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Biogeographic affinities of Dictyotales from Madagascar: a phylogenetic approach

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Abstract – During the *Atimo Vatae* research cruise of 2010, the seaweed flora of the southern coast of Madagascar was extensively sampled. Here we report on the species diversity and biogeographic affinities of the brown algal order Dictyotales, which was assessed using DNA-barcoding makers. Molecular identification resulted in 23 MOTU's belonging to 9 genera. From a biogeographic perspective Madagascar is considered to be part of the large tropical Western Indo-Pacific realm. However, only 3 out of 23 species confirmed this affinity. In contrast, species- and genus-level links to the more temperate coast of KwaZulu-Natal were as prominent (4 species) and 6 species represent endemic species. The remaining species were either widely distributed in tropical regions or their affinities were unclear. In conclusion, the Dictyotales data do not suggest the flora of southern Madagascar is unequivocally a part of the tropical Western Indo-Pacific realm, but rather a region of overlap, where more temperate species thrive in conjunction with some Indo West Pacific (IWP) elements.

Biogeography / Dictyotales / Madagascar / Western Indo-Pacific / South Africa

INTRODUCTION

Madagascar, the fourth largest island in the world, is positioned in the western Indian Ocean and separated from East-Africa by the Mozambique Channel. Originally Madagascar was landlocked within the supercontinent Gondwana but in the late Jurassic East-Gondwana (Madagascar, India, Antarctica and Australia) drifted apart from the African plate. India moved northward together with Madagascar in the late Cretaceous, but while India

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continued drifting toward Laurasia, Madagascar remained positioned alongside the East coast of Africa, isolated from the other landmasses (Smith *et al.*, 2004). This long lasting isolation (88 Mya) in combination with the presence of a rich variety of climatological and geological conditions has led to exceptionally high levels of biodiversity and endemism in terrestrial habitats. Much less attention however has been devoted to the diversity and biogeographic affinities of marine biota.

In a recent biogeographical classification of marine coastal environments (Spalding *et al.*, 2007) Madagascar together with the East-African coast, the Mascarene Islands and the Seychelles form the Western Indian Ocean province, which presents the western part of the large Western Indo-Pacific realm. Madagascar itself is divided into two ecoregions, consisting of the southeastern coast, and the western and northern coast, respectively (Fig. 1). Ecoregions are supposed to be homogenous in species composition, clearly distinct from adjacent systems and defined by the predominance of certain ecosystems or oceanographic or topographic features. Interestingly, the Malagasy ecoregions are classified in a different realm from South African ecoregions, despite their geographical proximity. In the Spalding *et al.* (2007) system, the South African coastal biota, from Namibia to the Mozambican border, form the Temperate Southern African realm.

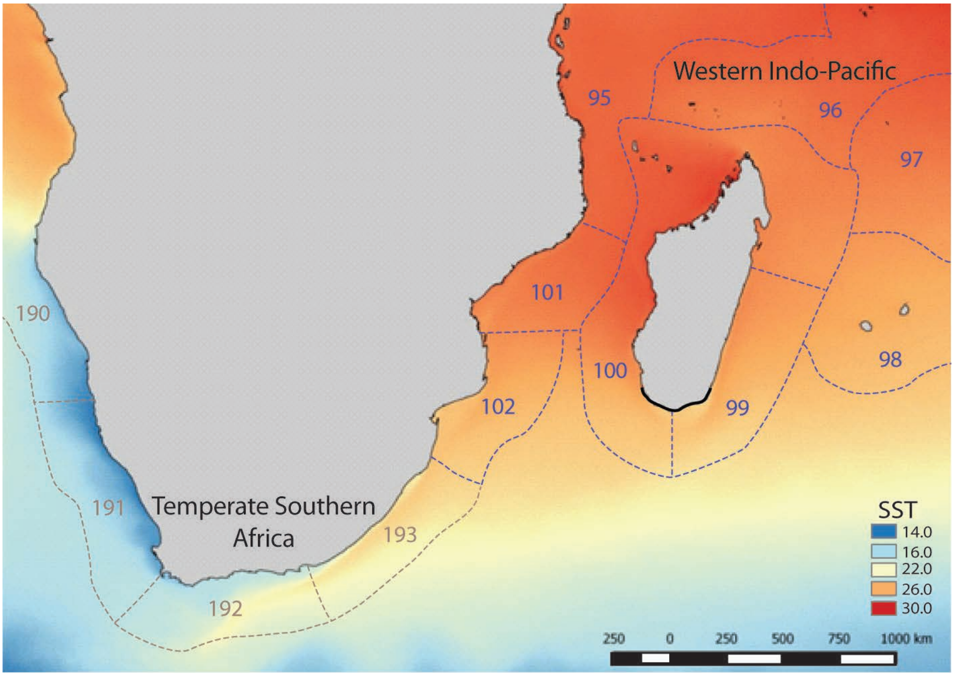

Fig. 1. Geographical setting of Madagascar with indication of the region sampled during the *Atimo Vatae* expedition. Mean sea surface temperatures are color-scaled. Dashed lines and associated numbers refer to ecoregions (Spalding *et al.* L2007): 95. East African Coral Coast; 96. Seychelles; 97. Cargados Carajos/Tromelin Island; 98. Mascarene Islands; 99. Southeast Madagascar; 100. Western and Northern Madagascar; 101. Bight of Sofala/Swamp Coast; 102. Delagoa; 190. Namib; 191. Namaqua; 192. Agulhas Bank; 193. Natal.

In van den Hoek's (1984) biogeographic classification of seaweed floras, Madagascar belongs to the tropical Indo West Pacific Region (IWP), which is approximately equivalent to a union of Spalding's Western, Central and Eastern Indo-Pacific realms (Spalding *et al.*, 2007). Van den Hoek's IWP also includes most of South Africa's KwaZulu-Natal province (30°S). This classification of Madagascar's marine flora confirms the earlier zoogeographical classification of Briggs (1974). By comparing species records listed in AlgaeBase, Bolton *et al.* (2007) confirmed a high species similarity within this larger region, but the eastern to southern African countries (Kenya, Tanzania, Madagascar, South Africa) displayed floras that were slightly different from the other Indian Ocean countries. However, Bolton *et al.* (2007) claimed that the classification of South Africa within this broader region is mainly due to the tropical nature of the flora of northern KwaZulu-Natal. Based on analyses of intertidal seaweed assemblages, Bolton *et al.* (2004) demonstrated that along the KwaZulu-Natal coast there is a gradual turn-over from a typical Indo West-Pacific flora to the flora characteristic of the southern temperate Agulhas province.

Reports on Malagasy seaweeds are relatively scarce. The *Catalogue of the Benthic Marine Algae of the Indian Ocean* (Silva *et al.*, 1996) includes 22 Dictyotales species recorded from Madagascar. Collections by Coppejans in 2003, yielded 8 additional species (Douterlungne, 2003). Based on these species records, the Malagasy flora indeed shows close affinities to the tropical Western Indo-Pacific. However, these records consist of morphological identifications and until now, such claims have not been tested using DNA-based identifications.

The samples analyzed within this study were taken during the *Atimo Vatae* expedition in 2010, which aimed exploring the largely unknown temperate deep south coast of Madagascar from Manantenina to Androka. The geographic sampling of the expedition spans two ecoregions, southeast Madagascar, and western and northern Madagascar. Madagascar has a north-south orientation and spans over 14°C of latitude. This leads to tropical marine conditions in the north of the country (mean annual SST 28°C), while the far south displays more temperate conditions (mean annual SST 24°) (Fig. 1). As temperature is one of the main factors determining seaweed distributions (van den Hoek, 1982), the question remains whether the marine flora in this region truly shows tropical Indo West Pacific affinities.

Using the brown algal order Dictyotales (Phaeophyceae) as a case study, we test whether the proposed marine biogeographic classification of Madagascar as part of the tropical Western Indo-Pacific realm remains valid by comparing DNA sequence data from specimens from the south coast with a global molecular Dictyotales dataset. As a second objective, we would like to infer whether the geographical isolation and geoclimatic diversity of Madagascar has led to the evolution of endemic seaweed species.

MATERIAL & METHODS

DNA sampling and extraction

Samples were collected by R. Anderson, E. Coppejans, B. de Reviers, L. Le Gall and F. Rousseau, and J. Tsarahevitra, in 2010 during the *Atimo Vatae* expedition which was land based at Lavanono and Fort Dauphin and on board of a research vessel to explore the deep southern coast of Madagascar. Tissue from apical tips was dried in silica gel for rapid dehydration and storage. Dry tissue was crushed with liquid nitrogen and incubated at 60°C in 5 µl proteinase K and 500 µl of CTAB for a total of 30-40 mins. The samples were centrifuged at full speed for 10 min. The supernatant was mixed with 25:24:1-phenolchloroform. The samples were shaken and centrifuged again. The supernatant was mixed with 24:1 chloroform – isoamyl – alcohol to remove protein residues and centrifuged for 5 mins. The supernatant was transferred to a clean tube and contains the DNA-extract. The extract is purified employing magnetic beads (MagAttract Suspension G, Qiagen). Plastid-encoded PSII reaction centre protein D1 (*psb*A) was amplified for *Canistrocarpus, Dictyota*, *Stypopodium*, *Rugulopteryx* and the large subunit of the Rubisco enzyme (*rbc*L) was amplified for *Dictyopteris.* Mitochondrial-encoded cytochrome oxidase subunit 3 (*cox*3) was amplified for the genus *Padina, Lobophora* and *Zonaria*. The choice of markers was based on the availability of sequenced specimens in existing datasets. PCR primers and conditions are described in De Clerck *et al.* (2006), Tronholm *et al.* (2010), Silberfeld *et al.* (2013) and Vieira *et al.* (2014).

Phylogenetic analyses and construction of maps

Sequences were edited and aligned in MEGA6 (Tamura *et al.*, 2013) and added to a global Dictyotales dataset (De Clerck, unpublished) in order to infer closest relatives. ML phylogenies were constructed in MEGA6 for the Malagasy records and their closest relatives employing a K2P-model. Molecular operational taxonomic units (MOTUs) were defined as clusters of sequences with no or little sequences divergence relative to other such clusters (Leliaert *et al.*, 2014). Presented phylogenies included unique sequences only and were rooted with the nearest taxon. Distribution maps were constructed in R (R core team, 2014) employing the *maps* and *mapdata* packages (Brownrigg & Minka, 2011, Becker *et al.*, 2013). Maps include all available species records from the global Dictyotales dataset. Full geographical data are available on www.phycology.ugent.be.

RESULTS & DISCUSSION

Sequence data were generated for 70 specimens. These represented 23 MOTUs that either correspond to species or distinct genetic entities of more widespread species complexes (Table 1). Below, we will discuss these results from a biogeographical rather than taxonomic point of view. General observed patterns are discussed.

Tropical Western Indo-Pacific distributions

Although Madagascar is traditionally considered to be part of the Western Indo-Pacific realm, we did not find an overwhelming imprint of this biogeographical classification within the total of Dictyotales. Only for *Lobophora* several Malagasy species most likely show clear Western Indo-Pacific affinities. This is clearest in *Lobophora crassa6*, a MOTU of the *Lobophora*

Table 1: Diversity of Dictyotales in Madagascar with indication of voucher specimens, Genbank accession numbers, sampling localities with coordinates and biogeographical affinities of the MOTUs

Species	*Voucher*	*Genbank*	*Locality*	*Latitude*	*Longitude*	*Biogeographical range*
Canistrocarpus cervicornis	MAD0061	LN831800	Anosy: Fort Dauphin: Plage monseigneur	– 25.0358	46.9983	pantropical
	MAD0139	LN831801	Anosy: Mahanoro: Chez Patrick	– 24.9683	47.1017	
	MAD0151	LN831802	Anosy: Mahanoro: Chez Patrick	– 24.9683	47.1017	
	MAD0228	LN831803	Anosy: Fort Dauphin: Plage monseigneur	– 25.0358	46.9983	
	MAD0481	LN831804	Anosy: Libanona: Cap d'Antsirabe	– 25.0433	46.9967	
	MAD1676	LN831805	Anosy: Baie des Galions	– 25.1467	46.7500	
	MAD1885	LN831806	Tuléar: Nosy Manitsa	– 25.2183	44.2333	
Dictyopteris sp1	MAD0279	LN831792	Anosy: Baie de Fort- Dauphin	– 25.0033	47.0250	Madagascar
	MAD0280	LN831793	Anosy: Baie de Fort-Dauphin	– 25.0033	47.0250	
	MAD0281	LN831794	Anosy: Baie de Fort-Dauphin	– 25.0033	47.0250	
	MAD0282	LN831795	Anosy: Baie de Fort-Dauphin	– 25.0033	47.0250	
	MAD1786	LN831796	Tuléar: Lavanono: Au large de Lavanono	– 25.4717	44.9267	
	MAD1813	LN831797	Beloha: Rocher de l'Albatros	– 25.4783	44.9467	
	MAD1943	LN831798	Tuléar: Cap Sainte Marie	– 25.5650	45.1150	
	MAD2107	LN831799	Anosy: Sainte Luce	– 24.7650	47.2133	
Dictyopteris sp2	MAD0098	LN831791	Anosy: Phare Flacourt: En face du phare	– 25.0217	47.0083	
Dictyota anastomosans	MAD0143	LN831807	Mahonoro: Chez Patrick	– 25.0667	47.2000	Southern Japan
	MAD0480	LN831808	Libanona: Cap d'Antsirabe	– 25.6333	47.7833	
	MAD0509	LN831809	Libanona: Cap d'Antsirabe	– 25.6333	47.7833	
	MAD1738	LN831810	Anosy: Baie des Galions	– 25.9333	46.7500	
Dictyota ceylanica5	MAD0751	LN831811	Anosy: Andavaka	– 25.2000	46.6333	pantropical
Dictyota humifusa	MAD1685	LN831812	Anosy: Baie des Galions	– 25.9333	46.7500	pantropical
Dictyota liturata	MAD0142	LN831813	Mahonoro: Chez Patrick	– 25.0667	47.2000	unclear
Dictyota rigida4	MAD0156	LN831814	Mahonoro: Chez Patrick	– 25.0667	47.2000	South Africa
	MAD0305	LN831815	Anosy: NE Phare Ephatra	– 25.2198	46.6221	
	MAD2157	LN831816	Anosy: Sainte Luce	– 25.6500	48.0000	
Dictyota sp1	MAD2178	LN831817	Anosy: Sainte Luce	– 25.5500	47.6000	Madagascar
Dictyota stolonifera	MAD0573	LN831818	Anosy: Lokaro	– 24.9500	47.6000	Western Indo Pacific + Central Pacific
Lobophora crassa6	MAD2138	LN831819	Anosy: Sainte Luce	– 24.7650	47.2133	Western Indo Pacific
Lobophora sp25	MAD0063	LN831820	Anosy: Fort Dauphin: Plage monseigneur	– 25.0358	46.9983	South Africa
Lobophora sp36	MAD0382	LN831821	Anosy: Pointe d'Ambero	– 25.1117	46.8317	Western Indo Pacific (Red Sea, Egypt)
	MAD1674	LN831822	Anosy: Baie des Galions	– 25.1467	46.7500	
	MAD1787	LN831823	Lavanono	– 25.4717	44.9267	

Table 1: Diversity of Dictyotales in Madagascar with indication of voucher specimens, Genbank accession numbers, sampling localities with coordinates and biogeographical affinities of the MOTUs *(continued)*

Species	*Voucher*	*Genbank*	*Locality*	*Latitude*	*Longitude*	*Biogeographical range*
	MAD1815	LN831824	Beloha: Rocher de l'Albatros	– 25.4783	44.9467	
	MAD1991	LN831825	Tuléar: Lagon de Baravo	– 25.2033	44.3217	
Lobophora sp44	MAD1788	LN831826	Tuléar: Lavanono	– 25.4717	44.9267	unclear
	MAD1956	LN831827	Tuléar: Cap Sainte Marie	– 25.5533	45.1100	
	MAD2183	LN831828	Anosy: Sainte Luce	– 24.7633	47.2067	
Lobophora sp45	MAD0109	LN831829	Anosy: Phare Flacourt: En face du phare	– 25.0217	47.0083	Western Indo Pacific
	MAD0110	LN831830	Anosy: Phare Flacourt: En face du phare	– 25.0217	47.0083	(Tanzania)
	MAD1641	LN831831	Anosy: Baie des Galions	– 25.1600	46.7433	
	MAD2068	LN831832	Anosy: Sainte Luce	– 24.7600	47.2067	
Padina boergesenii.c	MAD0036	LN831835	Anosy: Fort Dauphin: Plage monseigneur	– 25.0358	46.9983	pantropical (India,
	MAD0060	LN831836	Anosy: Fort Dauphin: Plage monseigneur	25.1833	47.8833	Carribean, Tanzania)
	MAD0268	LN831837	Anosy: Fort Dauphin: Plage monseigneur	25.1833	47.8833	
	MAD0423	LN831838	Anosy: Cap Ranavalona: Pointe Ehoala	– 25.0750	46.9617	
	MAD0636	LN831839	Anosy: Flacourt	– 25.0283	47.0017	
	MAD1704	LN831840	Anosy: Baie des Galions	– 25.9333	46.7500	
	MAD1887	LN831841	Tuléar: Nosy Manitsa	– 25.2183	44.2333	
	MAD1966	LN831842	Tuléar: Nosy Manitsa	– 25.3167	44.2333	
Padina gymnospora.e	MAD0316	LN831843	Anosy: NE Phare Ephatra	– 25.2198	46.6221	unclear
Padina melemele.i	MAD1789	LN831844	Tuléar: Lavanono: Au large de Lavanono	– 25.4717	44.9267	Madagascar
	MAD1957	LN831845	Tuléar: Cap Sainte Marie	– 25.7500	45.7000	
Padina antillarum.e	MAD0137	LN831833	Mahonoro: Chez Patrick	– 25.0667	47.2000	Madagascar
	MAD0564	LN831834	Anosy: Sainte Luce	– 24.8833	48.0833	
Rugulopteryx suhrii	MAD0166	LN831846	Anosy: Mahanoro: Chez Patrick	– 24.9683	47.1017	South Africa
Stoechospermum polypodioides	MAD0075	LN831847	Anosy: Fort Dauphin: Plage monseigneur	– 25.0358	46.9983	Pantropical
	MAD0304	LN831848	Anosy: Nord- Est du phare d'Evatra	– 24.9783	47.0983	
	MAD0582	LN831849	Anosy: Lokaro: Sud de la baie	– 24.9500	47.1083	
	MAD0729	LN831850	Anosy: Baie des Galions	– 25.1483	46.7567	
	MAD1675	LN831851	Anosy: Baie des Galions	– 25.1467	46.7500	
	MAD1992	LN831852	Tuléar: Lagon de Baravo	– 25.2033	44.3217	
	MAD2120	LN831853	Anosy: Sainte Luce	– 24.7650	47.2133	
Stypopodium multipartitum2	MAD0379	LN831854	Anosy: Pointe d'Ambero	– 25.8000	47.7167	South Africa
	MAD1927	LN831855	Tuléar: Cap Sainte Marie	– 25.5900	45.1300	
	MAD1948	LN831856	Tuléar: Cap Sainte Marie	– 25.5533	45.1100	
Zonaria subarticulata2	MAD0380	LN831857	Anosy: Pointe d'Ambero	– 25.8000	47.7167	South Africa
	MAD0717	LN831858	Anosy: Baie des Galions	– 25.9333	46.7500	
	MAD1642	LN831859	Anosy: Baie des Galions	– 25.7500	47.3333	
	MAD1814	LN831860	Beloha: Roche de l'Albatros	– 26.1667	45.7333	

crassa species complex (Fig. 2B). This species complex consists of 7 distinct clades, all from confined geographic regions, which may indicate that each MOTU might be revalued as a distinct species. *Lobophora crassa6* has sampling localities in Madagascar, Vietnam and the tropical coast of Oman. A species range extending from the south of Madagascar to the Red Sea in Egypt was found for *Lobophora sp36* (Fig. 2B). Apparently restricted ranges might be the consequence of scarce sampling throughout the north of the Indian Ocean for the genus *Lobophora*, but this distribution does not refute a Western Indo-Pacific distribution.

Surprisingly, the only species with a distribution confined to Madagascar and the tropical coast of East-Africa (thus excluding pantropically distributed species as listed below), was *Lobophora sp45* (Fig. 2C). Given the geographical proximity of this regions and their classification within the Western Indian Ocean province, we expected to encounter more species with such distributions. As the tropical East African coast is well covered by our dataset (> 350 specimens) we do not recognize this as a sampling artifact. Conversely, we found substantially more species-level similarity between the south Malagasy coast and the coast of KwaZulu-Natal in South Africa as described below.

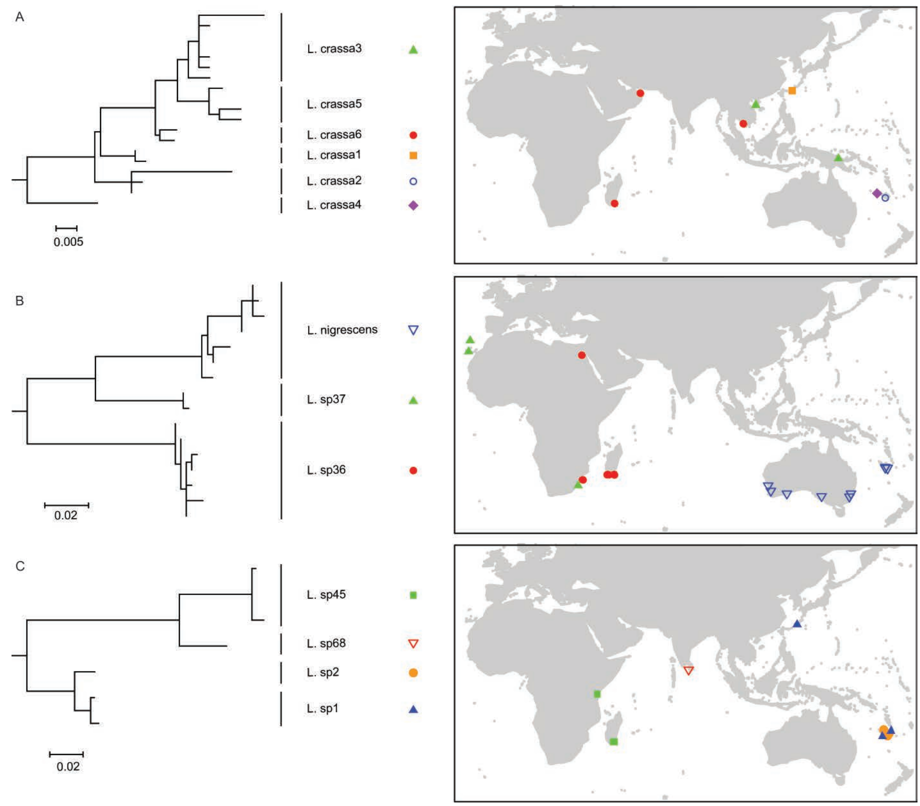

Fig. 2. Phylogenetic trees and distribution maps for species with a Western Indo-Pacific distribution: **A.** *Lobophora crassa* complex, **B.** *Lobophora* sp36, **C.** *Lobophora* sp45. Note: *Lobophora crassa*5 is reported from French Polynesia but not depicted on the map.

Biogeographical links between South Africa and Madagascar

Four of 23 Malagasy species show a clear biogeographical link to the coast of KwaZulu-Natal. In these cases, species are exclusively found in South Africa and southern Madagascar. In the case of *Rugulopteryx*, one shared species (*R. suhrii*) between the South African coast and Madagascar was found (Fig. 3A). *Rugulopteryx suhrii* was originally described by Suhr in 1839 from Madagascar, but Kützing subsequently changed the locality to Algoa Bay, Cape Province in South Africa, (Silva *et al.*, 1996) which was then later accepted as type locality for the species (De Clerck, 2003). Other *Rugulopteryx* species are found in clearly delineated geographical areas: *R. marginatus* and *R. radicans* in southwest and southeast Australia respectively and *R. okamurae* in Japan. Records of

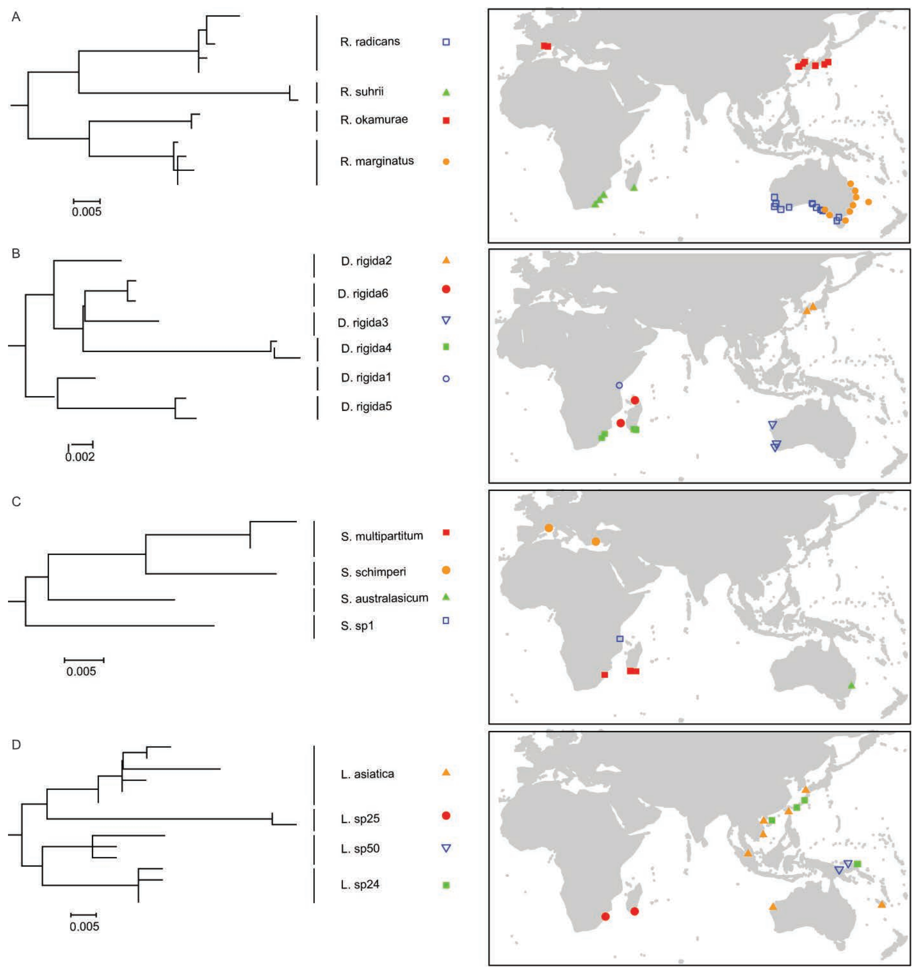

Fig. 3. Phylogenetic trees and distribution maps for Dictyotales with a strong biogeographic affinity to South Africa: **A.** *Rugulopteryx suhrii*, **B.** *Dictyota rigida* complex 4, **C.** *Stypopodium multipartitum*, **D.** *Lobophora* sp25. Note: *Dictyota rigida* 5 is reported from South Brazil and the Caribbean coast of Panama, but is not depicted on the map.

R. okamurae in Europe result from an introduction of the species in the Thau lagune most likely as a result of shellfish transport as described in Verlaque *et al.* (2009). A similar link between South Africa and Madagascar is apparent for the *Dictyota rigida4*. *Dictyota rigida* was originally described from Tanzania (De Clerck & Coppejans, 1999), but most likely represents a complex of at least six morphologically similar but genetically distinct lineages. Each of these six MOTUs appears to have a restricted distribution in the Indian Ocean and the Caribbean (Fig. 3B). Additional links between KwaZulu-Natal and the south of Madagascar are apparent in *Stypopodium multipartitum* and *Lobophora sp25* (Fig. 3C-D).

The biogeographical link between South Africa and Madagascar has until now hardly been described in the literature, mainly due to the fact that the seaweed flora of southern Madagascar is barely sampled. But for instance *Pseudocodium devriessi* shows a disjunct warm temperate distribution, with specimens from the coast of KwaZulu-Natal, southern Madagascar and southwestern Australia in the southern hemisphere, and within the temperate upwelling region of Dhofar in Oman (De Clerck *et al.*, 2008). Temperature conditions in KwaZulu-Natal and southern Madagascar are more temperate, and according to Bolton (2004) most of the KwaZulu-Natal flora is a mixture of temperate and tropical elements, lacking a significant amount of endemic shallow-water species. KwaZulu-Natal should therefore be treated as an overlap region between the Western Indian Ocean province and the temperate Agulhas province. However, in the case of south Madagascar and based on the dictyotalean data, there is no reason to assume that this region harbors an exclusive tropical Western Indo-Pacific flora either. The presence of typical warm-temperate species and the more temperate sea surface temperatures suggest that the southern Malagasy coast might also be considered an overlap region with both temperate and tropical species, similar to the KwaZulu-Natal coast.

Endemic species for Madagascar

Six of 23 MOTUs sampled from Madagascar did not yet occur in our global Dictyotales dataset (> 5000 specimens). We recovered a new MOTU (*Z. subarticulata2*) closely related to South-African specimens of "*Zonaria subarticulata*". *Zonaria subarticulata* (Fig. 4A) was first described from Madagascar (Silva *et al.*, 1996), but had until now only confirmed molecular records in South Africa. Sequence divergence between South African and Malagasy specimens could warrant species-level divergence. If so, the Malagasy specimens would retain the name *Z. subarticulata* and would represent an endemic species for Madagascar. For the South African specimens the earliest available name would be *Mycinema scandens* Suhr (Suhr, 1834), described from Algoa Bay and considered to represent the rhizoids of *Z. subarticulata* by Papenfuss (cit. in. Silva *et al.*, 1996). It seems evident that further studies including examination of the type material of *M. scandens* are required before any taxonomic changes are effected.

As depicted in Fig. 4B, a new clade in the *Padina melemele*-complex has been uncovered. This taxon did not exhibit any clear geographical link with any other region. In addition, each of the lineages in the complex appears to have a geographically restricted range in the Central Indo-Pacific and Temperate Australasia. In this respect, we believe that the Malagasy specimens might belong to a new undescribed species endemic to Madagascar. The same holds for a

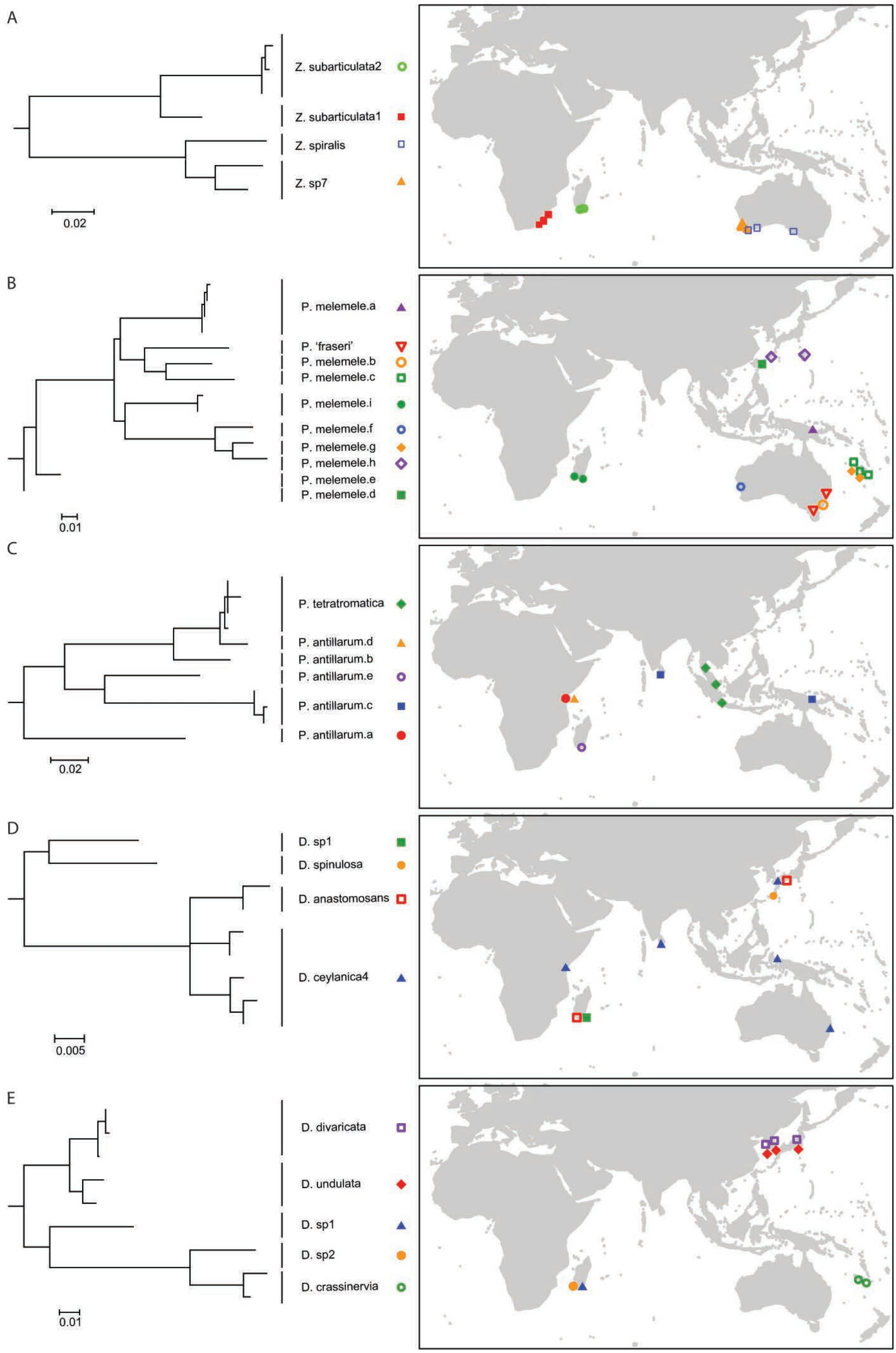

Fig. 4. Phylogenetic trees and sampling localities for presumed endemics: **A.** *Zonaria subarticulata,* **B.** *Padina melemele* species complex, **C.** *Padina antillarum* species complex, **D.** *Dictyota* sp1, **E.** *Dictyopteris* sp1 & *Dictyopteris* sp2.

previously unrecorded taxon within the *Padina antillarum* species complex (Fig. 4C). In the genus *Dictyota* a new species was sampled which is closely related to *Dictyota spinulosa* from Japan (Fig. 4D). Those species might be genuine endemics, or they might be the result of under sampling of the order in certain regions. With a total of 2840 specimens and over 850 localities for *Dictyota* and of 453 specimens and over 250 localities for *Padina*, our sampling is extensive and we are confident that these species are genuine endemics. In the case of *Dictyopteris* (Fig. 4E) however, our *rbc*L dataset is more restricted and as such, both species might wrongfully be perceived as endemics. On the other hand, in comparison to the total of Dictyotales species sampled in Madagascar, this represents a substantial subset of endemicity. Whether this is also the case for other marine macroalgal groups is not clear as literature, and in particular molecular studies, are scarce for this region.

Biogeographical link between Japan and Madagascar

Based on *psb*A gene sequences, *Dictyota anastomosans* is not resolved from *D. ceylanica4* (Fig. 4D). The species *D. anastomosans* is observed both in Japan and Madagascar. The same link between Japan and Madagascar is observed in the sister-species *D. spinulosa* and *Dictyota sp1* that are endemics from Japan and Madagascar respectively. Hommersand (1986) hypothesized the existence of similar biogeographical patterns between the red seaweed flora of South Africa and Japan. Hommersand postulated that some of these taxa probably evolved in the Tethyan Ocean and radiated species in two directions. Alternatively, others might well have originated in warm-temperate climates in East Asia and migrated through the tropics into South Africa during the Miocene. In the case of *Dictyota sp1* and *D. anastomosans* both of these explanations seem unlikely, given the very small evolutionary divergence between the Japanese and Malagasy samples that are either the same species or sister-species. In this case, a secondary introduction either with a Western Pacific or a Japanese origin might be more plausible.

Broader or pantropical distributions

Several of the species found in Madagascar have wide tropical distributions, occurring in both the tropical Indo-Pacific and Atlantic Oceans. These include *D. ceylanica5*, *Canistrocarpus cervicornis* and *Dictyota humifusa*. DNA-confirmed records of *Padina boergesenii.c* showed a wide distribution in the Western Indo-Pacific and one record from the Caribbean coast of Costa Rica. Additional sampling, especially in the Caribbean, should indicate whether this species is truly pantropical. The same is observed for *Lobophora sp44*, which has one record from Brazil and one from Atlantic Panama.

Dictyota stolonifera shows a Western Indo-Pacific distribution with extensions into Japan and the Central Pacific. The same holds true for the monospecific genus *Stoechospermum polypodioides*, which is reported from the whole Western Indo-Pacific but extending into the Central Pacific (NW-Australia) (Phillips *et al.*, 1993; De Clerck & Coppejans, 1997). In the case of *Dictyota liturata* and *Padina gymnospora.e*, a restricted taxon sampling did not allow us to make sound inferences.

CONCLUSIONS

In terms of sampling, the south coast of Madagascar has largely been ignored even though the different temperature conditions from the rest of the country might have led to a distinct seaweed flora relative to the rest of the island. In this regard, it is not surprising that during the *Atimo Vatae* expedition, unrecorded and undescribed Dictyotales species have been found. In the present study, we report a total of six MOTUs that might represent endemic species.

Only within the tropical genus *Lobophora* we do recognize a clear-cut biogeographical pattern concordant to the Western Indo-Pacific realm of Spalding *et al.* (2007). Additionally, despite the fact that Madagascar is accommodated in the Western Indian Ocean province, together with the East-African coast, we did not recover an unambiguous link between both geographical regions, except for *Lobophora sp45*. In contrast, we find some close biogeographical affinity between the South African coast of KwaZulu-Natal and the south of Madagascar. This may be due to the fact that both regions are subject to more temperate conditions in comparison to the tropical Western Indian Ocean. In this regard, the results of this study on the Dictyotales suggests that from a phycological point of view, it might be invalid to consider the south of Madagascar as belonging to a Western Indo-Pacific realm and neither to a tropical Western Indian Ocean Province, but rather as a region of overlap in which both tropical and temperate elements thrive. This statement however should be endorsed by comparative biogeographical studies on other seaweed taxa.

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