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3D chemotaxonomy of corals using fatty acid biomarkers: latitude, longitude and depth

Cátia Alexandra Alves Figueiredo

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“Sempre chegamos ao sítio aonde nos esperam.”

José Saramago

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RESUMO

A população mundial de corais tem vindo a diminuir ao longo dos anos, tanto em abundância como em diversidade. Esta diminuição deve-se à sobre-exploração dos recursos marinhos, à poluição, à acidificação dos oceanos e ao aquecimento global (principal responsável pelo processo de lixiviação). Sendo que aqueles organismos possuem grande importância ecológica e económica, o interesse no seu estudo tem vindo a aumentar, nomeadamente no que se refere à sua quimiotaxonomia

Os hexacorais possuem seis ou menos eixos de simetria na sua estrutura corporal e somente uma linha única de tentáculos. Estes organismos são formados de pólipos individuais, que em algumas espécies vivem em colónias, formando recifes, e podem possuir um esqueleto cálcico rígido, distinguem-se dos octocorais por estes terem um esqueleto interno excretado pela mesogleia e pólipos com oito tentáculos.

Entende-se por quimiotaxonomia o método de classificação biológica que se baseia na similaridade e/ou diferença no perfil de certos compostos e nas vias bioquímicas envolvidas na sua síntese, manutenção e obtenção. Estes compostos estudados podem ser proteínas, aminoácidos e lípidos, entre outros. Os lípidos constituem a base estrutural das membranas biológicas, podem atingir até cerca de 40% da biomassa seca de um coral e estão envolvidos numa série de processos bioquímicos e fisiológicos. Desta forma, alterações na composição lipídica reflectem alterações na ecologia, nutrição e saúde dos corais. Por exemplo, o catabolismo das ceras e triacilgliceróis pode fornecer a energia necessária para a respiração e crescimento do organismo quando a obtenção de alimento (ex. fitoplâncton, zooplânkton, matéria orgânica particulada) é reduzida.

Os ácidos gordos são os principais componentes dos lípidos e a sua composição é determinada, até um certo nível, pela predisposição genética de uma espécie para a sua biosintese. Apesar do perfil (composição) de ácidos gordos ser, de forma geral, específico de cada espécie de coral, este pode variar dependendo de condições ambientais, da disponibilidade e qualidade de alimento e da composição e presença de simbiontes (zooxantelas) e bactérias.

As zooxantelas são algas, geralmente dinoflageladas, que vivem em simbiose com vários invertebrados marinhos, especialmente cnidários. Estas fornecem compostos (maioritariamente lipídicos) aos coraisenquanto usufruem de um meio de suporte onde subsistir. Os corais são organismos politróficos, ou seja, que obtêm os nutrientes essenciais à sua sobrevivência simultaneamente através de uma variedade de mecanismos. Assim sendo, é actualmente aceite que corais zooxantelados podem satisfazer as suas necessidades energéticas por via heterotrófica (plankton e matéria orgânica em suspensão) e autotrófica (produção primária das zooxantelas), esta particularmente valiosa em águas pobres em nutrientes, onde a densidade de plâncton é insuficiente para suportar uma cadeia trófica robusta.

As zooxantelas podem apresentar uma composição de ácidos gordos diferente daquela o coral obtém através de outras fontes. Assim, corais zooxantelados e azooxantelados podem exibir diferenças significativas no que diz respeito à sua composição em termos de ácidos gordos. O carbono fixado fotossinteticamente pelas zooxantelas é rapidamente transformado em lípidos que, por sua vez, são transferidos para o tecido do hospedeiro na forma de triacilgliceróis, ceras, e ácidos gordos livres. Esta translocação é a principal fonte de ácidos gordos saturados, logo, a presença de ácidos gordos poli-insaturados é,

provavelmente, indicativo de uma fonte de alimentação externa, como de zoo- e fitoplâncton.

Muitas famílias de cnidários caracterizam-se pela presença de ácidos gordos pouco usuais. A composição de ácidos gordos é assim, útil em estudos de quimiotaxonomia neste grupo de organismos e torna possível uma clara distinção de espécimes de acordo com a sua ordem, família, e em alguns casos género.

Com o objectivo de contribuir para uma melhor compreensão das relações quimiotaxonómicas de: i)hexacorais e octocorais, ii) corais zooxantelados e azooxantelados, iii) corais costeiros e do mar profundo, compilou-se primariamente (numa meta-análise) os dados disponíveis (literatura científica) referentes à composição de ácidos gordos de 27 espécies (35 espécimenes) de hexacorais e 39 espécies (47 espécimenes) de octocorais.Posteriormente, analisou-se o perfil de ácidos gordos de 34 outras espécies de hexacoral e octocoral oriundas do Brazil, México, Seychelles, Portugal e Vietnam, e adicionou—se essa informação à meta-análise.

Numa primeira abordagem, compararam-se os perfis de ácidos gordos de hexa- e octocorais, obtendo-se uma clara separação entre estes dois grupos, principalmente através dos ácidos gordos 24:5*n*-6 e 24:6*n*-3, apenas presentes em octocorais. O ácido gordo 20:4*n*-6 também desempenhou um papel importante nesta separação, podendo ser adoptado como um marcador útil na quimiotaxonomia de hexa- e octocorais.

De seguida realizou-se uma análise dos hexacorais numa perspectiva espacial e taxonómica (Ordem). Não se obteve qualquer separação; i.e., os ácidos gordos utilizados naquela não foram úteis no estudo daquimiotaxonomia deste grupo de corais. No entanto, um cenário diferente foi observado para os octocorais. Neste grupo foi obtida uma clara separação entre alcionários, penatulários e gorgónias. As gorgónias apresentaram-se mais

próximas dos alcionários, enquanto os penatulários formaram um grupo bem individualizado e mais distante. Os alcionários são, desta forma, bioquimicamente mais próximos das gorgónias, indicando uma evolução divergente mais recente. Uma separação espacial foi também conseguida, revelando as espécies de regiões temperadas em costas Oeste de alta produção primária marinha (Portugal e Califórnia) como detentoras de uma geralmente maior quantidade de 20:5*n*-3, ácido gordo originário de fitoplâncton, disponível em maiores quantidades nestas regiões. Como esperado, o ácido gordo 18:4*n*-3, um dos principais ácidos gordos encontrados em zooxantelas, geralmente presente em maior quantidade nos alcionários com zooxantelas, contribuiu para a sua separação relativamente aos alcionários azooxantelados.

Por fim, uma separação espacial (incluindo a componente profundidade) foi conseguida com gorgónias. As gorgónias do mar profundo, quando comparadas com as de baixa profundidade da costa de Portugal, demonstraram uma menor percentagem quantitativa de todos os ácidos gordos estudados, confirmando que a temperatura, a ausência de luz e a disponibilidade de alimento afectam o perfil de ácidos gordos dos corais. Em conclusão, esta dissertação contribui significativamente para a compreensão da quimiotaxonomia de hexa- e octocorais oriundos de diferentes oceanos e tipos de habitat, incluindo diferentes zonas climáticas e batimétricas.

Palavras-chave:

Quimiotaxonomia; ácidos gordos; biomarcadores; Hexacorallia; Octocorallia; Zooxanthellae; baixa profundidade; mar profundo.

ABSTRACT

Corals have the ability to biosynthesize specific sets of fatty acids (FA) and their content is also known to be influenced by food intake, presence of symbiotic zooxanthellae and bacteria. Additionally, environmental conditions such as light intensity and water temperature were also shown to affect FA profiles of corals. To uncover differences in FA composition of corals from different climatic zones (e.g. temperate, subtropical and tropical) and distinct habitats (e.g. coral reefs, intertidal and subtidal zones, and deep-sea environments), we studied the FA profile of 41 species and performed a comparison with that of 66 species, available in the literature. Five (*n*-6) and five (*n*-3) PUFAs (18:2*n*-6, 18:4*n*-3, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-6, 22:5*n*-3, 22:6*n*-3, 24:5*n*-6, 24:6*n*-3) were used for the meta-analyses and consequent multivariated tests, namely Principal Component Analyses (PCA). We show a clear separation between hexa- and octocorals (mainly due to 20:4*n*-6, 24:5*n*-6 and 24:6*n*-3), but the selected PUFAs were not suitable for the separation of hexacorals at the order level (Zoanthidea and Scleractinia). On the other hand, a clear separation was achieved in octocorals. Within this group, gorgonians were placed closer to the other alcyonaceans because they are biochemically closer, indicating a recent evolutionary divergence within Octocorallia. Also, a clear separation between shallow and deep-sea gorgonians was achieved. The latter generally showed a lower content of the selected FAs, highlighting the different and scarcer sources of energy available to deep-sea organisms. Summing up, the present dissertation increased significantly the existing knowledge about the chemotaxonomy of corals, by expanding it to other oceanic regions (i.e. North and South Atlantic Ocean) and habitats (e.g. abyssal plains).

Key words: Chemotaxonomy; Fatty Acids; Biomarkers; Hexacorallia; Octocorallia; Zooxanthellae; shallow water; deep-sea.

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1. INTRODUCTION

Lipids constitute the structural base of biological membranes and perform protective and signalling functions (Spector and Yorek, 1985). In corals, these compounds can make up to 40 % of the dry weight and, thus, constitute the main source of stored energy (Stimson, 1987; Harland et al., 1993; Yamashiro et al., 1999). The principal components ("building blocks") of lipids - fatty acids (FAs; Fig. 1), are known to be involved in the majority of biochemical and physiological processes of those cnidarians (Ibarguren et al., 2014).

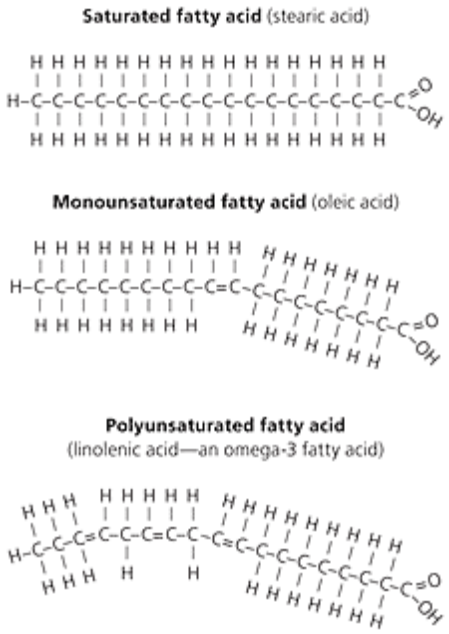


Figure 1. Chemical structure of saturated, mono- and polyunsaturated FAs

Corals are polytrophic organisms, i.e. they simultaneously obtain nutrients through a variety of sources, including FA from: i) prey items (plankton), ii) particulate organic matter, iii) symbiotic photosynthetic dinoflagellates (zooxanthellae - *Symbiodinium* group),

iv) bacteria (Volkman et al., 1998) and v) *de novo* biosynthesis pathways occurring in their tissues (Imbs et al., 2007a). This FA biosynthesis occurs in parallel both in zooxanthellae (Fig. 2) and the host (Oku et al., 2003; Imbs et al., 2010a).

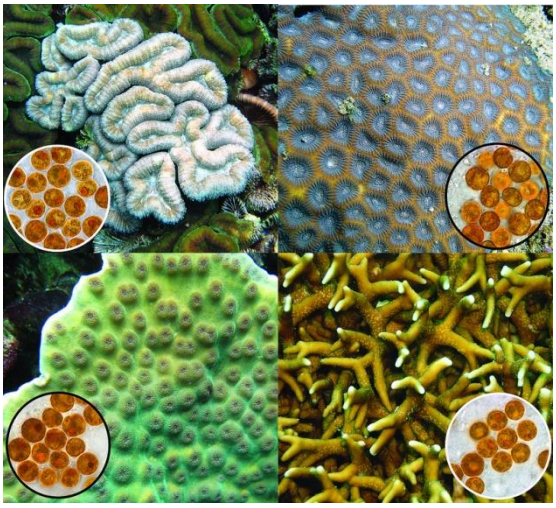


Figure 2. Photosynthetic symbiotic zooxanthellae in corals

It is worth noting that most animals cannot synthesize longer chain polyunsaturated fatty acids (PUFAs); instead, they are produced by phytoplankton and some bacteria and are transferred through the food web to higher trophic levels (Volkman et al., 1998).

There are certain differences in feeding behaviour between soft corals (mainly octocorals; Fig. 3) and reef-building corals (mainly hexacorals; Fig. 4), since soft corals, having a special anatomic structure, are believed to possess specific mechanisms of catching fine suspended food particles (Lewis, 1982; Imbs and Latyshev, 2012). These ecological dissimilarities can lead to differences in the FA profiles of hexa- and octocorals and, consequently, influence their chemotaxonomy classification.

Figure 3. Example of octocoral

Figure 4. Example of hexacoral

Regarding the symbiosis, the diversity and quantity of zooxanthellae in a specificcoral taxonomic group depends on environmental factors such as solar irradiance and water temperature (Fabricius et al., 2004). Consequently, the presence/absence of zooxanthellae should lead to significant differences in FA composition among coral species (Imbs et al., 2007c). Photosynthetically fixed carbon is quickly converted into lipids, which are then carried into 'host' tissues in the form of 'fat droplets', consisting of triglycerides, wax esters and free fatty acids (Patton et al., 1983). These 'fat droplet' lipids are the main source of saturated fatty acids (SFA), while the presence of PUFA is most probably indicative of external food sources such as zoo- and phytoplankton (Kellogg and Patton, 1983; Latyshev et al., 1991). Thus, by knowing the origin of such FAs, they can be used as chemotaxonomic markers (Imbs et al., 2010b).

Changes in the ecology, nutrition, food habits and health of corals, due to environmental pressures, for example, may become detectable through changes in FA composition. For instance, a decrease in temperature may cause changes in membrane fluidity. The integrity of living cells in response to thermal stress depends on the

biomolecular lipid layer and the associated non-lipid components (Neidleman, 1987). In fact, the maintenance of appropriate cell membrane fluidity is of serious importance for the function and integrity of the cell, mobility and function of embedded proteins and lipids, diffusion of proteins and other molecules laterally across the membrane for signalling reactions, and proper separation of membranes during cell division (Kates et al., 1984; Hazel, 1988; Murata and Los, 1997). A fundamental biophysical determinant of membrane fluidity is the balance between saturated and unsaturated fatty acids. The general trend is an increase in unsaturated FA at lower temperatures and an increase in saturated fatty acids at higher temperatures. This compositional adaptation of membrane lipids, called homeoviscous adaptation (Fig. 5), serves to maintain the correct membrane fluidity at the new conditions (Sinensky, 1974).

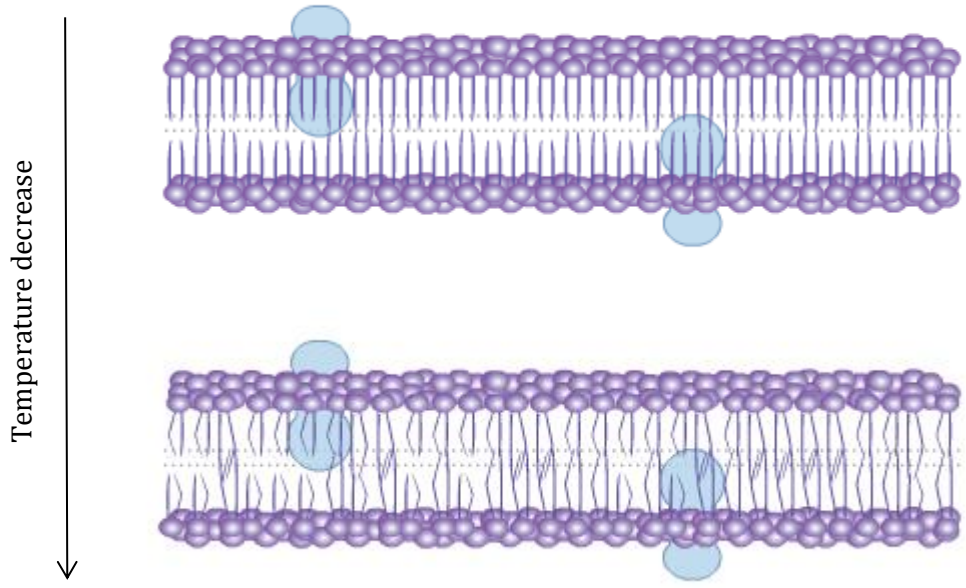


Figure 5. Effect of decreased temperature in the structure of cellular membranes

It is recognized that in ectothermic animals an increase in the content of unsaturated FA (UFA) occurs in response to cold temperatures (Hall et al., 2002), suggesting that

differences in the PUFA profile of corals may occur in relation to different climate conditions and depths.

Within this context, the aim of the present dissertation was to perform the most comprehensive examination of the chemotaxonomy of corals, by expanding the current knowledge on the subject, which is quite spatially limited (most species studied so far (85%) are from Vietnam, see Table 1). Here, I intended to uncover differences in FA composition of hexa- and octocorals from different climatic zones (temperate, subtropical and tropical) and distinct habitats (e.g. coral reefs, intertidal and subtidal zones, and deep-sea environments). More specifically, I studied the FA profile of 41 new species (19 hexacoral species from Mexico and Brazil, and 22 octocoral species from Azores Islands, Brazil, Portugal and also from Vietnam).

Table 1: Database of the coral species used (with presence or absence of zooxanthellae), respective region and collection sites.

Subclass	Order	Suborder	Species	Presence of zooxanthellae	Region	Collection site	Reference
Alcyonaria (Octocorallia)							
	Alcyonacea						
		Alcyoniina					
			Alcyonium digitatum	**Azooxanthellate**	**Portugal**	**Setúbal**	**PS**
			Cespitularia sp.	Zooxanthellate	Vietnam	Hong Island	1
			Chironephthya variabilis	Azooxanthellate	Vietnam	Nha Trang Bay	2
			Cladiella laciniosa	Zooxanthellate	Vietnam	Den Island	1
			Dendronephthya aurea I	Azooxanthellate	Vietnam	Cua Be Strait	1
			II		Vietnam	Den Island	1
			Dendronephthya crystallina I	Azooxanthellate	Vietnam	Cua Be Strait	1
			II		Vietnam	Den Island	1
			Dendronephthya gigantea	Azooxanthellate	Vietnam	Cua Be Strait	1
			Dendronephthya aff. *involuta*	Azooxanthellate	Vietnam	Maxfield Bank	1
			Dendronephthya sp. I	Azooxanthellate	Vietnam	Lon Island	1
			Dendronephthya sp. II	Azooxanthellate	Vietnam	Maxfield Bank	1
			Dendronephthya sp. III	Azooxanthellate	Vietnam	Maxfield Bank	1
			Dendronephthya sp. IV	Azooxanthellate	Vietnam	Maxfield Bank	1
			Litophyton sp.	Zooxanthellate	Vietnam	Den Island	1
			Lobophytum cf. *delectum*	Zooxanthellate	Vietnam	Tai Island	1

		Lobophytum pusillum	Zooxanthellate	Vietnam	Den Island	1
		Neospongodes atlantica	**Zooxanthellate**	**Brazil**	**Baía de Todos-os-Santos**	**PS**
		Paralemnalia thyrsoides	Zooxanthellate	Vietnam	Nha Trang Bay	2
		Sarcophyton acutum	Zooxanthellate	Vietnam	Cua Be Strait	1
		Sarcophyton buitendijki I	Zooxanthellate	Vietnam	Den Island	1
		II		Vietnam	Den Island	1
		Sarcophyton cinereum	Zooxanthellate	Vietnam	Lon Island	1
		Sarcophyton aff. *crassum*	Zooxanthellate	Vietnam	Den Island	1
		Sarcophyton elegans	Zooxanthellate	Vietnam	Cua Be Strait	1
		Sarcophyton trocheliophorum	Zooxanthellate	Vietnam	Cua Be Strait	1
		Sinularia cruciata	Zooxanthellate	Vietnam	Den Island	3
		Sinularia aff. *deformis*	Zooxanthellate	Vietnam	Den Island	3
		Sinularia densa	Zooxanthellate	Vietnam	Den Island	3
		Sinularia flexibilis	Zooxanthellate	Vietnam	Den Island	3
		Sinularia leptoclados	Zooxanthellate	Vietnam	Den Island	3
		Sinularia lochmodes	Zooxanthellate	Vietnam	Den Island	3
		Sinularia cf. *muralis*	Zooxanthellate	Vietnam	Den Island	3
		Sinularia notanda	Zooxanthellate	Vietnam	Den Island	3
	Calcaxonia					
		Ellisella plexauroides	Azooxanthellate	Vietnam	Nha Trang Bay	2
	Holaxonia					
		***Acanthogorgia armata* I**	**Azooxanthellate**	**Portugal - Azores**	**Banco D. João de Castro**	**PS**
		II		**Portugal - Azores**	**Furnas de Fora**	**PS**
		Acanthogorgia isoxya	Azooxanthellate	Vietnam	Nha Trang Bay	2
		Bebryce studeri	Azooxanthellate	Vietnam	Den Island	2
		Echinogorgia sp.	Azooxanthellate	Vietnam	Nha Trang Bay	2
		***Eunicea sp.* I**	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
		II		**México**	**Puerto Morelos Reef, Mexican Caribbean**	**PS**
		III		**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
		IV		**Portugal**	**Setúbal**	**PS**
		***Eunicella verrucosa* I**	**Azooxanthellate**	**Portugal**	**Setúbal**	**PS**
		II		**Portugal**	**Setúbal**	**PS**
		***Gorgonia sp.* I**	**Azooxanthellate**	**México**	**Puerto Morelos Reef, Mexican Caribbean**	**PS**
		II		**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
		***Leptogorgia sarmentosa* I**	**Azooxanthellate**	**Portugal**	**Setúbal**	**PS**
		II		**Portugal**	**Setúbal**	**PS**
		III		**Portugal**	**Setúbal**	**PS**
		Menella praelonga	Azooxanthellate	Vietnam	Nha Trang Bay	2
		Muricea sp.	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
		Unidentified	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
		Paramuricea biscaya	**Azooxanthellate**	**Portugal - Azores**	**Canal de S. Jorge**	**PS**
		c.f. Placogorgia sp.	**Azooxanthellate**	**Portugal - Azores**	**Canal de S. Jorge**	**PS**

			Plexaurella sp.	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
			***Pseudoplexaura sp.* I**	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
			II		**México**	**Puerto Morelos Reef, Mexican Caribbean**	**PS**
			Pseudopterogorgia sp.	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
			Rumphella aggregata	Zooxanthellate	Vietnam	Nha Trang Bay	2
		Scleraxonia	*Acabaria erythraea*	Azooxanthellate	Vietnam	Nha Trang Bay	2
		Stolonifera	**Carijoa riisei**	**Azooxanthellate**	**Brazil**	**Bahia Todos os Santos**	**PS**
			Clavularia sp.	Zooxanthellate	Vietnam	Tre Island	1
	Pennatulacea	Sessiliflorae	***Cavernularia obesa***	**Azooxanthellate**	**Vietnam**	**Unknown**	**PS**
			Pteroeides spp.	**Azooxanthellate**	**Vietnam**	**Unknown**	**PS**
			Veretillum cynomorium I	Azooxanthellate	Portugal	Sado Estuary	5
			II		Portugal	Sado Estuary	5
			III		Portugal	Sado Estuary	5
			IV		Portugal	Sado Estuary	5
			V		Portugal	Sado Estuary	5
			VI		Portugal	Sado Estuary	5
			Renilla koellikeri	Azooxanthellate	U.S.A.	Long Beach, California	4
Zoantharia (Hexacorallia)							
	Scleractinia		*Acropora cerealis*	Zooxanthellate	Vietnam	Mun Island	6
			Acropora florida	Zooxanthellate	Vietnam	Thotyu Island	7
			Acropora formosa	Zooxanthellate	Vietnam	Mun Island	6
			Acropora gemmifera	Zooxanthellate	Vietnam	Mun Island	6
			Acropora milepora I	Zooxanthellate	Vietnam	Thotyu Island	7
			II		Vietnam	Tyam Island	7
			Acropora nasuta I	Zooxanthellate	Vietnam	Thotyu Island	7
			II		Vietnam	Tyam Island	7
			Acropora nobilis	Zooxanthellate	Vietnam	Nha Trang Bay	6
			Acropora palifera	Zooxanthellate	Vietnam	Mun Island	6
			Acropora sp.	Zooxanthellate	Vietnam	Nha Trang Bay	6
			***Agaricia sp.* I**	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
			II		**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
			Caulastraea tumida	Zooxanthellate	Vietnam	Den Island	1
			Diploria sp.	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
			Diploria strigosa	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
			Echinophyllia orpheensis	Zooxanthellate	Vietnam	Nha Trang Bay	6
			Favia sp. I	Zooxanthellate	Vietnam	Nha Trang Bay	6
			II	Zooxanthellate	Vietnam	Nha Trang Bay	6
			Goniopora sp. I	Zooxanthellate	Vietnam	Tyam Island	7
			II	Zooxanthellate	Vietnam	Tyam Island	7

	Montastraea annularis	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
	Montastraea sp.	**Zooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
	Oculina sp.	**Zooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
	Pocillopora damicornis I	Zooxanthellate	Vietnam	Mun Island	6
	II		Vietnam	Thotyu Island	7
	III		Vietnam	Thotyu Island	7
	Pocillopora verrucosa	Zooxanthellate	Vietnam	Thotyu Island	7
	Porites cylindrica	Zooxanthellate	Vietnam	Mun Island	6
	Porites lobata	Zooxanthellate	Vietnam	Nha Trang Bay	6
	Porites lutea	Zooxanthellate	Vietnam	Thotyu Island	7
	Porites nigrescens	Zooxanthellate	Vietnam	Mun Island	6
	Porites porites	**Azooxanthellate**	**México**	**Puerto Morelos Reef, Mexican Caribbean**	**PS**
	Porites sp.	**Azooxanthellate**	**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
	Sandalolitha robusta	Zooxanthellate	Vietnam	Nha Trang Bay	6
	Scolymia cubensis	**Zooxanthellate**	**Brazil**	**Baía de Todos-os-Santos**	**PS**
	Scolymia sp.	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
	Scolymia wellsi	**Zooxanthellate**	**Brazil**	**Baía de Todos-os-Santos**	**PS**
	Seriatopora caliendrum	Zooxanthellate	Seychelles	Aldabra Island	7
	Seriatopora hystrix	Zooxanthellate	Vietnam	Mun Island	6
	Stylophora pistillata I	Zooxanthellate	Seychelles	Coetivy Island	7
	II		Seychelles	Coetivy Island	7
	III		Vietnam	Mun Island	6
	IV		Vietnam	Thotyu Island	7
	V		Vietnam	Tyam Island	7
	***Tubastraea coccinea* I**	**Azooxanthellate**	**Brazil**	**Baía de Todos-os-Santos**	**PS**
	II		Seychelles	Aldabra Island	7
	Tubastraea micrantha	Azooxanthellate	Seychelles	Aldabra Island	7
Zoanthidea	***Epizoanthus gabrieli* I**	**Zooxanthellate**	**Brazil**	**Baía de Todos-os-Santos – I**	**PS**
	II		**Brazil**	**Baía de Todos-os-Santos – II**	**PS**
	III		**Brazil**	**Baía de Todos-os-Santos – III**	**PS**
	***Palythoa caribaeorum* I**	**Azooxanthellate**	**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
	II		**México**	**Mahahual Reef, Mexican Caribbean**	**PS**
	III		**México**	**Puerto Morelos Reef, Mexican Caribbean**	**PS**
	Palythoa sp.	**Azooxanthellate**	**México**	**La Gallega Reef, Veracruz Reef System**	**PS**
	Protopalythoa variabilis	**Zooxanthellate**	**Brazil**	**Baía de Todos-**	**PS**

				os-Santos	
	***Zoanthus sociatus* I**	**Zooxanthellate**	**México**	**La Gallega Reef, Veracruz Reef System**	**PS**
	II		**México**	**Madagascar Reef, Yucatán Peninsula**	**PS**
	***Zoanthus sp.* I**	**Zooxanthellate**	**Brazil**	**Baía de Todos-os-Santos**	**PS**
	II		**Brazil**	**Baía de Todos-os-Santos**	**PS**

References: 1 - Imbs et al. 2007b; 2 - Imbs et al. 2009; 3 - Imbs and Latyshev 2011; 4 – Pernet et al.2002; 5 - Baptista et al. 2012; 6 - Imbs et al. 2007a; 7 - Latyshev et al. 1991.

2. MATERIAL AND METHODS

2.1. Sampling

2.1.1. Shallow-living corals

Specimens of shallow-living hexa- and octocorals were collected by scuba-divers in Mexico [La Gallega Reef (n=2 species); Madagascar Reef (n=10); Mahahual Reef (n=10); Puerto Morelos Reef (n=5)], Brazil [Baía de Todos-os-Santos (n=8)], Portugal [Setúbal (n=4)] and Vietnam (n=2) (Fig. 6) at depths between 0.5-6 m. Samples were placed in liquid nitrogen and, in the lab, stored at -80 °C.

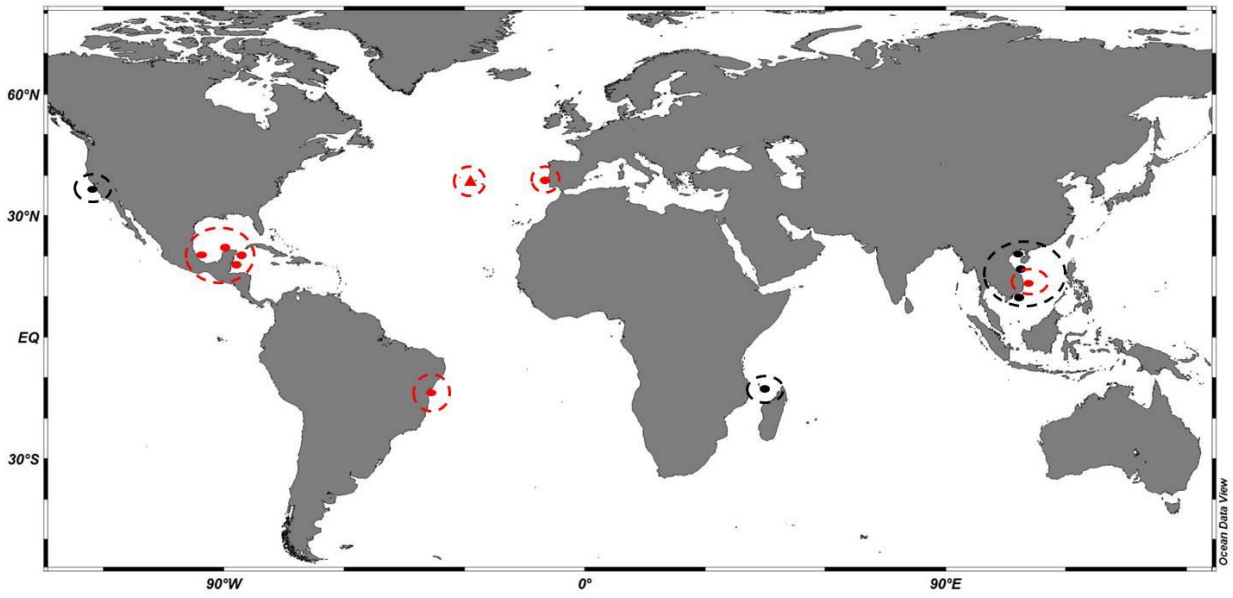


Figure 6. Sampling sites of coral specimens used in the present study (Azores, continental Portugal, Brazil, Mexico and Vietnam, red circles and red triangle representing deep-sea species from Azores) and those from the literature (Vietnam, Seychelles and California, black circles).

2.1.2. Deep-sea corals

Deep-sea gorgonian corals (n=4 specimens) were collected off the Portuguese Azores archipelago (Fig. 6; red triangle), namely in Furnas de Fora, Dom João de Castro Seamount and São Jorge Channel, at depths between 313-1077 m, with the working class ROV *Luso* model Bathysaurus XL (Fig. 7), operated from R/V Almirante Gago Coutinho. Following collection, samples were immediately stored in an on-board -80 °C freezer. Species belonging to the suborder Holaxonia, commonly designated as "gorgonians", were analysed as one group (i.e. gorgonians) for the sake of clarity and to allow comparison with previously published data.

Figure 7. ROV Luso model Bathysaurus XL.

2.2. Biochemical (fatty acid) analysis

Samples [145-301 mg for hexacorals and 300-301 mg for octocorals (dry mass)] were dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v; Merck), shaken for 30 sec, and heated (80 °C; 1 h). After cooling in room temperature for at least 30 min, 1 mL of Milli-Q distilled water and 2 mL of *n*-heptane pro analysis (Merck) were added and samples were

shaken, for 30 sec, and centrifuged (3000 g, 3 min) until phase separation. The organic content of the upper phase was filtered using an anhydrous sodium sulphate (Panreac) and cotton column. The filtered content was evaporated under a constant flow of nitrogen. Afterwards, 100µl of *n*-heptane were added to each replicate. Following, an aliquot (2 µL) was injected onto a gas chromatograph (Varian Star 3800 Cp, Walnut Creek, CA, USA) equipped with an autosampler and fitted with a flame ionization detector at 250 °C for FAME analysis. The separation was carried out with helium as carrier gas at a flow rate of 1 mL min^{-1}, in a capillary column DB-WAX (30 m length × 0.32 mm internal diameter; 0.25 µm film thickness; Hewlett– Packard, Albertville, MN) programmed at 180 °C for 5 min, raised to 220 at 4 °C min−1, and maintained at 220 °C for 25 min, with the injector at 250 °C. FAME identification was accomplished through comparison of retention times with those of Sigma standards. Quantitative data were obtained with Varian software using C21:0 FA (Sigma) as internal standard.

2.3. Meta-analysis

2.3.1. Database compilation

The FA profile of 27 hexacoral species (order Scleractinia) and 39 octocoral species (orders Alcyonacea, Gorgonacea and Pennatulacea) was compiled by means of a comprehensive search of primary literature (Latyshev et al., 1991; Pernet and Anctil, 2002; Imbs et al., 2007c; Imbs et al., 2009; Baptista et al., 2012; Imbs and Latyshev, 2012) (see Table 1). The taxonomic classification was performed following the World Register of Marine Species - WoRMS. Given that most authors only provide information on FAs

representing 0.2 % or more of total FA content, FA percent data below 0.2 % were not considered in the present study.

2.4. Statistical analysis

Principal component analysis (PCA) of FA profiles has been successfully applied to study the chemotaxonomy of hexacorals (Latyshev et al., 1991; Imbs et al., 2007a; Imbs et al., 2010b) and octocorals (Imbs et al., 2009; Imbs and Latyshev, 2012; Imbs, 2014). Moreover, it has also been shown that the use of a few selected PUFAs is more suitable for the determination of chemotaxonomic differences between corals than total FA matrix (Imbs et al., 2007a). Consequently, in the present study, PCA was applied in a FA matrix of ten PUFAs, namely, the five major *n*-3 series FAs (18:4*n*-3; 20:5*n*-3; 22:5*n*-3; 22:6*n*-3; 24:6*n*-3) and the five major *n*-6 series FAs (18:2*n*-6; 20:4*n*-6; 22:4*n*-6; 22:5*n*-6; 24:5*n*-6).

Additionally, differences in FA profile among coral groups were tested with analysis of variance (ANOVA) followed by multiple comparisons tests (Unequal N HSD). All statistical analyses were tested at 0.05 level of probability with the software STATISTICATM 12 (Statsoft, Inc., Tulsa, 167 OK 74104, USA).

3. RESULTS

A detailed overview of the coral species used in the present dissertation is provided in Table 1. The percentual content of the selected PUFAs for all species (present study and literature) is shown in Table 2.

Table 2: Fatty acid composition of selected PUFAs (% of total fatty acids, values greater than 0.2 % are shown)

Fatty Acids	*Alcyonium digitatum*	*Cespitularia* sp.	*Chironephthya variabilis*	*Cladiella laciniosa*	*Dendronephthya aurea* I
18:2*n*-6	0.20	1.90	1.37	2.30	0.90
18:4*n*-3	0.33	7.40	-	7.40	1.10
20:4*n*-6	0.85	15.80	40.43	12.90	29.40
20:5*n*-3	0.53	4.40	1.90	3.60	3.30
22:4*n*-6	-	-	0.70	0.20	0.00
22:5*n*-6	-	-	0.70	0.90	1.90
22:5*n*-3	0.76	-	-	-	0.50
22:6*n*-3	0.32	6.10	1.37	6.00	3.90
24:5*n*-6	-	5.40	12.33	4.30	11.60
24:6*n*-3	-	0.50	1.30	1.30	4.30
Fatty Acids	***Dendronephthya aurea* II**	***Dendronephthya crystallina* I**	***Dendronephthya crystallina* II**	***Dendronephthya crystallina* III**	***Dendronephthya gigantea***
18:2*n*-6	1.00	1.40	1.30	1.30	1.30
18:4*n*-3	-	1.10	0.30	0.30	0.30
20:4*n*-6	28.90	15.70	27.00	27.00	21.10
20:5*n*-3	1.90	3.30	3.50	3.50	1.50
22:4*n*-6	0.30	0.40	0.30	0.30	0.90
22:5*n*-6	0.40	1.20	0.60	0.60	0.90
22:5*n*-3	-	-	-	-	0.30
22:6*n*-3	0.90	4.10	2.30	1.30	2.10
24:5*n*-6	12.90	9.60	9.40	12.40	12.50
24:6*n*-3	2.30	3.60	2.20	4.70	2.40
Fatty Acids	***Dendronephthya* aff. *involuta***	***Dendronephthya* sp. I**	***Dendronephthya* sp. II**	***Dendronephthya* sp. III**	***Dendronephthya* sp. IV**
18:2*n*-6	-	1.00	1.80	1.80	1.90
18:4*n*-3	-	1.10	0.60	0.60	0.60
20:4*n*-6	25.00	37.70	30.90	23.40	21.30
20:5*n*-3	1.80	3.80	1.30	1.60	1.60
22:4*n*-6	-	0.80	-	-	-
22:5*n*-6	1.100	1.80	2.30	3.20	1.90
22:5*n*-3	1.30	-	0.90	0.70	0.70
22:6*n*-3	4.10	2.20	3.00	2.50	7.20
24:5*n*-6	15.00	16.50	12.30	15.20	5.00
24:6*n*-3	4.60	5.10	4.00	7.10	0.60

Fatty Acids	*Litophyton* sp.	*Lobophytum* cf. *delectum*	*Lobophytum pusillum*	*Neospongodes atlantica*	*Paralemnalia thyrsoides*
18:2*n*-6	-	1.60	0.50	0.66	1.55
18:4*n*-3	5.70	0.60	4.10	9.54	2.85
20:4*n*-6	15.00	30.40	18.40	12.39	25.55
20:5*n*-3	6.70	0.20	1.30	3.66	2.75
22:4*n*-6	-	-	-	-	0.15
22:5*n*-6	-	0.10	0.20	0.47	-
22:5*n*-3	-	0.40	-	-	-
22:6*n*-3	0.20	2.50	6.63	2.45	5.10
24:5*n*-6	14.90	7.20	-	7.40	8.80
24:6*n*-3	0.40	0.90	-	0.80	0.50

Fatty Acids	*Sarcophyton acutum*	*Sarcophyton buitendijki* I	*Sarcophyton buitendijki* II	*Sarcophyton cinereum*	*Sarcophyton* aff. *crassum*
18:2*n*-6	0.50	-	0.30	-	0.30
18:4*n*-3	8.90	4.70	8.10	4.80	2.40
20:4*n*-6	21.10	16.30	24.80	12.60	15.10
20:5*n*-3	5.00	2.20	5.20	1.50	1.00
22:4*n*-6	-	-	-	-	-
22:5*n*-6	0.20	-	-	-	-
22:5*n*-3	0.30	0.20	-	-	-
22:6*n*-3	3.10	5.50	2.50	1.30	1.50
24:5*n*-6	4.40	8.40	4.20	4.60	4.80
24:6*n*-3	0.90	0.80	0.60	0.50	0.40

Fatty Acids	*Sarcophyton elegans*	*Sarcophyton trocheliophorum*	*Sinularia cruciata*	*Sinularia* aff. *deformis*	*Sinularia densa*
18:2*n*-6	1.30	0.30	0.20	0.20	0.60
18:4*n*-3	3.70	6.70	6.20	5.60	4.80
20:4*n*-6	15.20	17.90	23.80	19.10	10.20
20:5*n*-3	6.20	2.20	2.20	2.40	0.80
22:4*n*-6	-	0.20	-	-	-
22:5*n*-6	-	-	-	-	-
22:5*n*-3	-	-	-	-	-
22:6*n*-3	3.80	3.90	2.90	1.90	3.00
24:5*n*-6	5.60	8.40	5.80	5.60	5.30
24:6*n*-3	0.80	1.20	1.80	1.20	1.10

Fatty Acids	*Sinularia leptoclados*	*Sinularia lochmodes*	*Sinularia* cf. *muralis*	*Sinularia notanda*	*Ellisella plexauroides*
18:2*n*-6	-	-	-	-	0.90
18:4*n*-3	1.10	4.40	4.50	5.20	-
20:4*n*-6	23.20	21.20	18.10	19.10	39.30
20:5*n*-3	1.00	2.30	1.50	2.20	1.97
22:4*n*-6	-	-	-	-	8.97
22:5*n*-6	-	-	-	-	0.90
22:5*n*-3	-	-	-	-	0.43
22:6*n*-3	2.50	2.70	3.40	3.10	2.90
24:5*n*-6	8.90	6.00	6.30	7.30	3.10
24:6*n*-3	0.90	0.90	1.20	1.20	1.40

Fatty Acids	*Acanthogorgia armata* I	*Acanthogorgia armata* II	*Acanthogorgia isoxya*	*Bebryce studeri*	*Echinogorgia* sp.
18:2*n*-6	0.51	0.70	1.03	0.70	0.70
18:4*n*-3	0.19	0.38	-	0.30	-
20:4*n*-6	-	10.54	38.77	21.70	47.60
20:5*n*-3	1.30	1.48	3.27	2.00	2.20
22:4*n*-6	-	0.64	3.83	1.10	0.40
22:5*n*-6	0.49	0.19	1.13	4.20	0.20
22:5*n*-3	-	0.71	-	0.50	-
22:6*n*-3	1.77	1.24	2.53	3.50	0.80
24:5*n*-6	3.21	1.10	7.50	7.20	8.90
24:6*n*-3	1.17	0.39	2.40	0.50	2.60

Fatty Acids	*Eunicea sp.* I	*Eunicea sp.* II	*Eunicea sp.* III	*Eunicea sp.* IV	*Eunicella verrucosa* I
18:2*n*-6	0.62	1.27	1.42	0.81	0.90
18:4*n*-3	1.76	0.74	0.69	0.60	1.11
20:4*n*-6	7.83	10.25	9.97	19.37	22.94
20:5*n*-3	1.75	1.67	2.24	2.66	3.02
22:4*n*-6	1.37	0.90	1.07	1.69	2.06
22:5*n*-6	0.29	0.31	0.24	0.57	0.79
22:5*n*-3	0.86	0.58	0.58	0.63	0.63
22:6*n*-3	3.66	3.86	3.97	1.29	2.32
24:5*n*-6	1.71	3.16	2.89	8.68	12.92
24:6*n*-3	0.44	2.21	2.15	1.74	2.92

Fatty Acids	*Eunicella verrucosa* II	*Gorgonia sp.* I	*Gorgonia sp.* II	*Leptogorgia sarmentosa* I	*Leptogorgia sarmentosa* II
18:2*n*-6	0.92	2.06	2.94	0.75	0.82
18:4*n*-3	1.01	3.94	2.17	0.67	0.54
20:4*n*-6	21.49	12.38	-	18.67	17.19
20:5*n*-3	3.47	2.74	2.92	3.13	1.75
22:4*n*-6	2.03	0.29	-	2.76	2.81
22:5*n*-6	0.75	0.24	-	0.90	1.16
22:5*n*-3	0.66	0.22	0.21	0.49	0.50
22:6*n*-3	2.38	2.98	9.83	2.07	1.82
24:5*n*-6	11.38	4.66	3.10	9.53	11.46
24:6*n*-3	2.91	0.80	1.74	3.17	2.22

Fatty Acids	*Leptogorgia sarmentosa* III	*Menella praelonga*	*Muricea sp.*	*Unidentified*	*Paramuricea biscaya*
18:2*n*-6	0.70	0.83	0.49	0.76	1.78
18:4*n*-3	0.70	0.27	2.28	2.64	1.12
20:4*n*-6	19.61	39.70	10.42	6.27	7.21
20:5*n*-3	2.68	3.67	2.80	1.19	1.76
22:4*n*-6	3.48	3.93	0.43	0.23	-
22:5*n*-6	1.15	1.27	0.60	0.45	0.55
22:5*n*-3	0.86	0.35	0.47	-	-
22:6*n*-3	2.50	2.60	18.42	11.77	0.98
24:5*n*-6	12.01	9.13	4.23	3.55	3.65
24:6*n*-3	3.21	2.93	3.69	2.77	0.65

Fatty Acids	*c.f. Placogorgia sp.*	*Plexaurella sp.*	*Pseudoplexaura sp.* I	*Pseudoplexaura sp.* II	*Pseudopterogorgia sp.*
18:2*n*-6	0.33	6.71	0.87	1.27	3.61
18:4*n*-3	-	2.08	2.85	1.52	1.03
20:4*n*-6	21.10	8.23	11.85	10.69	11.77
20:5*n*-3	4.71	3.66	4.21	4.14	5.13
22:4*n*-6	-	0.75	0.77	1.06	1.39
22:5*n*-6	-	-	0.29	0.32	0.64
22:5*n*-3	-	0.81	0.82	0.88	2.03
22:6*n*-3	0.43	4.31	8.07	7.08	8.38
24:5*n*-6	7.46	1.78	2.42	2.23	2.94
24:6*n*-3	0.36	0.66	1.39	0.79	2.13

Fatty Acids	*Rumphella aggregata*	*Acabaria erythraea*	Carijoa riisei	*Cavernularia obesa*	*Pteroeides spp.*
18:2*n*-6	0.65	0.90	0.83	2.58	0.79
18:4*n*-3	2.15	0.40	0.22	2.92	2.60
20:4*n*-6	13.15	37.20	24.18	3.71	14.52
20:5*n*-3	2.05	1.70	2.98	10.16	9.21
22:4*n*-6	0.65	0.60	2.82	1.22	5.61
22:5*n*-6	-	5.70	0.65	-	0.24
22:5*n*-3	-	0.30	0.24	0.36	0.65
22:6*n*-3	1.60	3.90	2.83	2.54	0.66
24:5*n*-6	3.40	14.50	-	-	-
24:6*n*-3	0.20	2.30	-	-	-

Fatty Acids	*Veretillum cynomorium* I	*Veretillum cynomorium* II	*Veretillum cynomorium* III	*Veretillum cynomorium* IV	*Veretillum cynomorium* V
18:2*n*-6	0.58	0.70	0.79	0.64	0.79
18:4*n*-3	0.39	0.53	0.86	1.18	0.64
20:4*n*-6	8.88	7.16	7.36	10.01	7.74
20:5*n*-3	9.58	8.99	12.42	11.42	9.76
22:4*n*-6	3.10	2.52	2.76	3.88	3.23
22:5*n*-6	0.12	0.22	0.30	0.25	0.29
22:5*n*-3	0.88	1.01	1.18	0.87	0.95
22:6*n*-3	0.79	0.98	2.23	1.88	1.25
24:5*n*-6	0.93	0.80	0.81	1.00	1.03
24:6*n*-3	7.72	8.00	11.54	10.31	11.10

Fatty Acids	*Veretillum cynomorium* VI	*Renilla koellikeri*	*Acropora cerealis*	*Acropora florida*	*Acropora formosa*
18:2*n*-6	0.87	0.80	0.90	1.30	1.00
18:4*n*-3	0.77	-	1.40	5.10	1.80
20:4*n*-6	6.22	36.65	6.70	11.00	14.70
20:5*n*-3	9.56	11.35	16.50	6.90	9.50
22:4*n*-6	2.78	3.75	5.50	6.30	7.20
22:5*n*-6	0.28	-	-	1.30	-
22:5*n*-3	1.12	1.45	3.90	1.20	3.10
22:6*n*-3	0.90	2.85	6.30	6.70	6.20
24:5*n*-6	1.02	-	-	-	-
24:6*n*-3	11.16	-	-	-	-

Fatty Acids	*Acropora gemmifera*	*Acropora milepora* I	*Acropora milepora* II	*Acropora nasuta* I	*Acropora nasuta* II
18:2*n*-6	1.20	1.70	1.10	2.10	0.70
18:4*n*-3	1.20	1.10	6.60	2.60	4.90
20:4*n*-6	10.40	8.00	7.20	7.10	3.20
20:5*n*-3	10.30	1.60	10.40	0.80	4.50
22:4*n*-6	4.10	1.00	6.00	4.30	2.40
22:5*n*-6	-	-	0.60	-	0.30
22:5*n*-3	2.60	0.50	3.00	0.90	1.30
22:6*n*-3	4.90	10.40	12.60	10.80	8.80
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Acropora nobilis*	*Acropora palifera*	*Acropora* sp.	*Agaricia sp.* I	*Agaricia sp.* II
18:2*n*-6	1.60	0.60	1.40	1.61	1.91
18:4*n*-3	2.70	1.00	1.60	0.82	1.33
20:4*n*-6	2.30	1.80	2.00	2.93	2.22
20:5*n*-3	3.00	9.90	1.70	2.13	1.96
22:4*n*-6	1.30	2.60	1.10	1.65	1.14
22:5*n*-6	-	-	-	-	-
22:5*n*-3	1.20	1.70	0.70	1.14	0.99
22:6*n*-3	4.20	3.30	4.10	7.90	8.26
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Caulastraea tumida*	*Diploria sp.*	*Diploria strigosa*	*Echinophyllia orpheensis*	*Favia* sp. I
18:2*n*-6	0.80	1.20	1.67	1.00	1.60
18:4*n*-3	0.80	0.78	1.09	1.10	1.20
20:4*n*-6	4.90	4.55	7.53	3.40	4.60
20:5*n*-3	2.90	1.19	1.97	1.70	0.80
22:4*n*-6	-	1.01	1.65	1.70	2.10
22:5*n*-6	-	-	-	-	-
22:5*n*-3	1.90	0.48	0.76	2.30	6.70
22:6*n*-3	10.10	5.99	4.58	9.20	3.60
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Favia* sp. II	*Goniopora* sp. I	*Goniopora* sp. II	*Montastraea annularis*	*Montastraea sp.*
18:2*n*-6	3.00	2.20	1.60	1.58	1.37
18:4*n*-3	1.10	2.30	4.30	0.88	2.17
20:4*n*-6	3.70	13.30	21.90	3.75	3.11
20:5*n*-3	1.00	4.10	4.60	1.72	2.40
22:4*n*-6	2.00	3.30	6.00	1.27	1.25
22:5*n*-6	-	-	-	-	-
22:5*n*-3	6.20	1.00	0.80	0.71	0.46
22:6*n*-3	2.90	15.70	11.50	6.10	5.18
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Oculina sp.*	*Pocillopora damicornis* I	*Pocillopora damicornis* II	*Pocillopora damicornis* III	*Pocillopora verrucosa*
18:2*n*-6	1.18	1.30	1.70	1.80	1.20
18:4*n*-3	4.09	2.10	2.20	0.80	3.30
20:4*n*-6	5.37	3.90	2.10	2.00	1.80
20:5*n*-3	4.54	3.00	1.80	1.40	3.20
22:4*n*-6	4.67	2.60	1.30	0.90	1.30
22:5*n*-6	2.58	-	-	-	-
22:5*n*-3	5.21	0.70	0.60	0.40	0.70
22:6*n*-3	2.53	12.30	14.00	9.50	10.40
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Porites cylindrica*	*Porites lobata*	*Porites lutea*	*Porites nigrescens*	*Porites porites*
18:2*n*-6	1.10	1.50	1.00	0.60	2.71
18:4*n*-3	1.70	0.60	2.90	1.60	1.33
20:4*n*-6	6.10	7.00	2.30	3.20	4.58
20:5*n*-3	4.10	2.00	3.30	4.80	3.14
22:4*n*-6	3.10	4.20	1.40	3.20	1.94
22:5*n*-6	-	-	-	-	-
22:5*n*-3	1.30	2.10	0.80	1.50	1.68
22:6*n*-3	8.70	5.50	5.30	11.60	5.23
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Porites sp.*	*Sandalolitha robusta*	*Scolymia cubensis*	*Scolymia sp.*	*Scolymia wellsi*
18:2*n*-6	0.92	1.40	2.58	0.58	2.57
18:4*n*-3	1.54	0.10	1.22	0.30	1.67
20:4*n*-6	4.08	4.20	7.73	3.50	8.72
20:5*n*-3	3.71	1.60	3.30	1.05	2.97
22:4*n*-6	2.15	1.40	2.87	-	3.26
22:5*n*-6	-	-	-	0.34	-
22:5*n*-3	1.14	0.70	1.42	4.81	1.54
22:6*n*-3	8.74	2.60	7.28	-	6.42
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Seriatopora caliendrum*	*Seriatopora hystrix*	*Stylophora pistillata* I	*Stylophora pistillata* II	*Stylophora pistillata* III
18:2*n*-6	1.70	0.50	1.70	0.80	0.60
18:4*n*-3	1.70	1.20	1.30	1.90	1.80
20:4*n*-6	4.80	3.50	4.30	7.60	5.10
20:5*n*-3	2.60	1.80	2.00	7.70	1.80
22:4*n*-6	1.50	1.20	1.80	3.80	1.90
22:5*n*-6	-	-	-	-	-
22:5*n*-3	1.20	1.10	1.30	4.50	1.10
22:6*n*-3	16.90	13.30	16.40	14.40	13.20
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Stylophora pistillata* IV	*Stylophora pistillata* V	*Tubastraea coccinea* I	*Tubastraea coccinea* II	*Tubastraea micrantha*
18:2*n*-6	1.90	1.70	0.33	2.00	1.80
18:4*n*-3	1.40	2.00	3.68	0.70	0.80
20:4*n*-6	1.70	3.10	7.16	7.80	6.60
20:5*n*-3	1.40	2.60	7.40	14.90	10.90
22:4*n*-6	1.00	1.70	10.21	4.70	5.50
22:5*n*-6	-	-	-	-	-
22:5*n*-3	4.50	0.70	13.40	16.40	17.30
22:6*n*-3	8.80	10.10	1.05	1.40	1.30
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Epizoanthus gabrieli* I	*Epizoanthus gabrieli* II	*Epizoanthus gabrieli* III	*Palythoa caribaeorum* I	*Palythoa caribaeorum* II
18:2*n*-6	0.46	-	-	0.84	1.14
18:4*n*-3	2.11	-	-	2.45	1.67
20:4*n*-6	3.78	0.59	0.51	8.31	10.72
20:5*n*-3	2.65	-	-	2.27	1.98
22:4*n*-6	1.96	-	-	3.34	3.59
22:5*n*-6	-	2.76	3.04	-	-
22:5*n*-3	9.84	-	-	5.92	4.06
22:6*n*-3	2.50	8.20	7.63	2.04	2.19
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

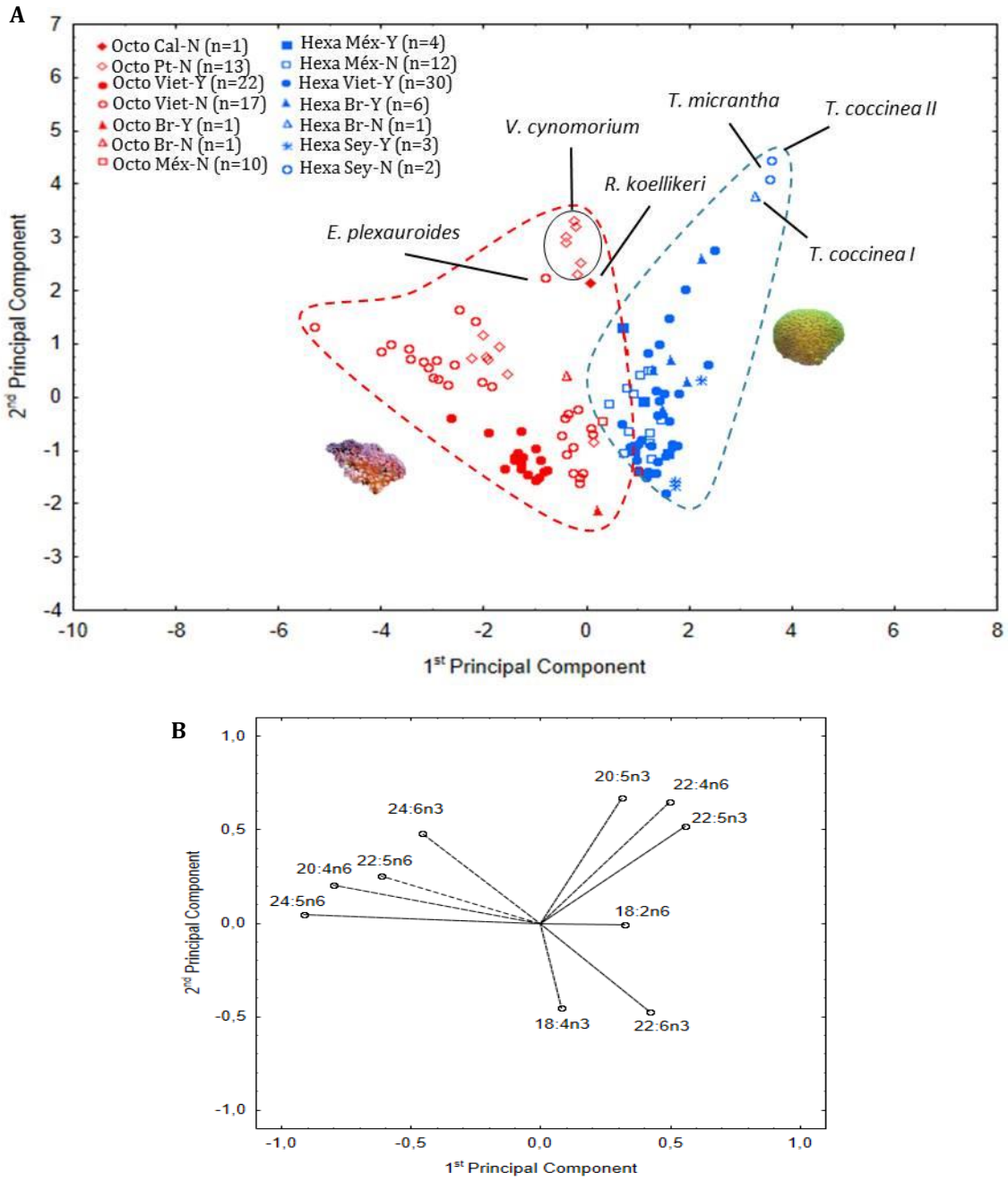
Fatty Acids	*Palythoa caribaeorum* III	*Palythoa sp.*	*Protopalythoa variabilis*	*Zoanthus sociatus* I	*Zoanthus sociatus* II
18:2*n*-6	1.56	0.53	0.33	1.03	2.09
18:4*n*-3	1.39	2.22	2.03	2.59	7.52
20:4*n*-6	9.23	7.51	5.40	5.21	1.68
20:5*n*-3	1.76	1.85	1.75	0.60	3.46
22:4*n*-6	2.81	4.07	2.91	3.40	1.04
22:5*n*-6	-	-	-	-	-
22:5*n*-3	2.73	4.95	6.99	3.72	4.08
22:6*n*-3	1.93	2.10	1.85	1.61	2.97
24:5*n*-6	-	-	-	-	-
24:6*n*-3	-	-	-	-	-

Fatty Acids	*Zoanthus sp.* I	*Zoanthus sp.* II
18:2*n*-6	1.69	3.78
18:4*n*-3	0.54	-
20:4*n*-6	8.08	3.46
20:5*n*-3	5.89	0.24
22:4*n*-6	7.34	1.88
22:5*n*-6	-	-
22:5*n*-3	6.98	7.10
22:6*n*-3	-	1.70
24:5*n*-6	-	-
24:6*n*-3	-	-

Values are means.

3.1. General differences between hexa- and octocorals

The results of the PCA (obtained with the 10 selected PUFAs) for the 45 hexa- and 59 octocoral species from different world regions (namely Brazil, USA, México, Portugal, Seychelles and Vietnam) are shown in Figure 8.



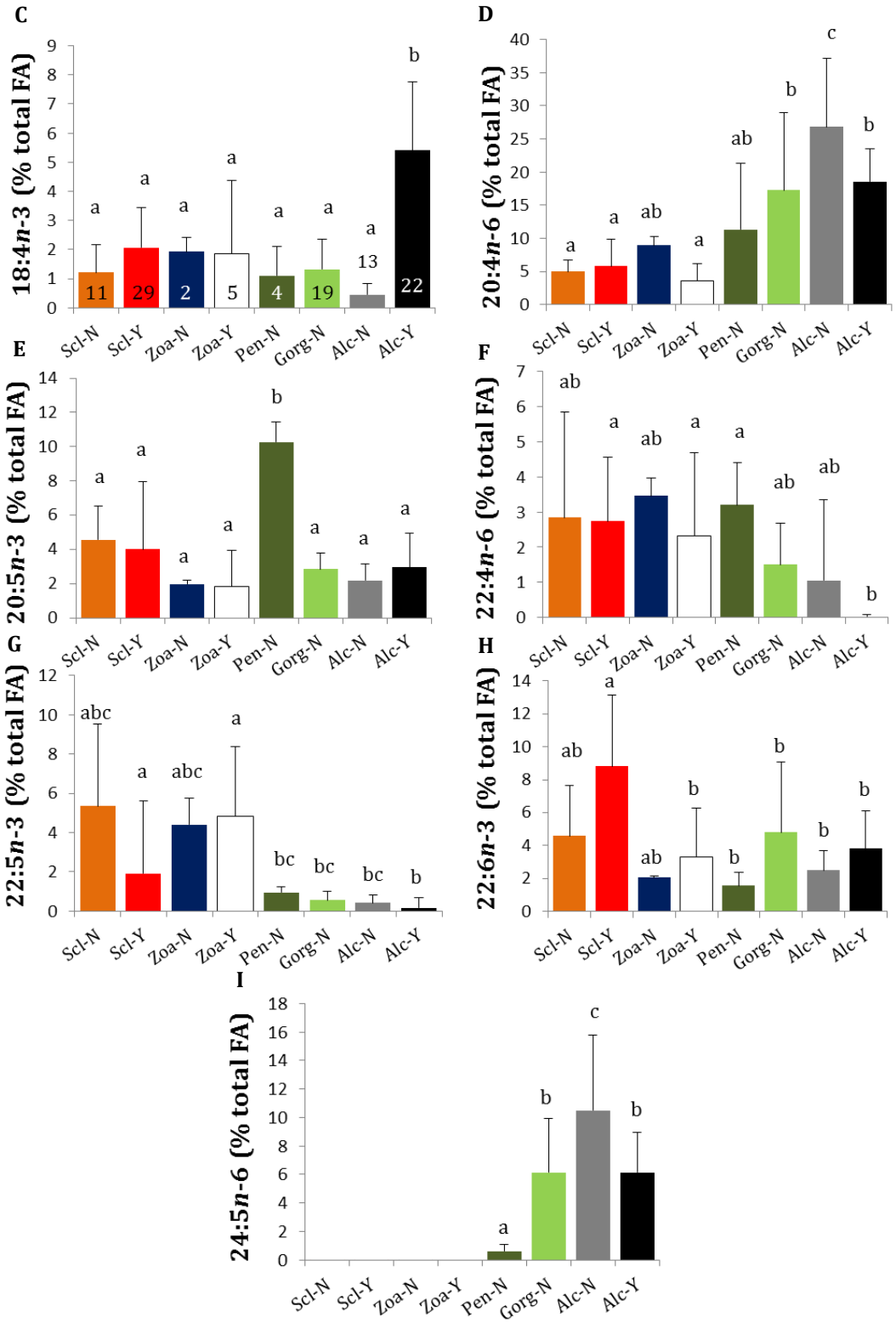


Figure 8: Principal component analysis based on the content of 10 PUFAs (18:2n-6, 18:4n-3, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3, 24:5n-6, 24:6n-3) of 104 hexacoral and octocoral species (123 specimens). A) Principal component plot; B) Loading plot of FAs and their contribution to the spread along PC1 and PC2; C) D) E) F) G) H) and I) Percentual content of different FAs in eight coral groups. Values are means (±SD). Different superscript letters represent significant differences between groups (P<0.05). Numbers on the bars of the plot for

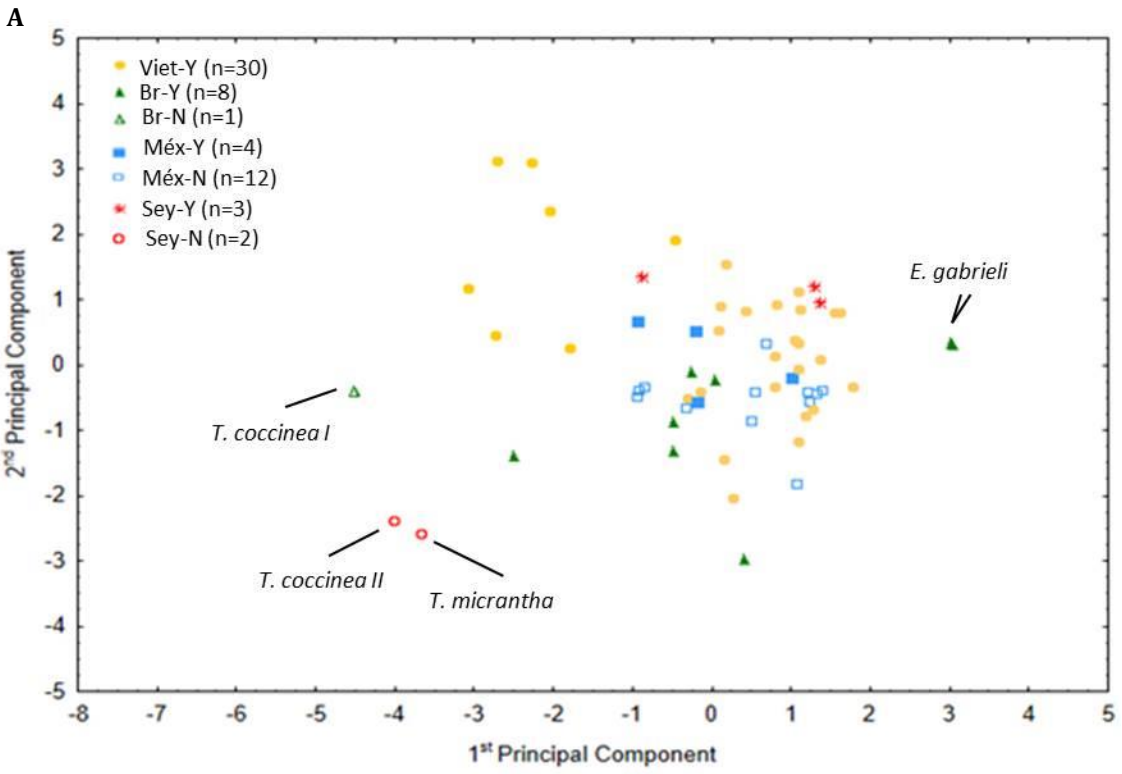
18:4n-3 represent number of species in each coral group. Legend of panel A: Octo Cal-N – azooxanthellae octocorals from California; Octo Pt-N – azooxanthellae octocorals from continental Portugal; Octo Viet-Y – zooxanthellae octocorals from Vietnam; Octo Viet-N – azooxanthellae octocorals from Vietnam; Octo Br-Y – zooxanthellae octocorals from Brazil; Octo Br-N – azooxanthellae octocorals from Brazil; Octo Mex-N – azooxanthellae octocorals from Mexico; Hexa Mex-Y – zooxanthellae hexacorals from Mexico; Hexa Mex-N – azooxanthellae hexacorals from Mexico; Hexa Viet-Y – zooxanthellae hexacorals from Vietnam; Hexa Br-Y – zooxanthellae hexacorals from Brazil; Hexa Br-N – azooxanthellae hexacorals from Brazil; Hexa Sey-Y – zooxanthellae hexacorals from Seychelles; Hexa Sey-N – azooxanthellae hexacorals from Seychelles; panel C, D, E, F, G, H and I: Scl-N – azooxanthellae scleractinians; Scl-Y – zooxanthellae scleractinians; Zoa-N – azooxanthellae zoanthidians; Zoa-Y – zooxanthellae zoanthidians; Pen-N – azooxanthellae pennatulaceans; Gorg-N – azooxanthellae gorgonians; Alc-N – azooxanthellae alcyonaceans; Alc – Y – zooxanthellae alcyonaceans.

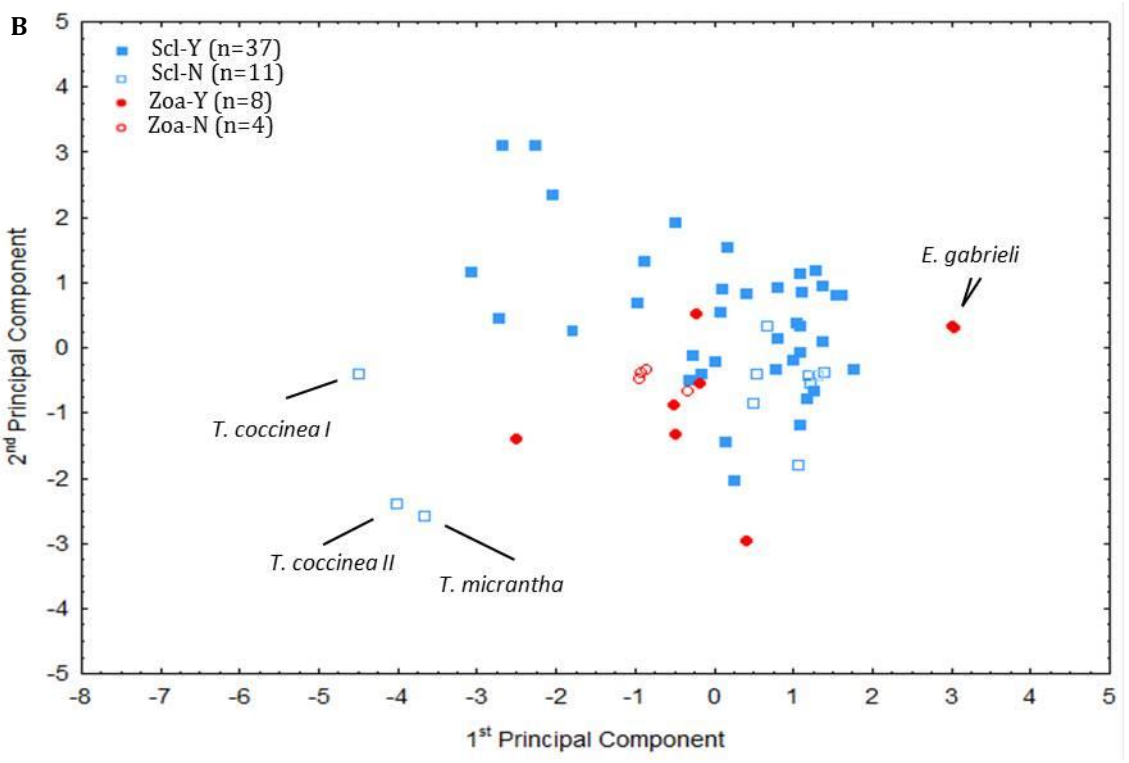
A clear separation was achieved between hexa- and octocorals along PC1 (explaining 30.04 % of the variance), with the former group to the right and the latter to the left. This separation was mainly caused by 20:4*n*-6 and 24:5*n*-6 (Fig. 8B). In terms of 20:4*n*-6, the separation occurred because octocorals generally exhibited a higher content of this PUFA. Yet, such differences were not always observed among members of the two coral groups (Fig. 8D). Regarding 24:5*n*-6, it is worth noting that this FA was only present in octocorals (Fig. 8I). The separation within each group was mainly achieved along PC2 (explaining 19,11 % of the variance). Among the hexacoral group, species belonging to the *Tubastraea* genera (from Seychelles and Brazil) were placed in an upper position relative to the other species. A similar result was observed in octocorals, where *Veretillum cynomorium*, *Ellisella plexauroides* and *Renilla koellikeri* were also placed in an upper position. Such differences were mainly driven by 20:5*n*-3 (Fig. 8E), 22:4*n*-6 (Fig. 8F) and 22:5*n*-3 (Fig. 8G). These PUFA were found in higher percentage in the above mentioned species. On the other hand,

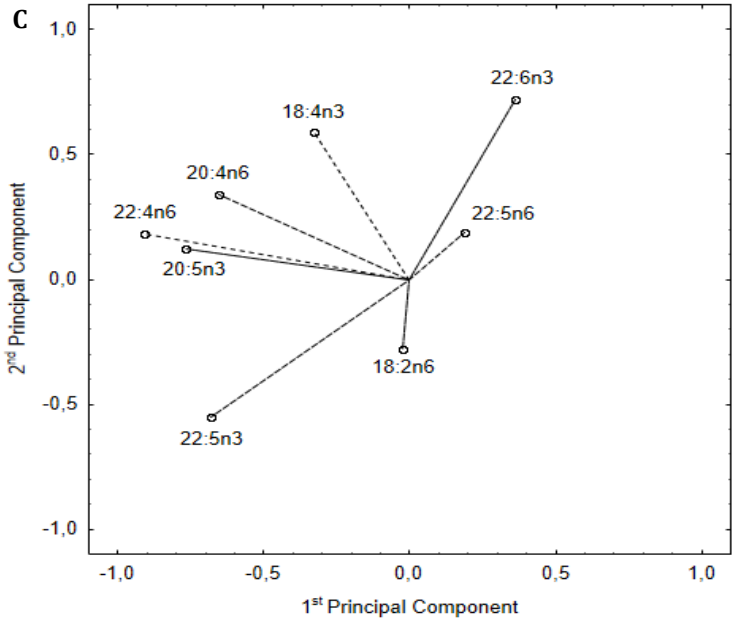
18:4*n*-3 (Fig. 8C) and 22:6*n*-3 (Fig. 8H) were mostly found in lower percentages on those species (Fig. 8B; Table 2).

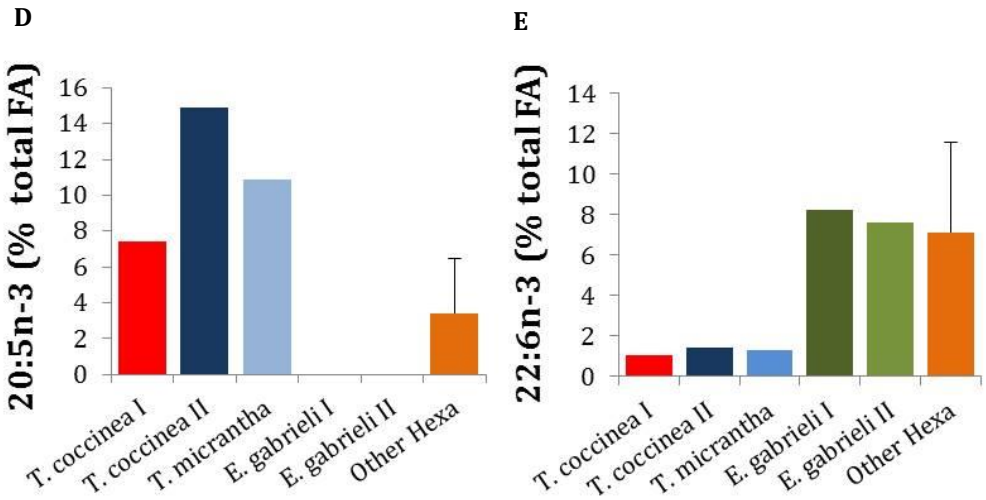
3.2. Differences among hexacorals

The results of the PCA of 44 species of Hexacorals (55 specimens belonging to Zoantharia and Scleractinia orders), from the different world regions (Brazil, México, Seychelles and Vietnam) are shown in Figure 9.




B
Scl-Y (n=37)
Scl-N (n=11)
Zoa-Y (n=8)
Zoa-N (n=4)
E. gabrieli
T. coccinea I
T. coccinea II
T. micrantha
2nd Principal Component
1st Principal Component


C
22:6n3
18:4n3
20:4n6
22:4n6
22:5n6
20:5n3
18:2n6
22:5n3
2nd Principal Component
1st Principal Component


D
20:5n-3 (% total FA)
T. coccinea I
T. coccinea II
T. micrantha
E. gabrieli I
E. gabrieli II
Other Hexa
E
22:6n-3 (% total FA)
T. coccinea I
T. coccinea II
T. micrantha
E. gabrieli I
E. gabrieli II
Other Hexa

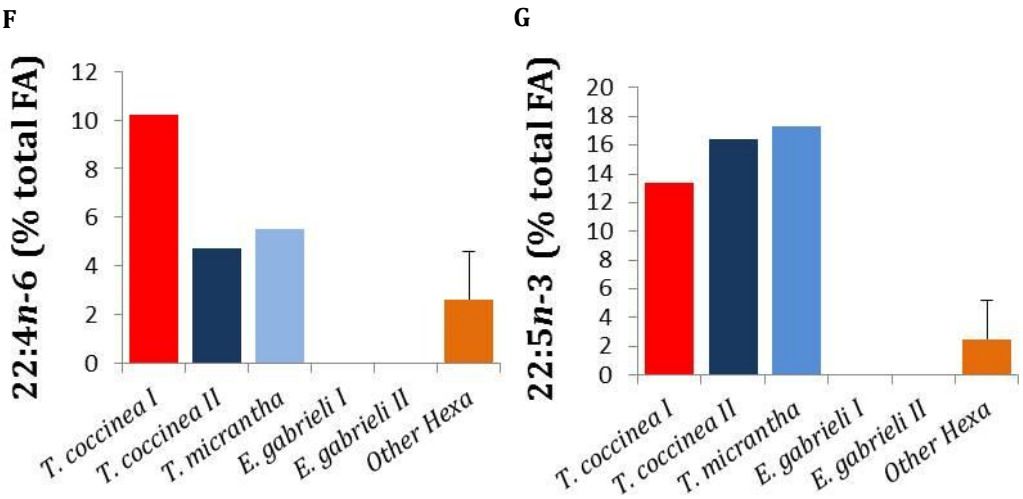


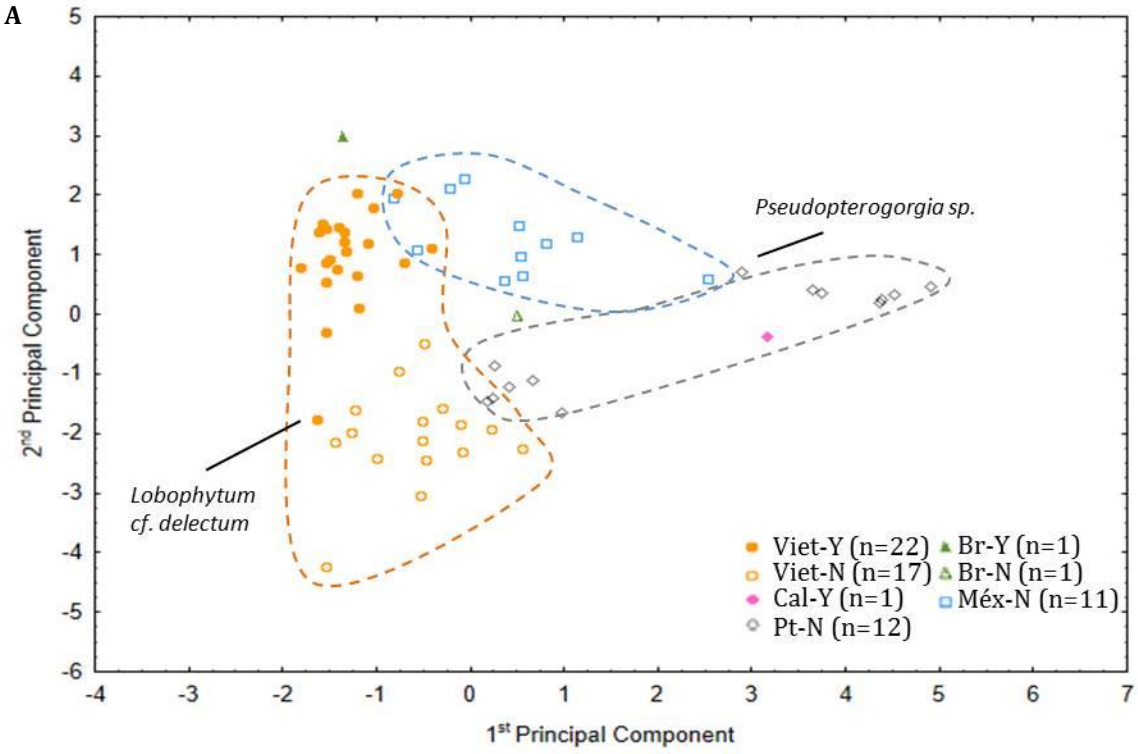
Figure 9: Principal component analysis based on the content of 10 PUFAs (18:2n-6, 18:4n-3, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3, 24:5n-6, 24:6n-3) of 44 hexacoral species (55 specimens). A) Principal component plot; B) Loading plot of FAs and their contribution to the spread along PC1 and PC2; C) D) E) F) and G) Percentual content of different FAs in three species (5 specimens) and other hexacorals. Values are mean (±SD), except in the case of the species (T. coccinea I, T. coccinea II, T. micrantha, E. gabrieli I and E. gabrieli II) where only one value is available. Legend of panel A: Viet-Y – zooxanthellae hexacorals from Vietnam; Br-Y – zooxanthellae hexacorals from Brazil; Br-N – azooxanthellae hexacorals from Brazil; Mex-Y – zooxanthellae hexacorals from Mexico; Mex-N – azooxanthellae hexacorals from Mexico; Sey-Y – zooxanthellae hexacorals from Seychelles; Sey-N – azooxanthellae hexacorals from Seychelles. Panel B: Scl-Y – zooxanthellae scleractininans; Scl-N – azooxanthellae scleractininans; Zoa-Y – zooxanthellae zoantharians; Zoa-N – azooxanthellae zoatharians. Panels D-G: Other Hexa – other hexacorals.

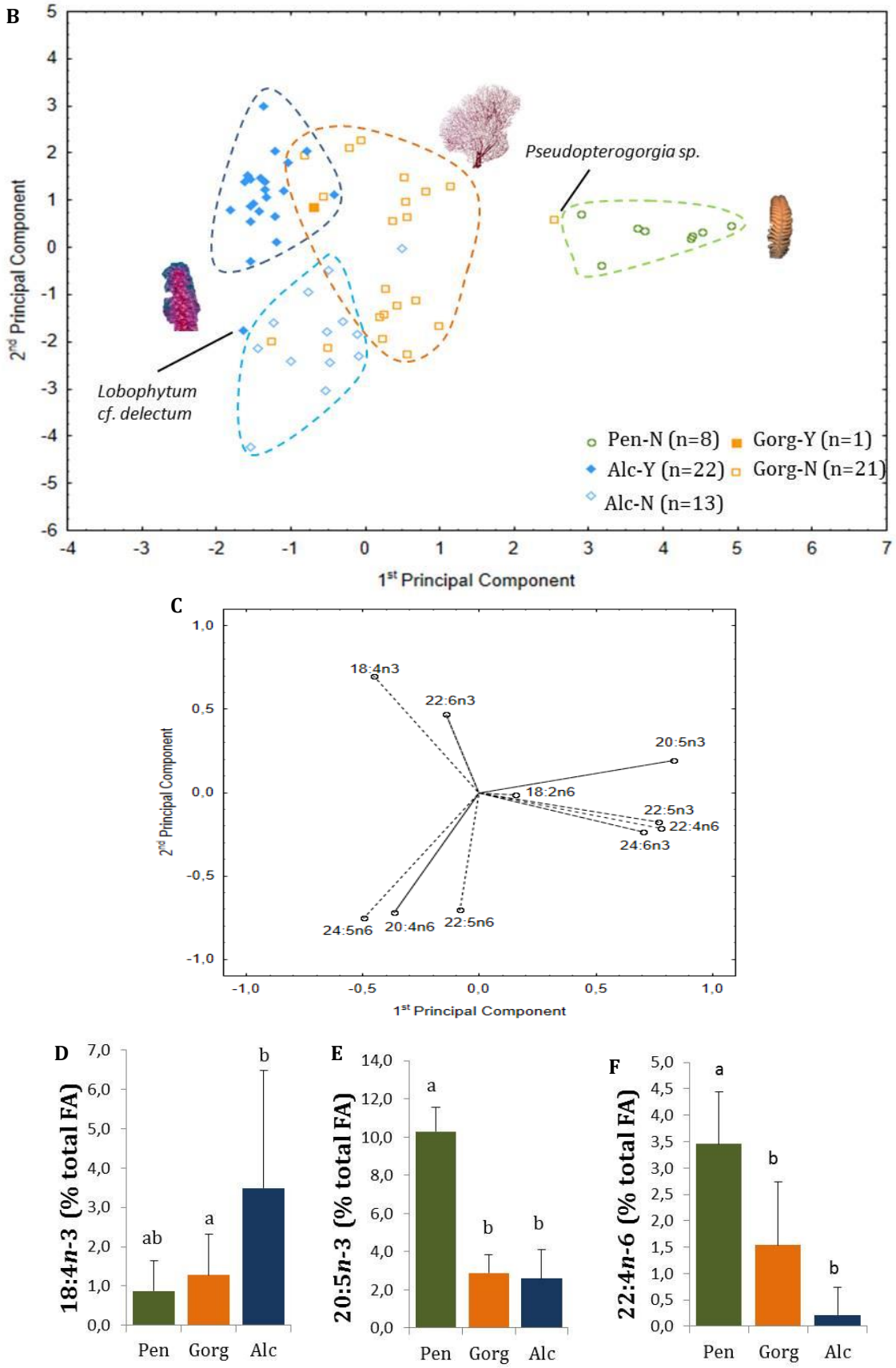
Distinct spatial (Fig. 9A) and taxonomic separations (Fig. 9B) among hexacorals were not observed (even though both axis explained 50.36% of the variance). However, it is noteworthy that *Tubastraea* species (*T. coccinea* and *T. micrantha*) from Seychelles and Brazil (placed upwards) were placed to the leftmost position and two *Epizoanthus gabrieli* specimens, from Brazil, appeared in the rightmost position. The marginal placement of *Tubastraea* species occurred due to the higher contents of 20:5*n*-3, 22:4*n*-6 and 22:5*n*-3, and a lower content of 22:6*n*-3, when compared to most other hexacorals. The placement of *E. gabrieli* to the right, on the other hand, was caused due to a high concentration of

22:6*n*-3, closer to that found in other hexacorals, and the lack of 20:5*n*3, 22:4*n*-6 and 22:5*n*-3.

3.3. Differences among octocorals

The results of the PCA with 58 octocoral species (65 specimens belonging to alcyonaceans, pennatulacenas and gorgonian alcyonaceans) from Brazil, México, Seychelles and Vietnam, are shown in Figure 10. Interestingly, clear separations in respect to sampling region (Fig. 10A), coral group (Fig. 10B) and presence/absence of zooxanthellae were achieved.




B
2nd Principal Component
1st Principal Component
Pseudopterogorgia sp.
Lobophytum
cf. delectum
Pen-N (n=8)
Gorg-Y (n=1)
Alc-Y (n=22)
Gorg-N (n=21)
Alc-N (n=13)
C
2nd Principal Component
1st Principal Component
18:4n3
22:6n3
20:5n3
18:2n6
22:5n3
22:4n6
24:6n3
24:5n6
20:4n6
22:5n6
D
18:4n-3 (% total FA)
ab
a
b
Pen
Gorg
Alc
E
20:5n-3 (% total FA)
a
b
b
Pen
Gorg
Alc
F
22:4n-6 (% total FA)
a
b
b
Pen
Gorg
Alc

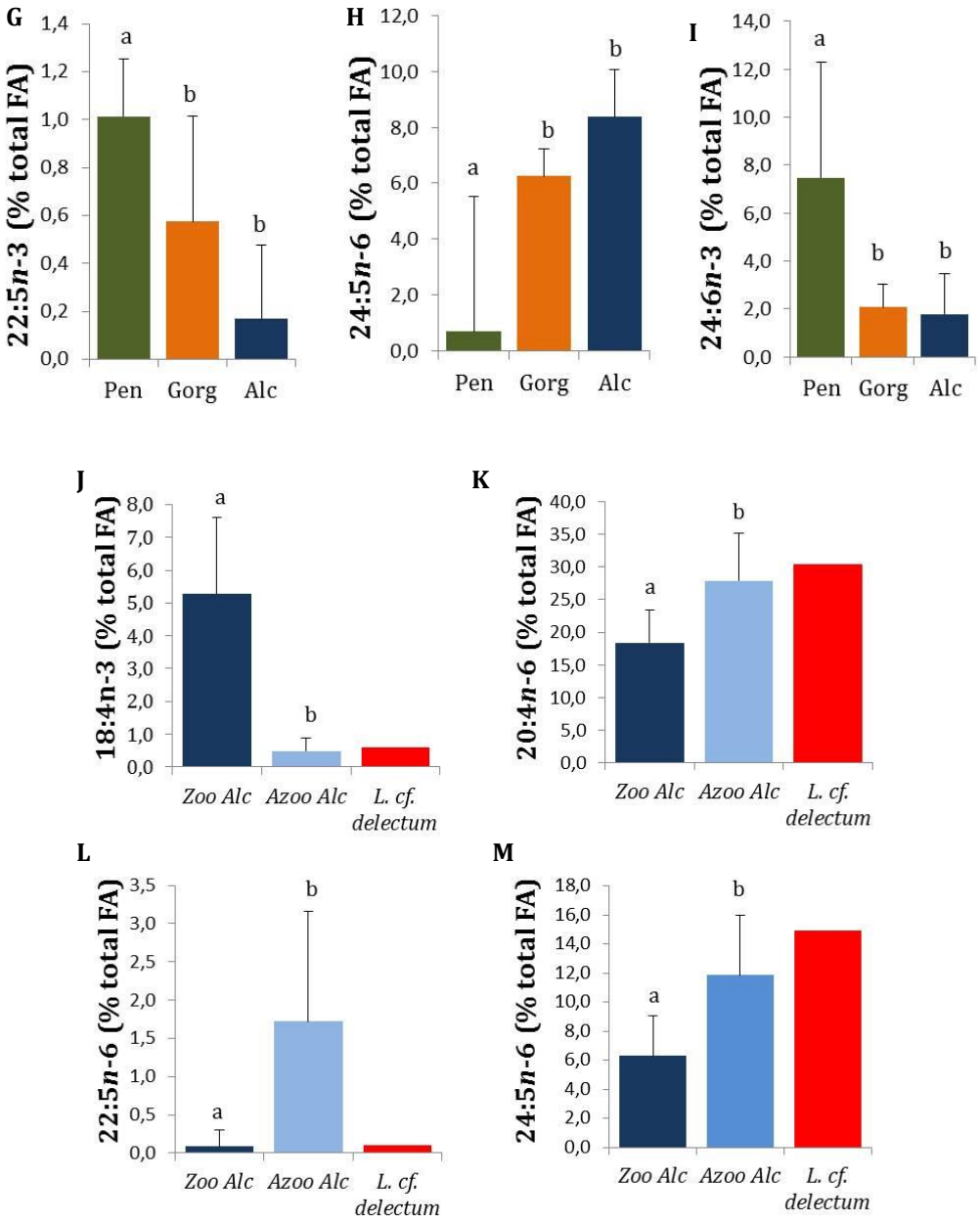


Figure 10: Principal component analysis based on 10 selected PUFAs (18:2n-6, 18:4n-3, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3, 24:5n-6, 24:6n-3) composition of 57 Octocoral species (66 specimens). A) Principal component plot showcasing different locations; B) Principal component plot showcasing different coral groups C) loading plot of fatty acids (FA) and their contribution to the spread along PC1 and PC2; D) E) F) G) H) I) Percentual content of different FAs in tree octocoral orders J) K) L) M) Percentual content of different FAs in alcyonaceans. Values are means (+-SD), except in the case of L cf. delectum where only one value is available Different superscript letters represent significant differences between groups (P<0.05). Legend panel A: Viet-Y – zooxanthellae octorocals from Vietnam; Viet-N – azooxanthellae octocorals from Vietnam; Cal-Y – zooxanthellae

octocoral from California; Pt-N – azooxanthellae octocoral from Portugal; Br-y – zooxanthellae octocorals from Brazil; Br-N – azooxanthellae octocorals from brazil; Mex-N – azooxanthellae from Mexico. Panel B: Pen-N – azooxanthellae pennatulaceans; Alc-y – zooxanthellae alcyonaceans; Alc-N – azooxanthellae alcyonaceans; Gorg-Y – zooxanthellae gorgonians; Gorg-N – azooxanthellae gorgonians. Panels D-I: Pen – pennatulaceans; Gorg – gorgonians; Alc – alcyonaceans. Panels J-M: Zoo Alc – zooxanthellae alcyonaceans; Azoo Alc – azooxanthellae alcyonaceans.

Regarding spatial origin, the octocorals sampled in México, Vietnam and Portugal/California were clearly separated along PC1 (explaining 30.27% of variance) and PC2 (explaining 24.51%). In respect to coral group, a clear individualization of the pennatulacean group occurred to the right. Additionally, while gorgonians were clustered in the central region, the alcyonaceans were placed to the left.

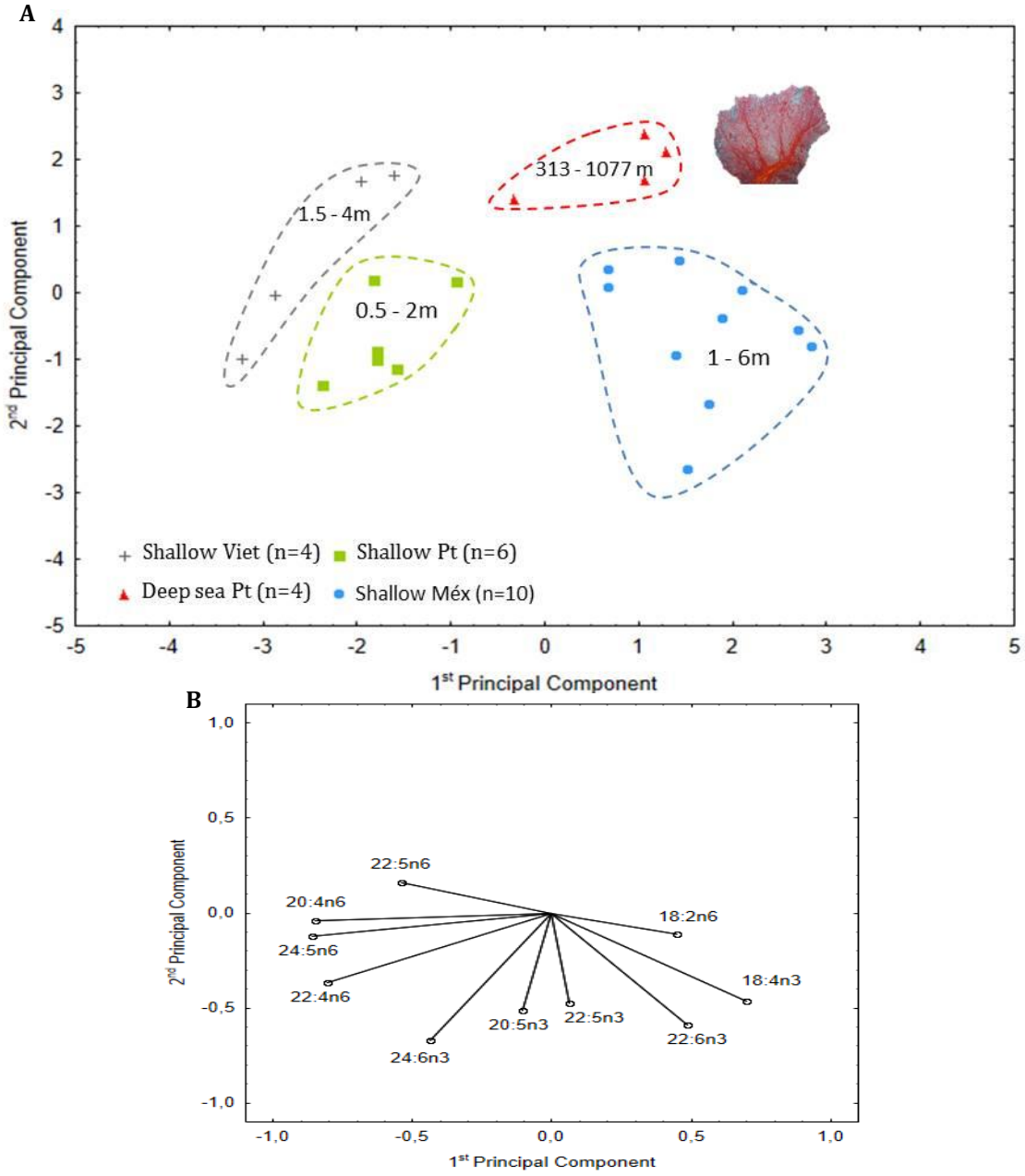
Regarding PC1, pennatulaceans generally displayed higher percentages of 20:5*n*-3 (Fig. 10E), 22:5*n*-3 (Fig. 10G), 22:4*n*-6 (Fig. 10F) and 24:6*n*-3 (Fig. 10I) and lower percentages of 18:4*n*-3, 24:5*n*-6, as opposed to alcyonaceans (Figs. 10D,H). Gorgonians, on the other hand, displayed relatively average levels of the mentioned FAs, hence being placed in a central position between pennatulaceans and alcyonaceans. An azooxanthellate gorgonian, *Pseudopterogorgia sp.,* was placed closer to pennatulaceans as it displayed a lower value of 20:4*n*-6 and higher values of 20:5*n*-3 and 22:5*n*-3, when compared to other gorgonians (i.e. resembling the values showed by pennatulaceans).

A separation between zooxanthellate and azooxanthellate alcyonacens was also achieved along PC2. This occurred because azooxanthellate alcyonaceans normally displayed higher contents of 20:4*n*-6 (Fig. 10K), 22:5*n*-6 (Fig. 10L) and 24:5*n*-6 (Fig. 10M) and a lower content of 18:4*n*-3 (Fig. 10J), in comparison to zooxanthellate alcyonaceans. One exception was *Lobophytum cf. delectum*, a zooxanthellate alcyonacean, which was

placed next to azooxanthellate ones (Fig. 10B). This positioning occurred because *L. cf. delectum* showed higher contents of 20:4*n*-6, 22:5*n*-6, 24:5*n*-6 and a lower content of 18:4*n*-3, similarly to azooxantellate alcyonaceans.

3.4. Differences among shallow-living and deep-sea gorgonians

The results of the PCA with 21 gorgonian alcyonacean species (25 specimens from deep-sea and shallow water habitats) are shown in Figure 11.



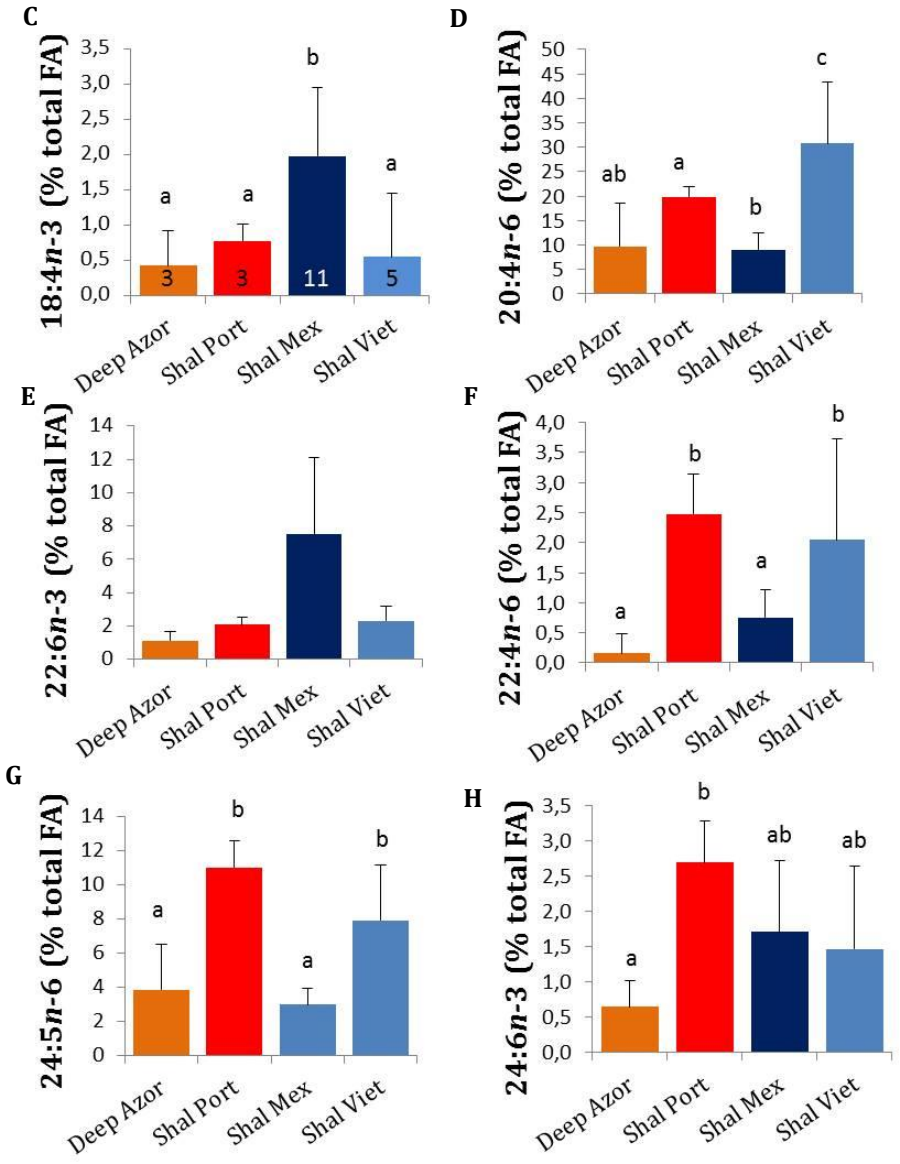


Figure 11: Principal component analysis based on 10 selected PUFAs (18:2n-6, 18:4n-3, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3, 24:5n-6, 24:6n-3) composition of 21 gorgonian species (25 specimens). A) Principal component plot showcasing different sampling depths; B) loading plot of fatty acids (FA) and their contribution to the spread along PC1 and PC2; C) D) E) F) G) H) Percentual content of different FAs in shallow and deep sea gorgonians from different locations. Values are means (±SD). Different superscript letters represent significant differences between shallow and deep sea gorgonians from different locations (P<0.05). Legend panel A: Shallow Viet – shallow-living gorgonians from Vietnam; Deep sea Pt – deep-sea gorgonians from Azores (Portugal); Shallow Pt – shallow-living gorgonians from Portugal; Shallow Mex – shallow-living gorgonians from Mexico; panels C-H: Deep Azor – deep-se gorgonians from Azores; Shal Port – shallow-living gorgonians

from Portugal; Shal Mex – shallow-living gorgonians from Mexico; Shal Viet – shallow living gorgonians from Vietnam.

Along PC1 (explaining 35.35% of variance), a clear separation between sampling regions was achieved. Concomitantly, a clear distinction between habitat depths was observed along the PC2 (explaining 19.04%).

Shallow living gorgonians from Portugal and Vietnam (placed to the left) revealed higher contents of 20:4*n*-6, 22:4*n*-6 and 24:5*n*-6 (Figs. 11 D,E,F) than shallow water gorgonians from México, which showed higher contents of 18:2*n*-6, 18:4*n*-3 and 22:6*n*-3 (Figs. 11 A,C). Also, shallow living gorgonians from Vietnam were separated from the ones from Portugal due to the higher contents of 20:4*n*-6 and 22:5*n*-6.

The separation of deep-sea gorgonians (from Azores archipelago) was mainly caused by 22:6*n*-3 and 24:6*n*-3 (Figs. 11 G,H). Though not statistically significant, these deep-sea octocorals exhibited lower contents of 22:4*n*-6, 22:6*n*-3 and 24:6*n*3 (Figs. 11 E,F,H), in comparison with those from the shallow habitats from Vietnam, Mexico and Portugal (Fig. 11A).

4. DISCUSSION

4.1. Chemotaxonomical differences between hexa- and octocorals

FAs have been used as chemotaxonomic biomarkers since the divergence of FA profiles between corals can be applied for the evaluation of the degree of biochemical variability between different taxonomical groups (Imbs et al., 2007b). As expected, hexacorals were separated from octocorals due to the lack of 24:5*n*-6 and 24:6*n*-3. In fact, these C24 PUFAs are the most useful biomarkers for the separation of these two coral groups (Svetashev and Vysotskii, 1998). Representatives from each group clustered together, with the exception of two hexacorals (*Tubastraea coccinea II* and *Tubastraea micrantha*) and three octocorals (*Veretillum cynomorium*, *Ellisella plexauroides* and *Renilla koellikeri*) which were placed away from other coral species. This separation occurred due to their generally higher contents of 20:5*n*-3, 22:4*n*-6 and 22:5*n*-3 and lower contents of 18:4*n*-3 and 22:6*n*-3. Both *V. cynomorium* (an azooxanthellate octocoral) and *R. koellikeri* (a zooxanthellate octocoral), were collected in coastal habitats located in marine temperate zones (Portugal and California, respectively). These areas, while subjected to upwelling events, often exhibit high levels of primary production and hence *V. cynomorium* and *R. koellikeri* are naturally expected to exhibit higher values of PUFAs deriving from phytoplankton and zooplankton intake, such as 20:5*n*-3 and 20:4*n*-6 respectively (Migne and Davoult, 2002; Palardy et al., 2005), and higher values of 22:5*n*-3 and 22:4*n*-6, that originate from the previously mentioned PUFAs (Sprecher, 2000). Both *T. coccinea II* and *T. micrantha*, on the other hand, while originating from a region of lower primary productivity (name of region), contain zooxanthellae and are therefore also expected to contain a high content of 20:5*n*-3

(Dalsgaard et al., 2003; Imbs et al., 2010a). Finally, E. *plexauroides* is an azooxanthellate octocoral from Vietnam, where monsoons periodically produce eutrophication episodes that result in high primary production and therefore high amounts of phytoplankton, which might also explain the high levels of 20:5*n*-3.

4.2. Hexacoral chemotaxonomy

A clear separation between zooxanthellate and azooxanthellate hexacorals was not achieved with PCA. However, differences between these groups were more noticeable through individual FA analysis. Zooxanthellate hexacorals showed a generally higher content of 18:4*n*-3; 20:5*n*-3 and 22:6*n*-3 when compared to azooxanthellate hexacorals (Table 2). This result is in accordance with the findings of Imbs et al. (2010a) hence corroborating the usefulness of these FAs as biomarkers for the separation of hexacorals in regard to the presence of photosynthetic symbionts. Azooxanthellate hexacorals, on the other hand, showed a higher content of 22:5*n*-3 than zooxanthellate ones (see also Latyshev et al. (1991) and Bishop and Kenrick (1980)).

Five species were placed away from the main cluster of hexacorals: *T. coccinea I*, *T. coccinea II*, *T. micrantha*, *E. gabrieli I* and *E. gabrieli II*. The species *T. coccinea I (from Brazil)* and *T. coccinea II* and *T. micrantha* (from Seychelles) do not contain zooxanthellae and were separated from other hexacorals as a result of exhibiting higher values of 20:5*n*-3; 22:4*n*-6; 22:5*n*-3 and a lower value of 22:6*n*-3. This seems to indicate that phytoplankton is the preferred food source for these species. Still, a significant difference in the contents of 20:5*n*-3 (Fig.3D) and 22:4*n*-6 (Fig. 3F) was observed between *Tubastraea* species from Brazil and Seychelles. Species from Brazil exhibited higher amounts of 22:4*n*-6 (originating

from 20:4*n*-6, a biomarker of zooplankton), while species from Seychelles contained higher values of 20:5*n*-3. An eventual higher availability of zooplankton in Baía de Todos-os-Santos (Brazil) (Paredes et al., 1980) when compared to sampling-site-in-Seychelles may explain these findings.

Epizoanthus gabrielli I and II, zooxanthellate hexacorals, displayed a content of 22:6*n*-3 higher than that found in *Tubastraea* species. This FA is associated with dinoflagellates (Dalsgaard 2003) and hence *E. gabrielli I* and *II* should obtain this FA from the photosynthetic symbionts not present in *Tubastraea* species. Interestingly, 20:5*n*-3, 22:4*n*-6 and 22:5n-3 were absent in *Epizoanthus gabrielli I* and *II* but present in *Tubastraea coccinea*, collected in the same geographical region even if in different areas of Baía de Todos-os-Santos reef. The absence of those FAs probably derives from phylogeny-related biochemical differences between those genera, but could also have occurred as a result of a bleaching event experienced by *E. gabrielli I* and *II*, for it is known that corals consume FAs when experiencing such events (Bachok et al., 2006).

The PUFAs used in this study were not suitable to distinguish hexacorals at the Order level (a separation at this taxonomic level was, however, obtained for octocorals; see next section). Imbs et al. (2009) alleged that zooxanthellae from reef-building and soft corals are attributed to the same *Symbiodinium* group of dinoflagellates. However, genetic analyses based on ribosomal DNA have shown that zooxanthellae populations of Scleractinian (hexacorals) and soft corals are not homogeneous and contain different proportions of several symbiodinium phylotypes (Fabricius et al., 2004; Van Oppen et al., 2005). Moreover, Mansour et al. (1999) have demonstrated a substantial variability in terms of FA profile of different species belonging to the same genus of the free-living dinoflagellates. Inter-individual and inter-populational variability are also expected since the diversity of

zooxanthellae in a specific coral taxonomic group can depend on environmental factors such as solar irradiance and water temperature (Fabricius et al., 2004). In fact, seasonal variations in the photosynthetic activity of zooxanthellae were shown by Pernet and Anctil (2002). Thus, we may argue that such biotic and abiotic sources of variability can help explain the lack of significant differences between zoo- and azooxanthellate hexacorals.

4.3. Octocoral chemotaxonomy

On the other hand, a clear spatial and taxonomical separation of octocorals as well as a separation in terms of presence/absence of zooxanthellae in respect to alcyonaceans was observed, mainly due to the variation of common FAs biomarkers for phyto- and zooplankton, 20:5*n*-3 and 20:4*n*-6, respectively, and 18:4*n*-3. The latter is related to the presence of zooxanthellae since this FA has been shown to be the general marker of zooxanthellae in corals (Bishop and Kenrick, 1980).

4.3.1. Spatial (geographical) differences

Octocorals from México and Vietnam showed a higher percentage of 18:4*n*-3, while those from Portugal (mainland) showed a higher content of 20:5*n*-3 and 22:5*n*-6. It is worth noting that Portuguese coastal (temperate) waters are more productive than those from Atlantic Mexican and Vietnamese (both tropical) coasts. In fact, the Portuguese western coast is situated in the Western Iberia Upwelling Ecosystem (WIUE), which comprises the northern limit of the Canary Current Upwelling System (one of the four major Eastern Boundary Currents of the world). The main feature of the region is the occurrence of coastal upwelling during spring and summer in response to the

intensification of northerly winds (Fiúza et al., 1982). As such, a significantly higher availability of phyto- and zooplankton is observed in the Portuguese coastal waters and higher levels of 20:5*n*-3 (biomarker of phytoplankton) and 22:5*n*-6 (originating from 20:4*n*-6, a biomarker of zooplankton) are expected to occur in specimens inhabiting such waters. On the other hand, octocorals from Mexico and Vietnam may compensate for lower food availability with richer symbiotic relationships (e.g. greater diversity and/or abundance of symbiotic species). In line with this suggestion, prior studies showed that 18:4*n*-3 (Sprecher, 2000) is one of the main PUFAs isolated from zooxanthellae of reef-building corals (Bishop and Kenrick, 1980; Latyshev et al., 1991; Zhukova and Titlyanov, 2003). The octocoral *Renilla koellikeri* (family Renillidae), collected at Long Beach, California, was placed near species from Portugal (family Veretillidae). The two geographical regions are found at similar latitudes, under temperate climate regimes and associated to upwelling systems. These common environmental features might explain the considerable degree of similarity in FA profiles. Thus, in this case, the close positioning of species from both regions appears to indicate that the food source contribution plays a more important role in the biochemical properties (i.e. FA profile) of these octocorals, than the genetic background and FA biosynthesis capability.

4.3.2. Zooxanthellae and FA profiles

Zooxanthellate alcyonaceans showed a significantly higher content of 18:4*n*-3 (5.27 ± 2.34 % total FA, mean ± SD) than azooxanthellate alcyonaceans (0.49 ± 0.41 % total FA, mean ± SD). This FA is therefore a good biomarker for the distinction of alcyonaceans and

other octocorals in terms of zooxanthellae presence as already observed in previous studies (Bishop and Kenrick, 1980; Latyshev et al., 1991; Zhukova and Titlyanov, 2003).

The species *Lopophytum c.f. delectum*, a zooxanthellae alcyonacean, was placed in the proximity of the bulk of azooxanthellate alcyonaceans mainly as a result of a lower content of 18:4*n*-3 and a higher content of 20:4*n*-6, 22:5*n*-6 and 24:5*n*-6, in respect to other zooxanthellate alcyonaceans. This result may indicate that even though *L. c.f. delectum* is a zooxanthellate species, it may acquire FAs from external food sources, especially zooplankton, the main source of 20:4*n*-6 (Palardy et al., 2005). The azooxanthellate gorgonian, *Pseudopterogorgia sp.,* on the other hand, was placed closer to pennatulaceans as it displayed a lower value of 20:4*n*-6 and higher values of 20:5*n*-3 and 22:5*n*-3, when compared to other azooxanthellate gorgonians. Such result may derive from the fact that this Mexican species has greater access to microalgae rather than zooplankton.

4.4. Shallow water and deep-sea gorgonians chemotaxonomy

There is very little information available about the life strategies of deep-sea cnidarians. Here we showed, for the first time, that deep and shallow living octocorals (namely gorgonians) exhibit a certain degree of physiological similarity. Shallow living gorgonians from Portugal and Vietnam showed a higher content of 20:4*n*-6, 22:4*n*-6 and 24:5*n*-6, while species from Mexico showed a higher content of 18:4*n*-3. The high content of 20:4*n*-6 (triggering the *n*-6 biosynthesis pathway and hence the production of 22:4*n*-6 and 24:5*n*-6) in the Portuguese and Vietnamese gorgonians may be caused by the existence of an abundant zooplankton community (Dalsgaard et al., 2003) resulting from the high primary productivity occurring in those areas (discussed above). Mexican gorgonians

appear to supplement their FA requirements with a rich symbiotic relationship with dinoflagellates, for 18:4*n*-3 is a typical dinoflagellate marker (Dalsgaard et al., 2003).

To further reduce the degree of variability and obtain a clearer notion on the effect of depth on gorgonian FA profiles, a comparison between deep-sea gorgonians (Azores archipelago) and shallow living ones (from the continental coast of Portugal) was performed. Deep-sea gorgonians were found to have a lower content of all selected PUFAs, especially 20:4*n*-6 (Fig. 11D), 22:4*n*-6 (Fig. 11F) and 24:5*n*-6 (Fig. 11G). The low content of these PUFAs may be related to the lower availability of food sources at greater depths, resulting from extremely low levels of primary productivity (i.e. chemoautotrophy) and reduced rates of particle deposition originating from the surface (Bühring and Christiansen, 2001). Deep-sea benthopelagic plankton depends predominantly on detritus and/or predation on other organisms. Moreover, the nutritional quality of deep-sea detritus depends on its origin, its sinking rate, water temperature and on the bacteria associated with the aggregate particles (Bühring and Christiansen, 2001). As such, a considerable lesser availability of PUFA sources is bound to impact PUFAs intake and, consequently, PUFA biosynthesis pathways in deep sea gorgonians.

It is worth noting that a decrease in temperature may cause changes in membrane fluidity, and that the integrity of living cells in response to environmental stresses (such as temperature) depends on the stability of the biomolecular lipid layer and the associated non-lipid components (Neidleman, 1987). While an increase in saturated fatty acids (SFA) increases the rigidity of biological membranes, PUFAs increase the fluidity of the membranes (Papina et al., 2007). In other words, cold stress causes unsaturation of the membrane lipids. Mironov et al. (2012) and Hulbert (2003) also stated that highly polyunsaturated membranes are associated with adaptation to cold environments and the

effect of low temperatures slowing down physiological processes. Yet, it is worth noting that there is no clear empirical proof of the relation between unsaturated fatty acids and membrane fluidity in marine organisms (Hall et al., 2002). Still, within this context, one could expect an increase of PUFAs in gorgonians from the harsh and cold deep-sea habitats. This trend, however, was not noticed. It is possible that the perennial low PUFA availability in the deep sea environment does not allow for homeoviscous adaptation of cell membranes in these organisms.

4.5. Fatty Acids challenging current taxonomic classification

Our multivariate analysis and consequent taxonomical separation of octocorals corroborates the presently outdated taxonomic classification that contemplated the existence of a separated Gorgonacea order (Gerhart, 1983; Song and Won, 1997). Gorgonians displayed average FA concentrations in respect to those of the other otocorals and the placement of this group closer to alcyonaceans highlighted a biochemical similarity between these groups. Still, the bulk of gorgonians clustered away from alcyonaceans and this result may indicate a recent evolutionary divergence within Octocorallia. As a matter of fact members of Scleraxonia, Stolonifera, and Alcyoniina (suborders of the order Alcyonacea) can be found within both major clades of Octocorallia (Holaxonia-Alcyoniina and Calcaxonia-Pennatulacea). More, the suborder of Holaxonia ("gorgonians") and Alcyoniina frequently appear as sister taxa (McFadden et al., 2010).

Pennatullaceans (sea pens) were found to exhibit a lower content of 18:4*n*-3 and 24:5*n*-6 and a higher percentage of 20:5*n*-3, 22:5*n*-3, 22:4*n*-6 and 24:6*n*-3 when compared

to gorgonians and alcyonaceans. Being the azooxanthellae octocorals examined in this study mostly from Portugal, the high content of 22:4*n*-6 may derive from increased zooplankton intake while the high content of 20:5*n*-3 and 22:5*n*-3 and 24:6*n*-3 (originating from 20:5*n*-3) may derive from increased phytoplankton intake. The main source of PUFAs in the phytoplankton are diatoms which biosynthesise mostly C20:5 (Volkman et al., 1998). On the other hand alcyonaceans were distinguished from the other octocorals due to higher contents of 18:4*n*-3 and 24:5*n*-6 and lower contents of 20:5*n*-3, 22:5*n*-3, 22:4*n*-6 and 24:6*n*-3. The high content of 18:4*n*-3 may be related to the presence of zooxanthellae since that FA has been shown to be the general marker of zooxanthellae in corals (Bishop and Kenrick, 1980). In respect to 24:5*n*-6, the obtained results indicate a higher content of this FA as a discriminating trait of alcyonaceans.

5. Final remarks

This study presents the most comprehensive meta-analysis (to date) on the chemotaxonomy of hexa- and octocorals (originating from different latitudes and longitudes). Despite the complexity and high number of variability sources, this study alsoprovides the first glance on the FA chemotaxonomical differences between shallow and deep-sea corals, but further studies are still needed on this topic. Still, a better understanding of the reproductive biology, symbiosis, bleaching events and lipid metabolism of corals is required in order to accurately interpret chemotaxonomical data in this lower branch of the marine tree of life.

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