haslin et al. 2001), antiprotozoal activity (Genovese et al. 2009) and cytotoxic activity against cancer cells (Zubia et al. 2009). In addition, *Asparagopsis* gametophytes have been proposed as the source of preservatives and solvents in industrial applications (Marshall et al. 2000, Kraan and Barrington 2005) and the *Falkenbergia* phase has been successfully tested as a rapidly growing species useful for bioremediation of animal effluent in marine aquaculture (Schuenhoff et al. 2006, Mata et al. 2007, Figueroa et al. 2008). This could provide an alternative to the green seaweed *Ulva*, which is currently used for bioremediation purposes in South African abalone aquaculture (Robertson-Andersson et al. 2008, Bolton et al. 2009).

Due to outstanding growth rates and biomass production, domesticated invasive species under controlled cultivation conditions may represent natural resources of considerable economic value. Kraan and Barrington (2005) discussed the possible benefits and drawbacks of commercial cultivation of introduced *Asparagopsis*. In this context, the potential use of selected lineages of *Asparagopsis* species may be able to compensate somewhat the negative impact of the invasion process to the local coastal ecosystems.

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