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Review

Biological and taxonomic perspective of triterpenoid glycosides of sea cucumbers of the family Holothuriidae (Echinodermata, Holothuroidea)

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ABSTRACT

Since the discovery of saponins in sea cucumbers, more than 150 triterpene glycosides have been described for the class Holothuroidea. The family Holothuriidae has been increasingly studied in search for these compounds. With many species awaiting recognition and formal description this family currently consists of five genera and the systematics at the species-level taxonomy is, however, not yet fully understood. We provide a bibliographic review of the triterpene glycosides that has been reported within the Holothuriidae and analyzed the relationship of certain compounds with the presence of Cuvierian tubules. We found 40 species belonging to four genera and 121 compounds. Holothurin A and B are the most common saponins for *Actinopyga*, *Holothuria*, and *Pearsonothuria*. The genus *Bohadschia* presents mainly bivittoside C and D. *Actinopyga* has only sulfated saponins mainly oxidized, *Bohadschia* non-sulfated ones mainly non-oxidized, *Holothuria* and *Pearsonothuria* contain both types of compounds, mainly oxidized. Within the genus *Holothuria*, the subgenus *Panningothuria* only has non-sulfated saponins. The presence of sulfated and non-sulfated compounds seemingly relates to the expellability or the absence of Cuvierian tubules and the temporal or permanent concealing habits of the species. Our study concludes that better insights into the systematic distribution of saponins in Holothuriidae will only be possible if the identifications of the investigated species are confirmed by a taxonomist, especially in this group wherein cryptic species and variation between life-history stages are common and yet poorly understood. Understanding of saponin distribution within the Holothuriidae would also benefit from a stabilization of triterpene glycoside nomenclature.

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Contents

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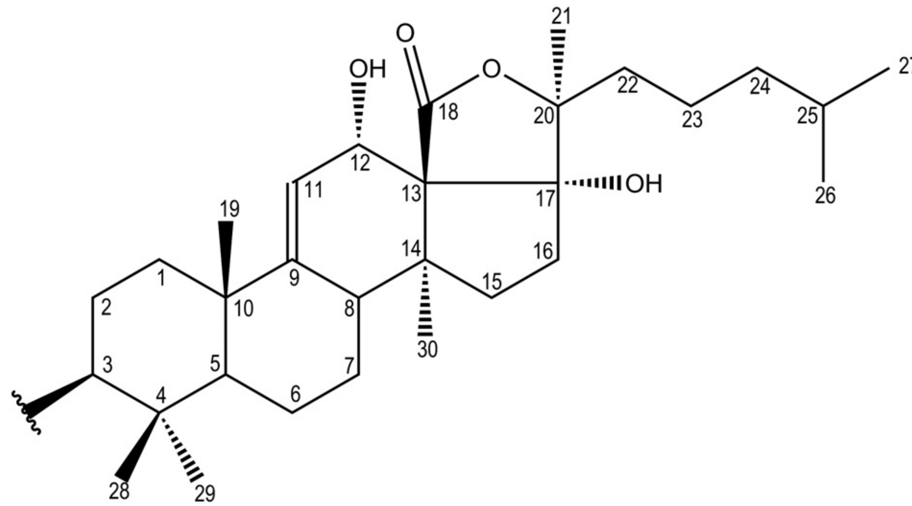

Fig. 1. Structure of characteristic holostane of the family Holothuriidae.

1. Introduction

Saponins are a group of secondary metabolites first discovered in vascular plants (Osbourn, 1996), but they have also been reported in marine organisms such as soft corals (He et al., 2002), sea stars (Mackie and Turner, 1970; Maier, 2008), and sponges (Campagnuolo et al., 2001; Kubanek et al., 2002). These compounds were first discovered in sea cucumbers by Nigrelli in 1952 (Nigrelli and Zahl, 1952)

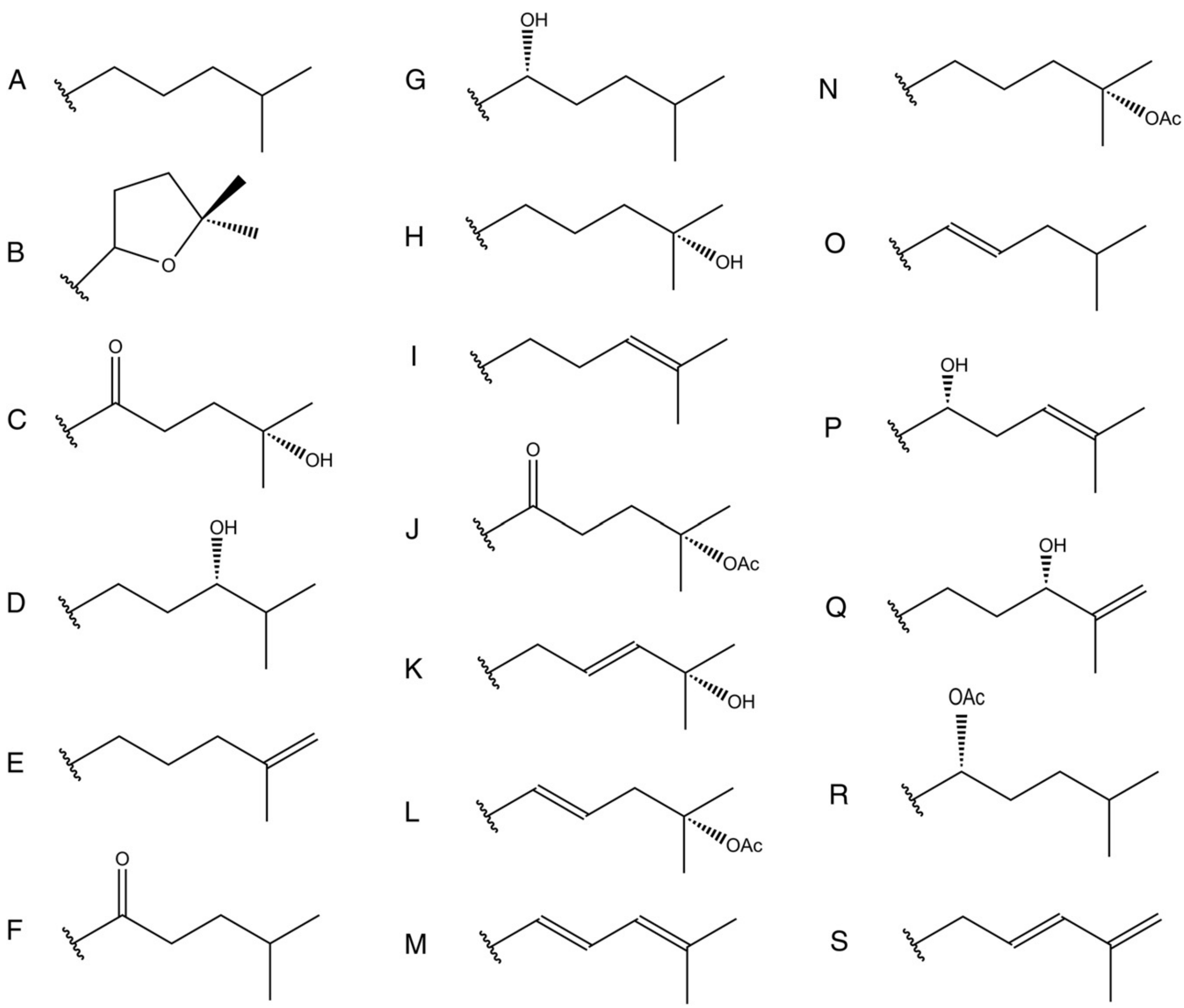

Fig. 2. Diversity of aliphatic chains that join in the C-20 of cyclic system of holostane documented for sea cucumbers of the family Holothuriidae.

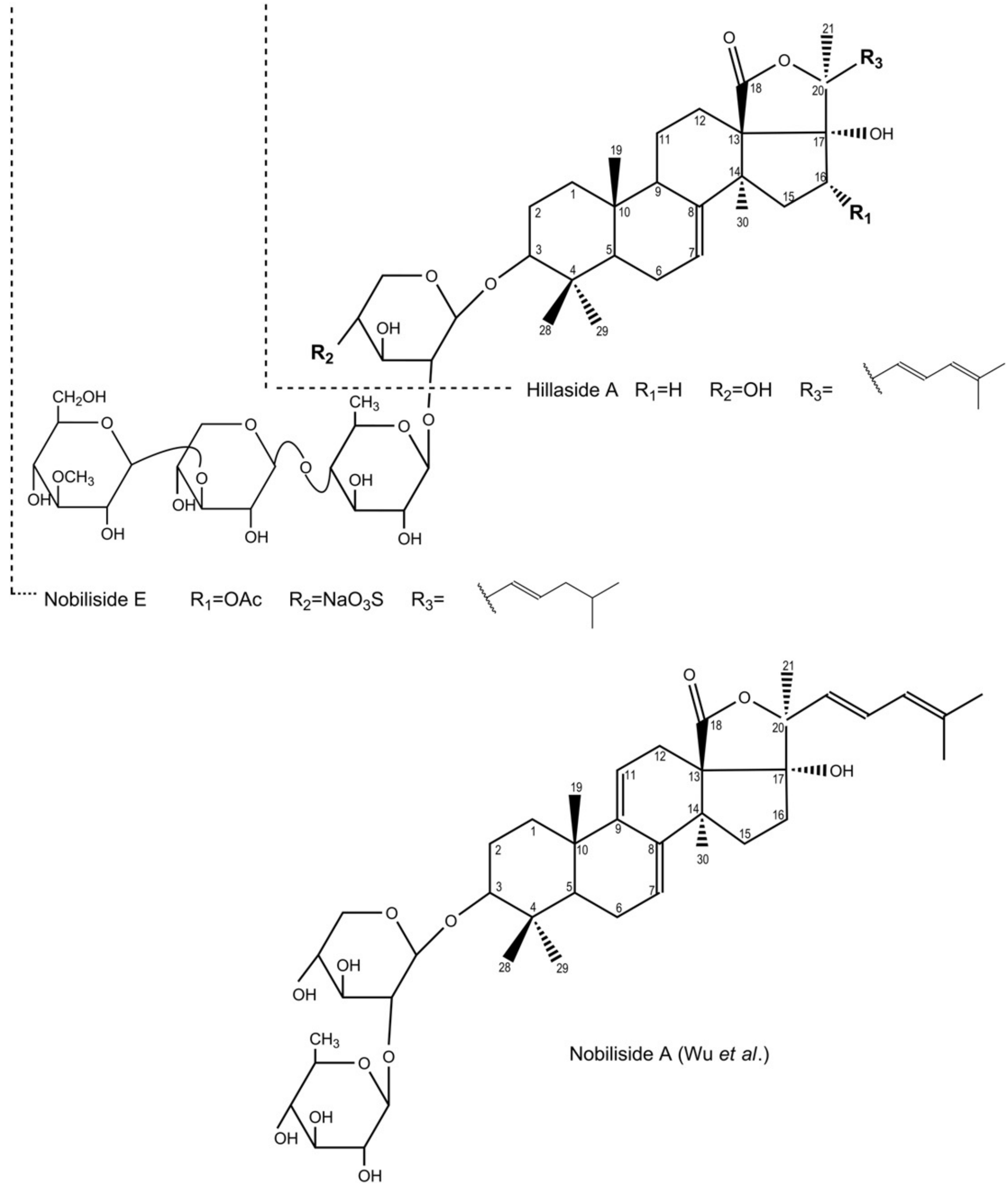

Fig. 3. Sulfated and non-sulfated triterpene glycosides with a 7-9 double bond uncommon in sea cucumbers of the family Holothuriidae. The structure of nobiliside A is the one described by Wu et al. (2006a,b,c) (see text).

and Yamanouchi (1955) from two different species. These independent studies established the glycosidic nature of these compounds and nominated them holothurins; its ichthyotoxic properties were also studied. Since then, more than 150 triterpene glycosides have been identified for the class Holothuroidea, mainly in the orders Dendrochirotida and Aspidochirotida (Kalinin et al., 2005). The saponins in sea cucumbers exist abundantly in the body wall, but they are also present in high concentration in the Cuvierian organs (Van Dyck et al., 2009) and the gonads (Kalinin et al., 2008). Although as secondary metabolites they have no explicit role in the internal metabolism, a variety of biological activities have been reported leading to an increased survival fitness of the producing organisms (Williams et al., 1989). Saponins are known for their cytotoxic (Zhang et al., 2006a; Pislyagin et al., 2012), antifungal (Kumar et al., 2007; Lakshmi et al., 2011), bactericidal (Haug et al., 2002), hemolytic (Kalinin et al., 1996), virucidal (Maier et al., 2001), and antiparasitic (Melek et al., 2012; Mona et al., 2012) properties. Some glycosides can inhibit the growth (Aminin et al., 2010; Li et al., 2010; Zhao et al., 2010), survival, invasion (Al Marzouqui et al., 2011), and metastasis (Attoub et al., 2013) of cancerous cells; others have immunomodulatory effects (Aminin et al., 2009), or act as inhibitors of sodium–potassium ATPase (Gorshkova et al., 1999), and even induce apoptosis (Li et al., 2008; Jin et al., 2009; Yun et al., 2012).

The majority of the saponins in sea cucumbers are triterpene glycosides with an aglycone derived from the lanostane with an 18(20)-lactone (Kalinin et al., 2005). The wide range of biological activities of the triterpene glycosides is caused by a strong membranolytic action due to the interaction with 5,6-unsaturated sterols of the cellular membrane that causes a saponification that lyses the cell (Kalinin, 2000). The membranotropic action of these compounds may serve as defense mechanism against predators; the glycosides increase in amount in the surrounding water as an aposematic signal, warning potential predators of the unpalatability of the sea cucumber tissues (Van Dyck et al., 2011). However, the sea cucumber saponins can also attract symbionts

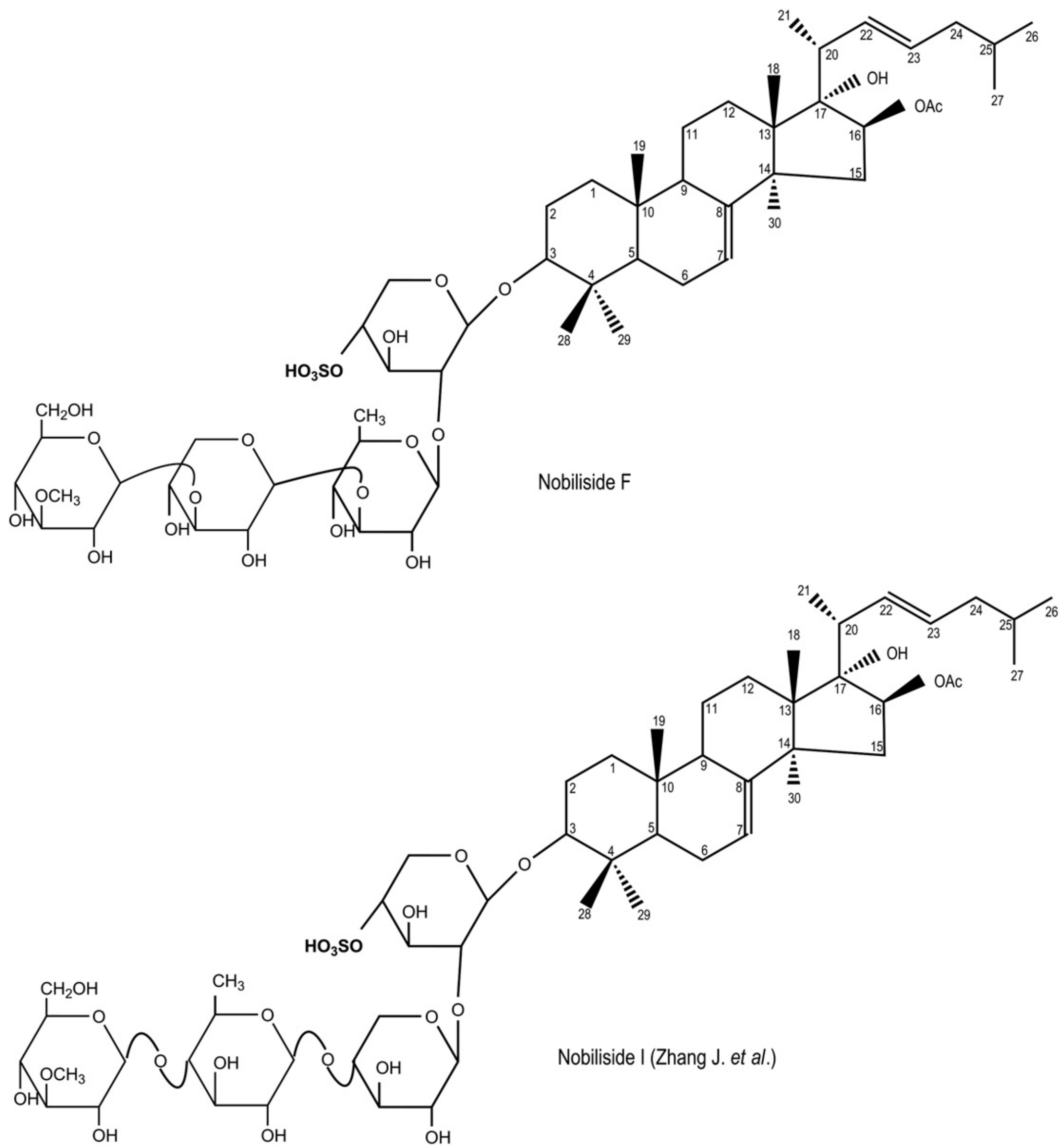

Fig. 4. Sulfated triterpene glycosides uncommon in sea cucumbers of the family Holothuriidae with an aglycone different from holostane, non-standard bonds between monosaccharides and different order of glycosides. The letter I in nobiliside I refers to capital i, compound described by Zhang J. et al. (2008) (see text).

as demonstrated by Caulier et al. (2013a) who called them kairomones and can also regulate oocyte maturation (Kalinin et al., 2008).

One family of sea cucumbers currently studied for its triterpene glycoside content is Holothuriidae (e.g. Silchenko et al., 2005). Five genera are traditionally recognized in this taxon, *Actinopyga* Bronn, 1860; *Bohadschia* Jaeger, 1833; *Holothuria* Linneaus, 1767; *Labidodemas* Selenka, 1867, and *Pearsonothuria* Levin in Levin et al., 1984. A unique trait of this family is the presence of Cuvierian tubules (Rowe, 1969) in several species. These structures are attached to the base of the left respiratory tree and can in some species be expelled through the cloacum if the sea cucumber is disturbed. The tubules in contact with seawater expand in size; when in contact with a surface they turn sticky and get attached to it (VandenSpiegel and Jangoux, 1987). In the past five years, more species belonging to this family have been analyzed for the saponin content in the body wall and Cuvierian tubules (Van Dyck et al., 2009, 2010; Mona et al., 2012; Bondoc et al., 2013; Bahrami et al., 2014; Elbandy et al., 2014).

Currently, the family Holothuriidae is being intensively studied in terms of the taxonomic status and phylogenetic relationships of several of its species (Samyn et al., 2010) and substantial changes in classification at the generic level can be expected that will occur over the next decade. So far, some published reviews of the saponin content of species of this family have focused only on the biochemical activity and/or the structure of the compounds discovered (Chludil et al., 2003; Zou et al., 2004; Sun et al., 2007a; Kalinin et al., 2008, 2005; Kwon and Himaya, 2012). Some inferences have been made for the presence of certain saponins in the Cuvierian tubules, but with only a few species (Van Dyck et al., 2010). However, no overall review has been conducted from a chemotaxonomic viewpoint, probably because of identification uncertainties by non-taxonomists, but also because of the presence of cryptic species, invasive species differences in life-history stages and/or any spatio-temporal factors.

This family together with family Stichopodidae is heavily exploited around the World as food for Asian countries (Purcell et al., 2012). The identification of specimens and correct nomenclature both have been promoted so that the fisheries may recognize and preserve the resource. Many of the species fished are those in which triterpene glycosides have been studied, so it is important to standardize nomenclature and establish good interdisciplinary communication. The aim of this paper is to gather bibliographic data on triterpene glycosides described for sea cucumbers of the family Holothuriidae and to find patterns of distribution of these compounds among genera and subgenera. The taxonomic

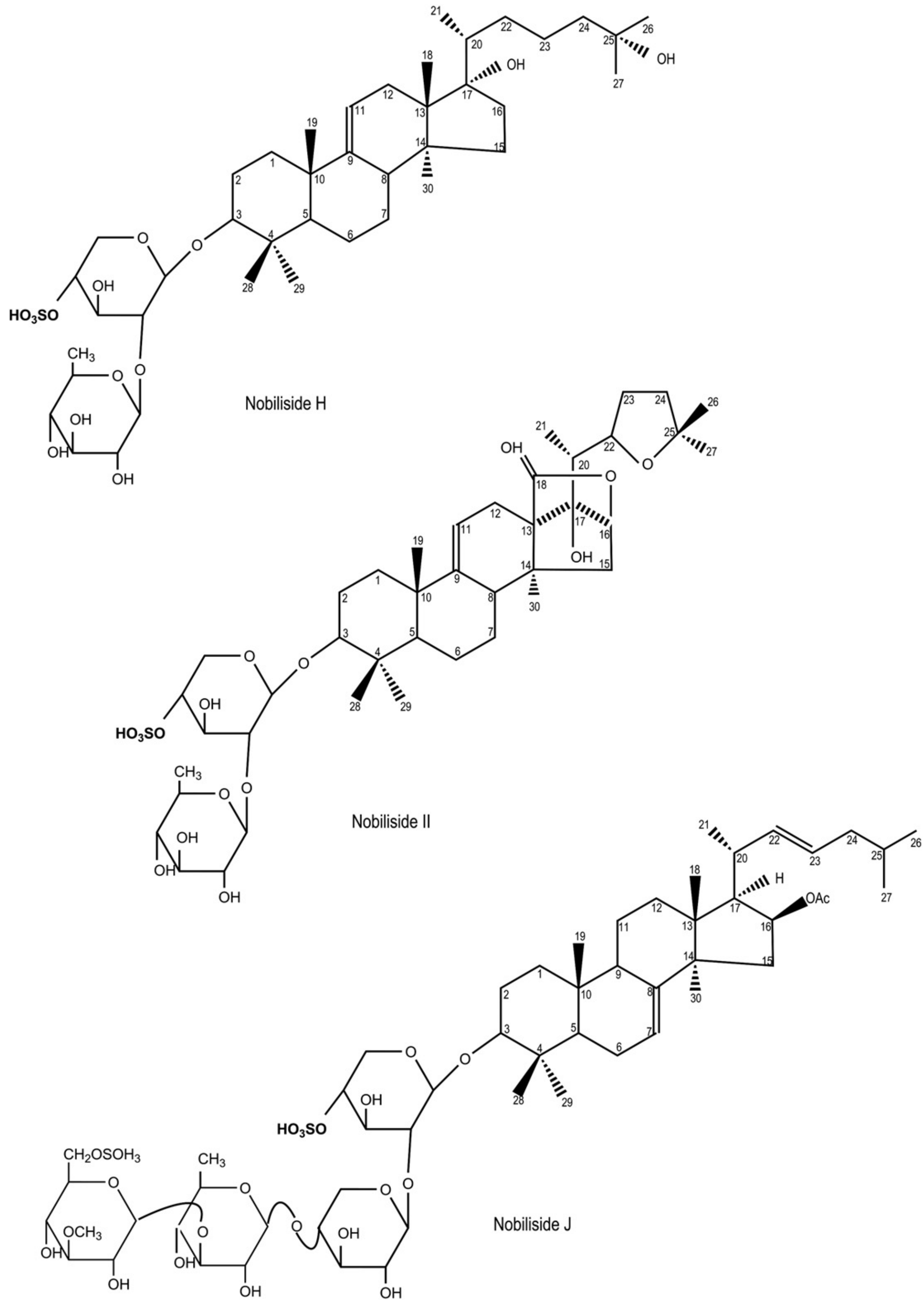

Fig. 5. Sulfated triterpene glycosides with an aglycone different from holostane uncommon in sea cucumbers of the family Holothuriidae.

identification and nomenclature of the species were verified with recent taxonomical literature of this group and the database in World Register of Marine Species (http://www.marinespecies.org/). The structure and names of the compounds were checked through comparison with previous reviews (Chludil et al., 2003; Zou et al., 2004; Sun et al., 2007a; Kalinin et al., 2008, 2005; Kwon and Himaya, 2012) and with each other to look for differences in positions of double bonds, hydroxyl, sulfate, and acetate groups on the aglycone and side chains, as well as number, type, and position of monosaccharides.

2. Structure of triterpenoid glycosides

The triterpenoid glycosides of the Holothuriidae have two parts, the carbohydrate chains and the aglycone, which is a derivative from the lanostane, called holostane, that can also be divided into the cyclic system and the aliphatic chain (Figs. 1 and 2).

The structure of the cyclic system of the holostane is composed of 19 carbons, with an 18(20)-lactone and a 9(11)-double bond (Fig. 1). C-3 is where the carbohydrate chains join the cyclic system and the side chain

Table 1
Triterpene glycosides of species of genus *Actinopyga*. The column Figure refers to figure number with a scheme of the compound. *unique for the species; NA = not available; CT = Cuvierian tubules; BW = Body wall and X = body compartment not specified.

	Actinopyga						
	agassizii	*echinites*	aff. *flammea*	*lecanora*	*mauritiana*	*miliaris*	Figure
Echinoside A (Holothurin A_2)		X/BW	X		X		8
Echinoside B (Holothurin B_1)		X/BW			X		8
24-dehydroxyechinoside A	CT		X		X		8
24-dehydroxyechinoside B*					X		8
22-hydroxy, 24-dehydroechinoside A*			X				9
24-hydroxy, 25-dehydroechinoside A*			X				9
22-acetoxyechinosideA*			X				9
25-hydroxydehydroechinoside A*			X				9
Holothurin A (Nobiliside I, Zhang J.)	X/CT	X/BW	X	X	X	X	7
HolothurinA_1			X	X			9
Holothurin B		X	X	X	X	X	7
Holothurin B_2		BW					9
HolothurinB_3		BW					7
Lecanoroside A*				X			7
Lecanoroside B*				X			7
Isomer 1 Fuscocineroside B/C		BW/CT					NA
Isomer 2 Fuscocineroside B/C		BW					NA
Isomer 3 Fuscocineroside B/C*		BW					NA
Isomer Holothurin B_2*		BW					NA
Isomer Echinoside A (Holothurin A_2)		BW					NA
Isomer Holothurin B/B_4		BW					NA
References	Elyakov et al. (1975); Kitagawa et al. (1982); Kobayashi et al. (1991)	Elyakov et al. (1973); Kitagawa et al. (1980, 1985); Kobayashi et al. (1991); Van Dyck et al. (2010); Melek et al. (2012)	Bhatnagar et al. (1985)	Elyakov et al. (1973); Kumar et al. (2007); Zhang et al. (2008a)	Elyakov et al. (1973); Kobayashi et al. (1991); Parameswaran et al. (1991); Radhika et al. (2002)	Elyakov et al. (1973)	

of the aglycone is joined at C-20. The current bibliographic revision showed that some differences among the compounds of the Holothuriidae are the presence of hydrogen or hydroxyl group at C-12 and C-17, and, more rarely, the presence of an acetoxy group at C-16 (Fig. 1). These findings do not support the statement that the glycosides in the family Holothuriidae contain only hydroxyl groups at C-12 and C-17 (Kalinin et al., 2005). Approximately half the compounds verified for this review have hydrogen in these positions. Few triterpenoid glycosides found in few species of the Holothuriidae have a 7(8)-double bond instead of the 9(11) one (Fig. 3). The presence of such a double bond alone is more characteristic of glycosides present in the family Stichopodidae (Kalinin et al., 2005), so this could represent a parallelism. Only one compound (Nobiliside A) was described with the presence of 7(8), 9(11)-diene system (Wu et al., 2006b; Fig. 3). Aglycones with such double bonds are artifacts due to the strong acidic conditions during hydrolysis of the compound (Habermehl and Krebs, 1990), so this formula is very doubtful and should not be considered. Another five compounds present in a single species [*Holothuria* (*Microthele*) *whitmaei*] do not have the structure of the holostane (Figs. 4 and 5); glycosides of this type have only been discovered in sea cucumbers from the order Dendrochirotida, organisms distantly related to the Holothuriidae.

The side chain of the aglycone consists of six carbons arranged in a chain or in a cyclic molecule, and this is where the triterpenoid glycosides have great variation. Nineteen different structures have been described (Fig. 2). The most common structure is the cyclic one (Fig. 2, B), present in almost half of the glycosides described until now. Another common one (Fig. 2, A) is the linear chain with no double bonds, hydroxyls or ketone moieties. Other differences in structure include double bonds at C-22, C-23, C-24 or C-25, sometimes two at the same time (C-22 and C-24, or C-23 and C-25), hydroxyl groups at C-22, C-24 or C-25, ketone groups at C-22 or C-24, and the presence of an acetoxy group at C-25 (Fig. 2).

The carbohydrate chain may contain from one to six monosaccharides, in a linear or branched chain. The linear chain includes one to four monosaccharides. The first sugar is always xylose, the second usually quinovose (sometimes it is glucose, rarely another xylose), the third one is usually glucose (more rarely a xylose or a quinovose) and the fourth is always a 3-O-methylglucose. Almost half the triterpenoid glycosides identified until now have a sulfate group at the C-4 of the first xylose, which are known as sulfated glycosides. The glycosides with five or six sugars have a chain branched out from the first sugar (xylose). One branch is the one described above with four monosaccharides, and the second consists of one or two sugars. The fifth sugar is always glucose and the sixth is regularly 3-O-methylglucose, more rarely quinovose. The glycosides with a branched chain of monosaccharides never have a sulfated group, while most of the compounds with a linear branch have it; most of these sulfated groups contain sodium, and rarely lack it.

The different combinations of the parts of the triterpenoid glycosides described above, and their variations, form about 121 compounds in total for the Holothuriidae that are present in the body wall, the Cuvierian tubules or the viscera of the sea cucumbers. Kalinin et al. (2008) noticed that the differences in the composition of the triterpenoid glycosides affect their hemolytic, antifungal, cytotoxic, and ATPase inhibition activities. For the glycosides with a 9(11)-double bond the presence of an 18(20) lactone and one oxygen group close to the double bond is critical for biological activity. Also, glycosides with a 7(8)-double bond are more active than those with such a double bond and a C-16 ketone group. The linear tetrasaccharide fragment is significant for membranotropic action, as well as the presence of quinovose as the second unit in the chain for fungal activity. The activity is lower in the absence of a sulfate at C-4 of the first xylose of a linear chain than in its presence.

Table 2
Triterpene glycosides of species of genus *Bohadschia*. The column Figure refers to figure number with a scheme of the compound. * unique for the species; ** unique for the genus; NA = not available; CT = Cuvierian tubules; BW = body wall and X = body compartment not specified.

	Bohadschia					
	argus	*cousteaui*	*marmorata*	*subrubra*	*vitiensis*	Figure
Arguside A*	X					13
Arguside B	X					10
Arguside C	X	BW		CT	X	13
Arguside D*	X					13
Arguside F		BW				13
Bivittoside A*					BW/CT	10
Bivittoside B*					BW/CT/X	10
Bivittoside C**	X/CT		X	CT/X	BW/CT	10
Bivittoside D	BW/CT	BW	X	CT	BW/CT	10
25-acetoxybivittoside D*			X			10
Cousteside A		BW				15
Cousteside B		BW				15
Cousteside C		BW				15
Cousteside D		BW				15
Cousteside E		BW				15
Cousteside F		BW				15
Cousteside G		BW				15
Cousteside H		BW				15
Cousteside I		BW				15
Cousteside J		BW				15
Des-Holothurin A		BW				11
Des-Holothurin A_1 (Arguside E)	X					12
Holothurinoside A		BW			X	11
17-dehydroxyholothurinoside A (Holothurinoside E)		BW				11
Holothurinoside F				BW	X	11
Holothurinoside G				X	X	11
Holothurinoside H		BW		BW/CT/X	X	11
Holothurinoside I		BW		BW/CT		11
Holothurinoside H_1				BW/CT		12
Holothurinoside I_1				BW/CT		12
Holothurinoside J_1				BW/CT		12
Holothurinoside K_1				BW		12
Impatienside A		BW	X	BW/CT		10
Impatienside B		BW				10
17-hydroxyimpatienside A*			X			10
Marmoratoside A*		BW	X			10
Marmoratoside B*			X			10
Isomer Bivittoside D*				CT		NA
Isomer 1 Holothurinoside H_1*				BW		NA
Isomer 2 Holothurinoside H_1*				BW		NA
Isomer 1 Holothurinoside I_1*				BW		NA
Isomer 2 Holothurinoside I_1*				BW		NA
Isomer 3 Holothurinoside I_1*				BW		NA
Isomer 1 Impatienside A*				CT		NA
Isomer 2 Impatienside A*				CT		NA
References	Antonov and Stonik (1986); Kobayashi et al. (1991); Liu et al. (2007, 2008a, 2008b)	Elbandy et al. (2014)	Antonov and Stonik (1986); Yuan et al. (2009a)	Van Dyck et al. (2010); Caulier et al. (2013a,b)	Kitagawa et al. (1981a, 1989a); Antonov and Stonik (1986); Kobayashi et al. (1991); Lakshmi et al. (2008, 2011); Caulier et al. (2013a,b)	

3. Patterns of distribution of saponins

Sums up, 40 holothuriid species belonging to four genera are included in this review as well as 121 triterpene glycosides, 55 of them sulfated and 66 non-sulfated. Where specified, a distinction between body wall (BW), Cuvierian tubules (CT) or viscera (V) was scored, although most data did not specify a body compartment (see Tables 1 to 7). Most of the species included in this review have an Indo-Pacific distribution, but some stem from the Atlantic [mainly the Caribbean, *Actinopyga agassizii* (Selenka, 1867); *Holothuria* (*Cystipus*) *cubana* Ludwig, 1875; *Holothuria* (*Halodeima*) *floridana* Pourtalès, 1851; *Holothuria* (*Halodeima*) *grisea* Selenka, 1867; *Holothuria* (*Halodeima*) *mexicana* Ludwig, 1875] and the Mediterranean [*Holothuria* (*Panningothuria*) *forskali* Delle Chiaje, 1823; *Holothuria* (*Rowethuria*) *poli* Delle Chiaje, 1824; *Holothuria* (*Holothuria*) *tubulosa* Gmelin, 1791].

3.1. Genus Actinopyga

Six of the nineteen species that constitute the genus have been studied for their glycoside content in body wall and Cuvierian tubules (Table 1). Twenty-one triterpene glycosides have been isolated, all of them sulfated and nine unique for this genus (Table 1).

3.2. Genus Bohadschia

For this genus, in total 45 triterpenoid non-sulfated glycosides were isolated from five species of the eleven currently recognized (Kim et al., 2013). Of the 45 compounds, 27 are unique for the genus (Table 2). Of these 27 glycosides, ten were discovered in a single species, *Bohadschia cousteaui* (Elbandy et al., 2014). Additionally, Yuan et al.

Table 3
Triterpene glycosides of species of genus *Holothuria* (Part I). The column Figure refers to figure number with a scheme of the compound. * unique for the species; ** unique for the genus. NA = not available; BW = body wall and X = body compartment not specified.

		Holothuria									
		(*Acanthotrapeza*)	(*Cystipus*)	(*Halodeima*)						(*Holothuria*)	
		coluber	*cubana*	*atra*	*edulis*	*floridana*	*grisea*	*mexicana*	*pulla*	*tubulosa*	Figure
Sulfated	Echinoside A (Holothurin A_2)			X	X	X					8
	Echinoside B (Holothurin B_1)			X/BW		X					8
	Holothurin A (Nobiliside I, Zhang J.)	X	X	X	X		X	X	X	BW	7
	Holothurin A_1					X	X				9
	Holothurin B	X		X	X		X	X	X	BW	7
	Holothurin B_2			BW							9
	Holothurin B_3			BW							7
	Isomer Holothurin B/B4			BW							NA
Non-sulfated	Des-Holothurin B**			X							11
	Griseaside A**						X+				10
	17-dehydroxyholothurinoside A (Holothurinoside E)**						X+				11
	References	Elyakov et al. (1973).	Elyakov et al. (1975)	Elyakov et al. (1973); Stonik et al. (1979); Oleinikova and Kuznetsova (1986); Kobayashi et al. (1991); Anjaneyulu and Raju (1996); Van Dyck et al. (2010)	Elyakov et al. (1973); Kalinin and Stonik (1982b); Kobayashi et al. (1991)	Oleinikova et al. (1981, 1982a,b); Elyakov et al (1982); Kuznetsova et al. (1982)	Elyakov et al. (1975); Oleinikova et al. (1982b); Sun G.-Q. et al. (2008)	Elyakov et al. (1975)	Elyakov et al. (1973); Pocsidio (1987)	Silchenko et al. (2005)	

Table 4
Triterpene glycosides of species of genus *Holothuria* (Part II). The column Figure refers to figure number with a scheme of the compound. * unique for the species; ** unique for the genus; N/A = not available; CT = Cuvierian tubules; BW = body wall; X = body compartment not specified; V = viscera.

		Holothuria					
		(*Mertensiothuria*)		(*Metriatyla*)			
		hilla	*leucospilota*	*martensii*	*lessoni*	*scabra*	Figure
Sulfated	Echinoside A (Holothurin A_2)		CT		X/V	X/BW	8
	Echinoside B (Holothurin B_1)					X	8
	24-dehydroxyechinoside A				V	X/BW	8
	Fuscocineroside B				V		8
	Fuscocineroside C (17-dehydroxyholothurin A)**				V	X	7
	17-hydroxyfuscocineroside B (Scabraside B)				X/V	X/BW	8
	25-hydroxyfuscocineroside B				V		8
	Hillaside C (Nobiliside D)**	X					14
	Holothurin A (Nobiliside I, Zhang J.)	X	X/BW/CT	X	V	X/BW	7
	Holothurin A_1				V	X	9
	Holothurin A_3**				V	X/BW	9
	Holothurin A_4**				V	X	9
	Holothurin B	X	X/BW/CT	X	V	X	7
	Holothurin B_2		X		V		9
	Holothurin B_3		BW/CT		V		7
	Holothurin B_4**				V		9
	Leucospilotaside A**		X		V		8
	Leucospilotaside B**		X		V		8
	Leucospilotaside C*		X				7
	Leucospilotaside D*		X				8
	Nobiliside B**				V		7
	Pervicoside C**					BW	9
	Scabraside A**				V	X/BW	9
	Scabraside C*					X	NA
	Scabraside D**				V	X	9
	Isomer Holothurin B/B_4		BW/CT				NA
	Isomer Fuscocineroside A**					BW	NA
	Isomer Pervicoside C**					BW	NA
Non-sulfated	Arguside B				V		10
	Arguside C				V		14
	Bivittoside D		BW/CT			BW	10
	Des-Holothurin A (Nobiliside 2a)		CT		X/V	X/BW	11
	Des-Holothurin A_1 (Arguside E)				V		12
	Des-Leucospilotaside A*		X				8
	Griseaside A**				V		10
	Hillaside A*	X					3
	Hillaside B*	X					13
	17-dehydroxyholothurinoside A (Holothurinoside E)				V		11
	Holothurinoside A				V		11
	Holothurinoside A_1				V		12
	Holothurinoside C				V	X/BW	11
	Holothurinoside C_1**				V		12
	Holothurinoside E_1**		CT		V		12
	Holothurinoside G					BW	11
	Holothurinoside H					BW	11
	Holothurinoside J_1				V		12
	Holothurinoside K_1				V		12
	Holothurinoside M**				V		15
	Impatienside B				V		10
	Isomer 3 Impatienside A**					BW	NA
	References	Elyakov et al. (1973); Wu et al. (2006a, 2007)	Elyakov et al. (1973); Kitagawa et al. (1981b,c); Kobayashi et al. (1991); Han et al. (2007a,b, 2008, 2010a, 2012a); Van Dyck et al. (2010)	Minh et al. (2005)	Caulier et al. (2013a,b); Bahrami et al. (2014)	Elyakov et al. (1973); Kobayashi et al. (1991); Yan et al. (2005); Thanh et al. (2006); Dang et al. (2007); Han et al. (2009a,b, 2010b, 2012b); Bondoc et al. (2013); Caulier et al. (2013a,b)	

(2008a,b) described five more sulfated triterpene glycosides for the species *B. marmorata* (fuscocineroside A and B, 17-hydroxyfuscocineroside B, 25-hydroxyfuscocineroside B, and marmoroside C; Figs. 3, 5, and 8). Since it is apparent that this genus lacks sulfated compounds, and other authors (Elyakov et al., 1973; Antonov and Stonik, 1986) did not find this type of glycosides in this species, it is highly controversial that Yuan et al. (2008a,b) found sulfated glycosides in *B. marmorata*. A thorough revision of the specimens used is needed to discard a possiblemis-identification. *Bohadschia vitiensis* can be found in the references under its

Table 5
Triterpene glycosides of species of genus *Holothuria* (Part III). The column Figure refers to figure number with a scheme of the compound. * unique for the species; ** unique for the genus. CT = Cuvierian tubules; BW = body wall and X = body compartment not specified.

		Holothuria						
		(*Microthele*)			(*Panningothuria*)	(*Platyperona*)	(*Rowethuria*)	
		fuscopunctata	*nobilis*	*whitmaei*	*forskali*	*difficilis*	*poli*	Figure
Sulfated	Echinoside A (Holothurin A_2)	X		X				8
	Echinoside B (Holothurin B_1)						X	8
	Hillaside C (Nobiliside D)**			X				14
	Holothurin A (Nobiliside I, Zhang J.)	X		X		X	BW	7
	Holothurin B	X	X				BW	7
	Holothurin B_2						BW	9
	Holothurin B_3						BW	7
	Holothurin B_4*						BW	9
	Nobiliside A (Zhang)*			X				13
	Nobiliside B (Wu and Zhang J.)**			X				7
	Nobiliside E*			X				3
	Nobiliside F*			X				4
	Nobiliside G (Axilogoside)**	X		X				7
	Nobiliside H*			X				5
	Nobiliside J*			X				5
	Nobiliside I (Zhang, 2011)*			X				4
	Nobiliside II (Zhang J.)*			X				5
Non-sulfated	Arguside F*	X						13
	Des-Holothurin A (Nobiliside 2a)			X	BW/CT/X			11
	Des-Holothurin A_1 (Arguside E)				CT			12
	Holothurinoside A*				BW/CT			11
	17-dehydroxyholothurinoside A (Holothurinoside E)**				BW/CT			11
	Holothurinoside B*				BW			10
	Holothurinoside C				BW/CT/X			11
	Holothurinoside D*				BW			11
	Holothurinoside F				BW/CT/X			11
	Holothurinoside G				BW/CT/X			11
	Holothurinoside H				BW/CT			11
	Holothurinoside I				BW/CT			11
	Holothurinoside A_1**				CT			12
	Holothurinoside C_1**				CT			12
	Holothurinoside E_1**				CT			12
	Holothurinoside F_1*				CT			12
	Holothurinoside G_1*				CT			12
	Holothurinoside H_1				CT			12
	Holothurinoside I_1				CT			12
	Holothurinoside L (Holothurinoside N)**				X			15
	Holothurinoside M**				X			15
	Impatienside B*	X						10
	Nobiliside 1a*			X				14
	Nobiliside A (Wu)*			X				3
	Nobiliside C (Wu and Zhang J.)*			X				11
	Pervicoside D*	X						10
	References	Kobayashi et al. (1991); Yuan et al. (2008c, 2009b)	Radhika et al. (2002)	Elyakov et al. (1973); Wu et al. (2006b,c); Zhang et al. (2008b); Li et al. (2010); Zhang (2011)	Rodríguez et al. (1991); Van Dyck et al. (2009, 2011); Caulier et al. (2013a,b)	Elyakov et al. (1973)	Silchenko et al. (2005); Melek et al. (2012)	

subjective synonyms *Bohadschia bivittata* or *Bohadschia tennuissima*.

3.3. Genus Holothuria

Of some 200 species that constitute the genus, 28 have been studied for triterpenic glycoside content. They represent fourteen of the eighteen subgenera currently accepted for the genus. Eighty of the 121 glycosides reported here for the family Holothuriidae have been found in this genus, and most of them (42) are sulfated ones. Of the compounds described for this genus, 49 are exclusive to it (Tables 3, 4, 5, and 6). The species with most compounds recorded is *Holothuria* (*Metriatyla*) *lessoni* for which Bahrami et al. (2014) isolated 34 known compounds (Table 4) plus 39 unidentified ones. Bondoc et al. (2013) recorded nine additional non-identified compounds for *H.* (*Metriatyla*) *scabra* and seven for *H.* (*Thymiosycia*) *impatiens* and *H.* (*Stauropora*) *fuscocinerea*. Van Dyck et al. (2009) also recorded additional fourteen unidentified glycosides for *H.* (*Panningothuria*) *forskali*. Two non-sulfated compounds recorded for *H.* (*Halodeima*) *grisea* collected in Guangzhou, Guangdong Province, China (Sun G.-Q. et al., 2008) must be treated carefully because the species is most probably misidentified as the geographic distribution of *H.* (*H.*) *grisea* is restricted to the Gulf of Mexico, Caribbean Sea and West Africa (Panning, 1934). The species *Holothuria* (*Microthele*) *whitmaei* in many papers is identified as *Holothuria nobilis* and its unique glycosides are named nobiliside with a distinct letter or number assigned to differentiate them. Distinction should be made between nobiliside A sensu Wu et al. (2006b) and nobiliside A sensu Zhang J. et al. (2008), which have different formulas. The same for nobiliside I sensu Zhang J. et al. (2008) and nobiliside I (roman number one) sensu Zhang (2011); the latter has the same structure as holothurin A. *Holothuria* (*Microthele*) *fuscopunctata* Jaeger, 1833, is reported in the references as *Holothuria axiologa* H. L. Clark, 1921, and *Holothuria* (*Mertensiothuria*) *leucospilota* as *Holothuria vagabunda*.

Table 6
Triterpene glycosides of species of genus *Holothuria* (Part IV). The column Figure refers to figure number with a scheme of the compound. * unique for the species; ** unique for the genus. NA = not available; CT = Cuvierian tubules; BW = body wall and X = body compartment not specified.

		Holothuria								
		(*Semperothuria*)		(*Stauropora*)		(*Theelothuria*)	(*Thymiosycia*)			
		cinerascens	*surinamensis*	*fuscocinerea*	*pervicax*	*squamifera*	*arenicola*	*gracilis*	*impatiens*	Figure
Sulfated	Arenicolaside A*						X			7
	Fuscocineroside A			X/BW						8
	Fuscocineroside B			X/BW						8
	Fuscocineroside C (17-dehydroxyholothurin A)**			X/BW			X		X	7
	Holothurin A (Nobiliside I, Zhang J.)	X	X	X/BW	X	X	X	X	X/BW	7
	Holothurin A_1								X	9
	Holothurin B			X	X		X	X		7
	Pervicoside A*				BW/CT					9
	Pervicoside B*				X/BW					9
	Pervicoside C**			X/BW	X/BW				X	9
	Isomer 24-dehydroechinoside A								BW	NA
	Isomer Fuscocineroside A								BW	NA
	Isomer Holothurin A_3**			BW					BW	NA
	Isomer Pervicoside C**								BW	NA
Non-sulfated	Bivittoside D			BW					X/BW	10
	Des-Holothurin A			BW						11
	Des-Holothurin B**			X						11
	Holothurinoside C			BW					BW	11
	Holothurinoside H			BW					BW	11
	Impatienside A								X/BW	10
	Isomer 3 Impatienside A**			BW						NA
	References	Elyakov et al. (1973)	Elyakov et al. (1975)	Elyakov et al. (1973); Zhang et al. (2006b,c); Bondoc et al. (2013)	Elyakov et al. (1973); Kitagawa et al. (1989b); Kobayashi et al. (1991)	Ivanova et al. (1984)	Elyakov et al. (1973); Weng et al. (2007)	Elyakov et al. (1973)	Elyakov et al. (1973); Sun et al. (2006, 2007b, 2008b); Bondoc et al. (2013)	

Table 7
Triterpene glycosides of species of genus *Pearsonothuria*. The column Figure refers to figure number with a scheme of the compound. * unique for the species. N/A = not available; CT = Cuvierian tubules; BW = body wall and X = body compartment not specified.

		Pearsonothuria graeffei	Figure
Sulfated	Echinoside A (Holothurin A_2)	BW/CT	8
	24-dehydroxyechinoside A	X	8
	Holothurin A (Nobiliside I, Zhang J.)	BW/CT	7
	Holothurin A_1	X	9
	Holothurin B	X	7
	Isomer 1 Fuscocineroside B/C	BW/CT	NA
	Isomer 2 Fuscocineroside B/C	BW	NA
	Isomer Echinoside A (Holothurin A_2)	CT	NA
	Isomer Holothurin B/B_4	BW/CT	NA
Non-sulfated	Des-Echinoside A (des-Holothurin A_2)*	X	8
	Des-Holothurin A (Nobiliside 2a)	BW/CT	11
	Holothurinoside C	BW	11
	References	Elyakov et al. (1973); Kalinin and Stonik (1982a); Ivanova and Kuznetsova (1985); Kobayashi et al. (1991); Dong et al. (2008); Van Dyck et al. (2010); Zhao et al. (2010, 2011a,b, 2012)	

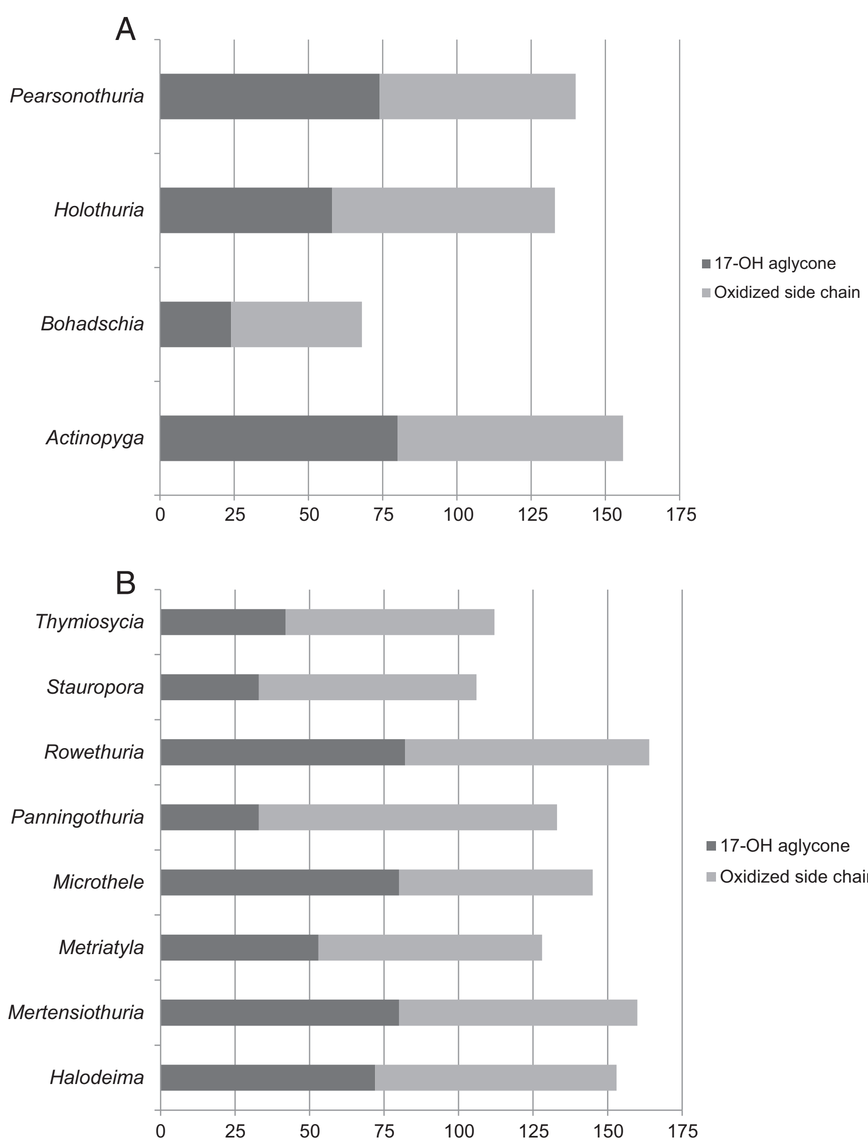

Fig. 6. Percentage of triterpene glycosides with hydroxyl group in C-17 of the aglycone or oxygen functions in the side chain for each genus of the family Holothuriidae (A) and eight subgenera of genus *Holothuria* (B). Only subgenera with more than five compounds are shown.

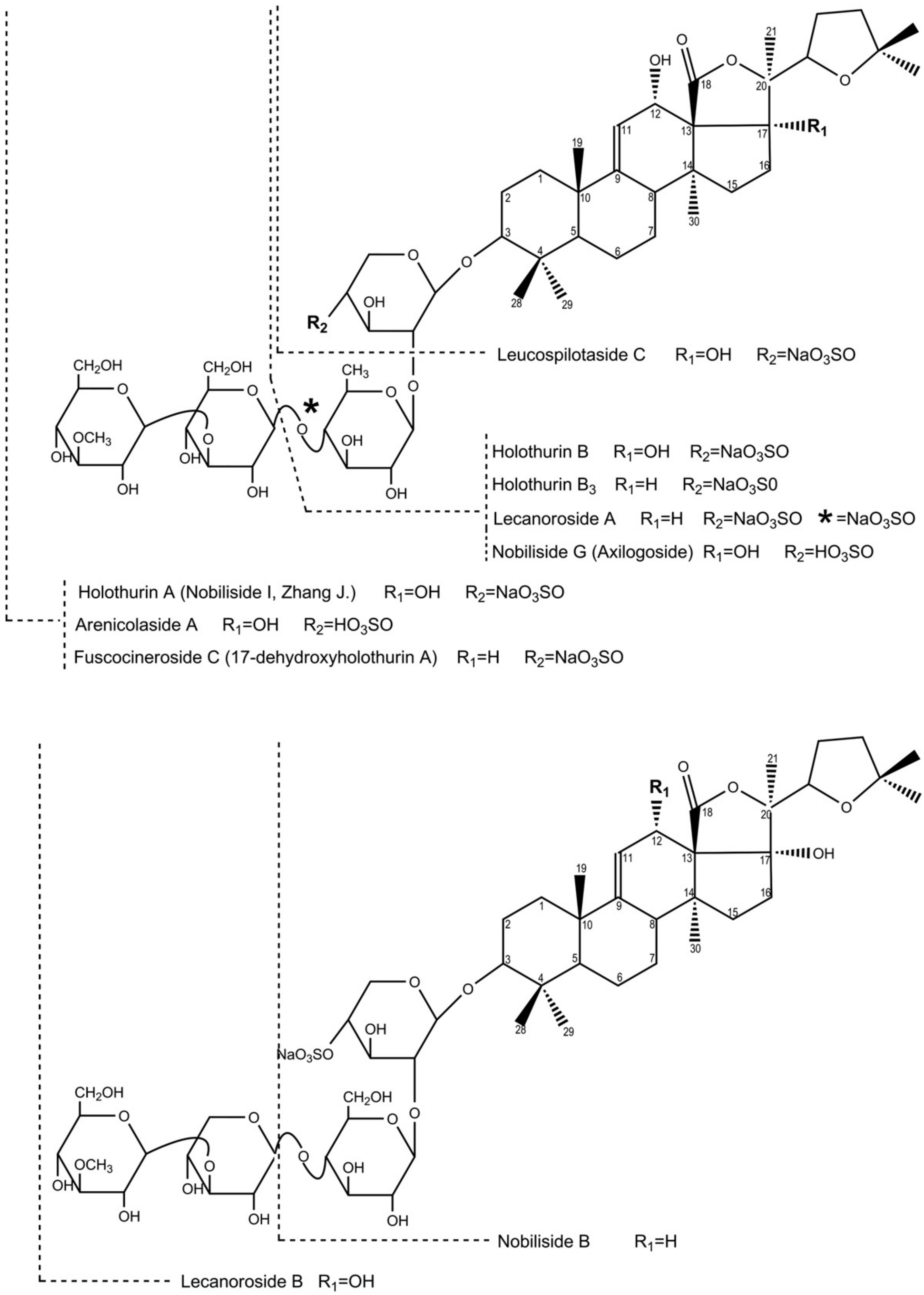

Fig. 7. Sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae. Lecanoroside A contains a sulfated group in each monosaccharide. The letter I in nobiliside I refers to roman number 1, compound described by Zhang in (2011) (see text).

3.4. Genus Pearsonothuria

This monotypic genus had its species split from the genus *Bohadschia* because of its strikingly different glycoside composition (Levin et al., 1984). *Pearsonothuria graeffei* (Semper, 1868) has a total of twelve triterpenoid glycosides described from body wall and Cuvierian tubules, and only one is unique for the species and the genus (Table 7).

The present bibliographic review revealed diversity of triterpene glycosides in the Holothuriidae, the most abundant being holothurin A and B, which are present in *Pearsonothuria*, all investigated species of

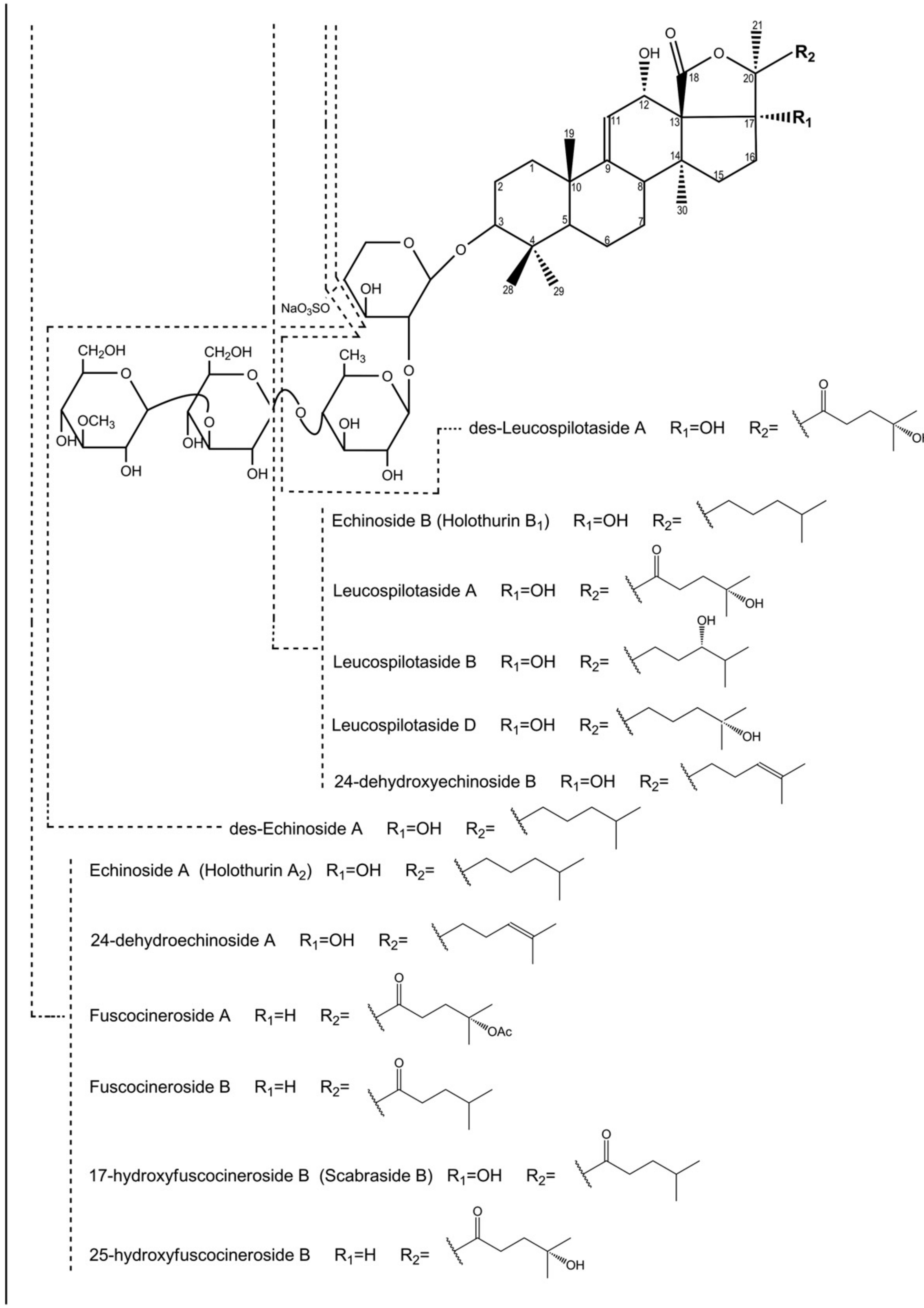

Fig. 8. Sulfated and non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae. The suffix des- refers to desulfated compounds.

Actinopyga and almost all species of *Holothuria* checked from literature, but absent from the genus *Bohadschia*. For the latter genus, the most common compounds are bivittosides C and D, and arguside C. The compounds in common for *Pearsonothuria*, *Actinopyga*, and *Holothuria* are 24-dehydroechinoside A, echinosides A and B, holothurins A, A_1 and B, and isomer of holothurin B/B_4. The genera *Actinopyga* and *Holothuria* share two glycosides, holothurins B_2 and B_3. The glycosides that *Actinopyga* has in common with the genus *Pearsonothuria* are three, isomers 1 and 2 of fuscocineroside B/C, and isomer of echinoside A. For the genera *Holothuria* and *Pearsonothuria*, the triterpenoid glycoside in common is holothurinoside C. The genus *Bohadschia* does not have saponins in common with the genus *Actinopyga*. It has one in common

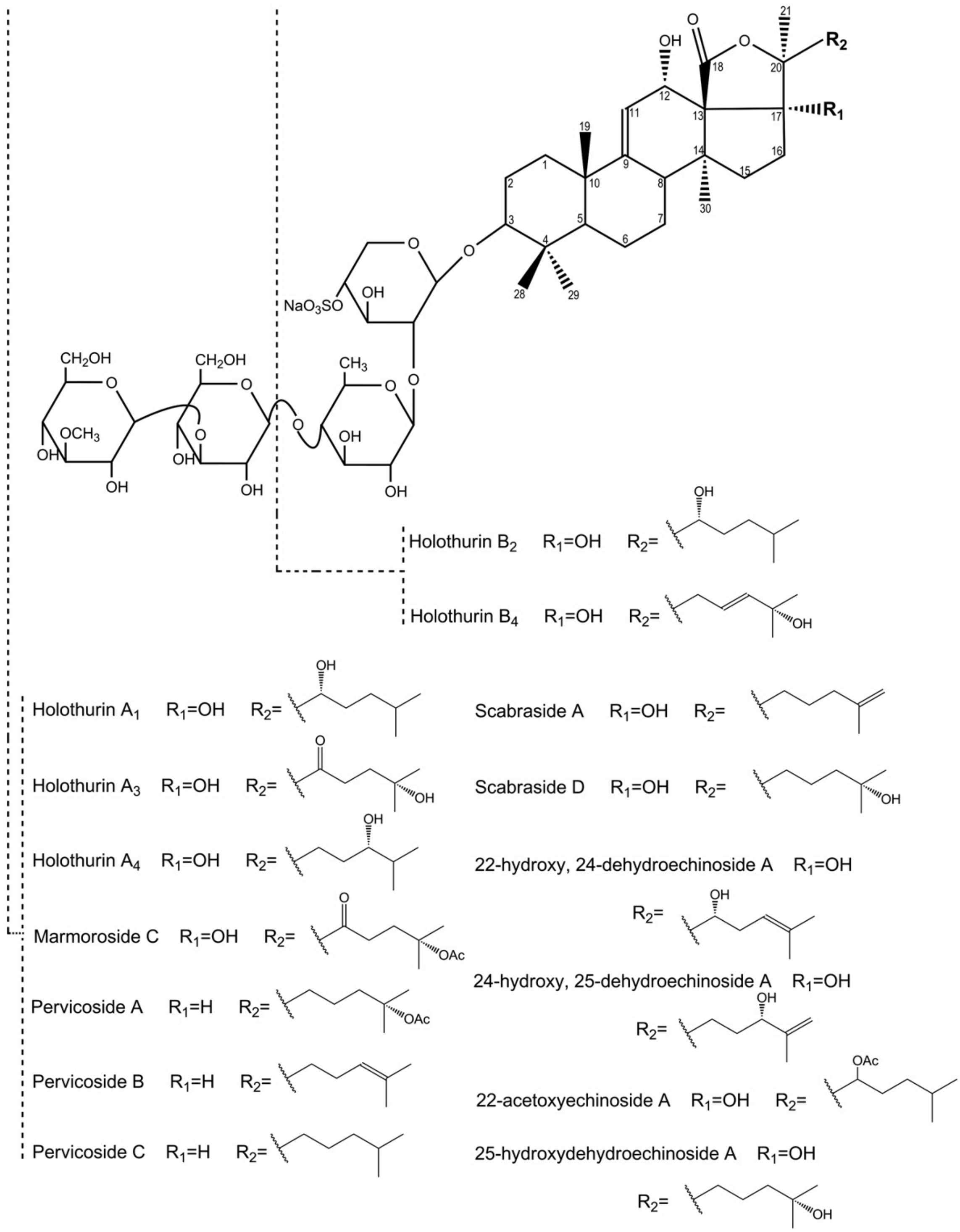

Fig. 9. Sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae.

with *Pearsonothuria* (desulfated holothurin A) and eighteen with the genus *Holothuria*, argusides B, C and F, bivittoside D, desulfated holothurins A and A$_1$, holothurinosides A, F, G, H, I, H$_1$, I$_1$, J$_1$ and K$_1$, 17-dehydroxyholothurinoside A, and impatiensides A and B.

Now, if we analyze the distribution of sulfated and non-sulfated triterpene glycosides some clear patterns can be found. *Actinopyga* presents only sulfated compounds, *Bohadschia* presents non-sulfated ones, *Pearsonothuria* has few non-sulfated saponins, and the genus *Holothuria* has both sulfated and non-sulfated compounds. These patterns corroborate recent findings (Caulier et al., 2011). Within the subgenera of *Holothuria*, seven contain only sulfated triterpene glycosides (*Acanthotrapeza*, *Cystipus*, *Holothuria*, *Platyperona*, *Roweothuria*, *Semperothuria*, and *Theelothuria*), one subgenus contains only non-sulfated saponins (*Panningothuria*), two subgenera have few non-sulfated ones (*Halodeima* and *Thymiosycia*), and the remaining four subgenera contain both sulfated and non-sulfated compounds in equal number approximately (*Mertensiothuria*, *Metriatyla*, *Microthele*, and *Stauropora*).

If we consider the degree of oxidation of the enlisted triterpene glycosides by the presence of hydroxyl group in C-17 of the aglycone or oxygen functions in the side chain, another clear pattern can be observed. The genera *Actinopyga*, *Pearsonothuria*, and *Holothuria* in general, have

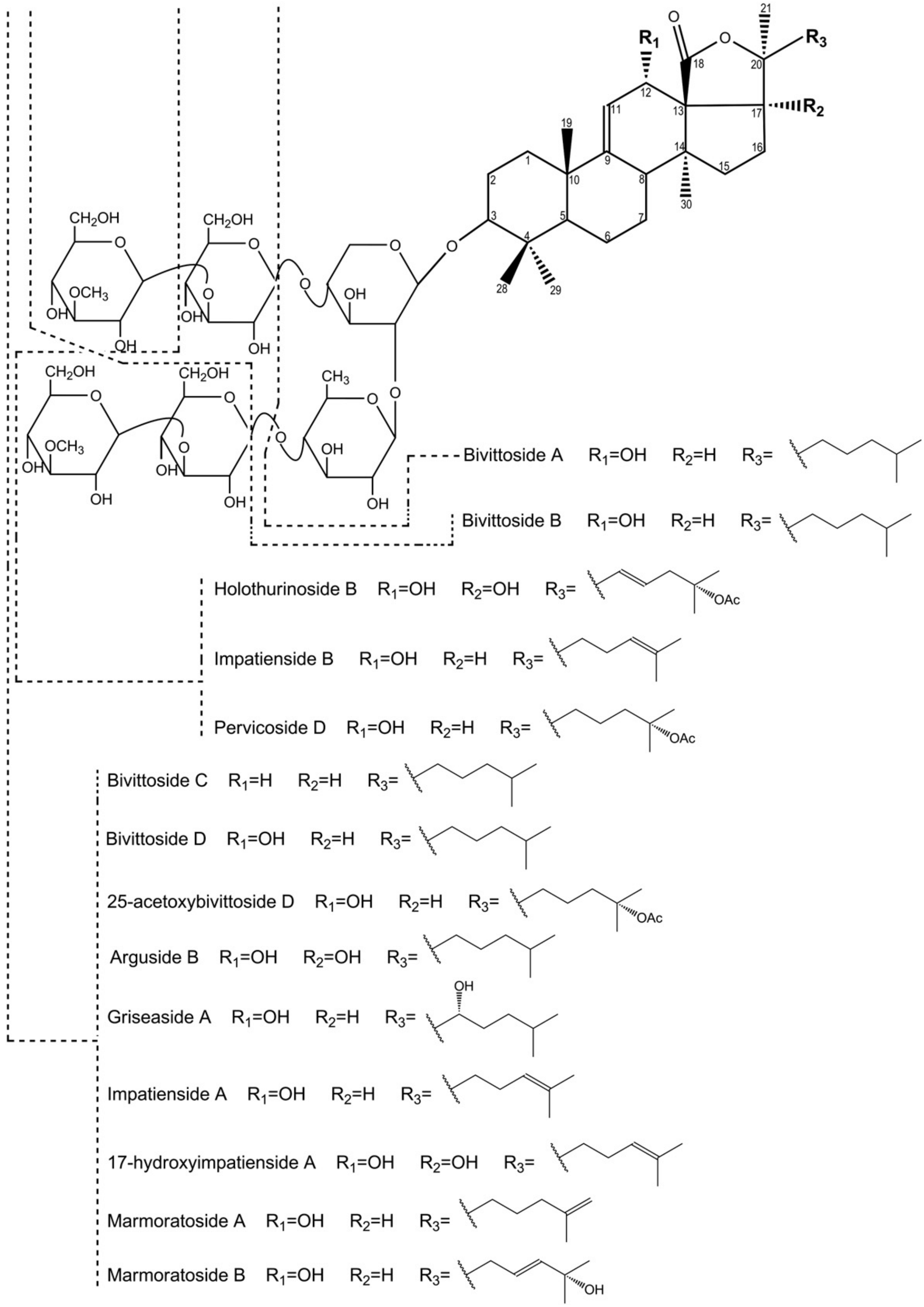

Fig. 10. Non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae.

greater amount of oxidized glycosides than genus *Bohadschia* (Fig. 6, A). The percentage of compounds with 17-OH in the aglycone for the genus *Holothuria* is barely above half percent and is due to the contribution each subgenus makes. Of the eight subgenera with more than five compounds described (Fig. 6, B), most of them have values above 50% for the presence of 17-OH in the aglycone, while three subgenera (*Panningothuria*, *Stauropora* and *Thymiosycia*) have percentages below 50. Nonetheless, the values in the eight subgenera for oxygen functions

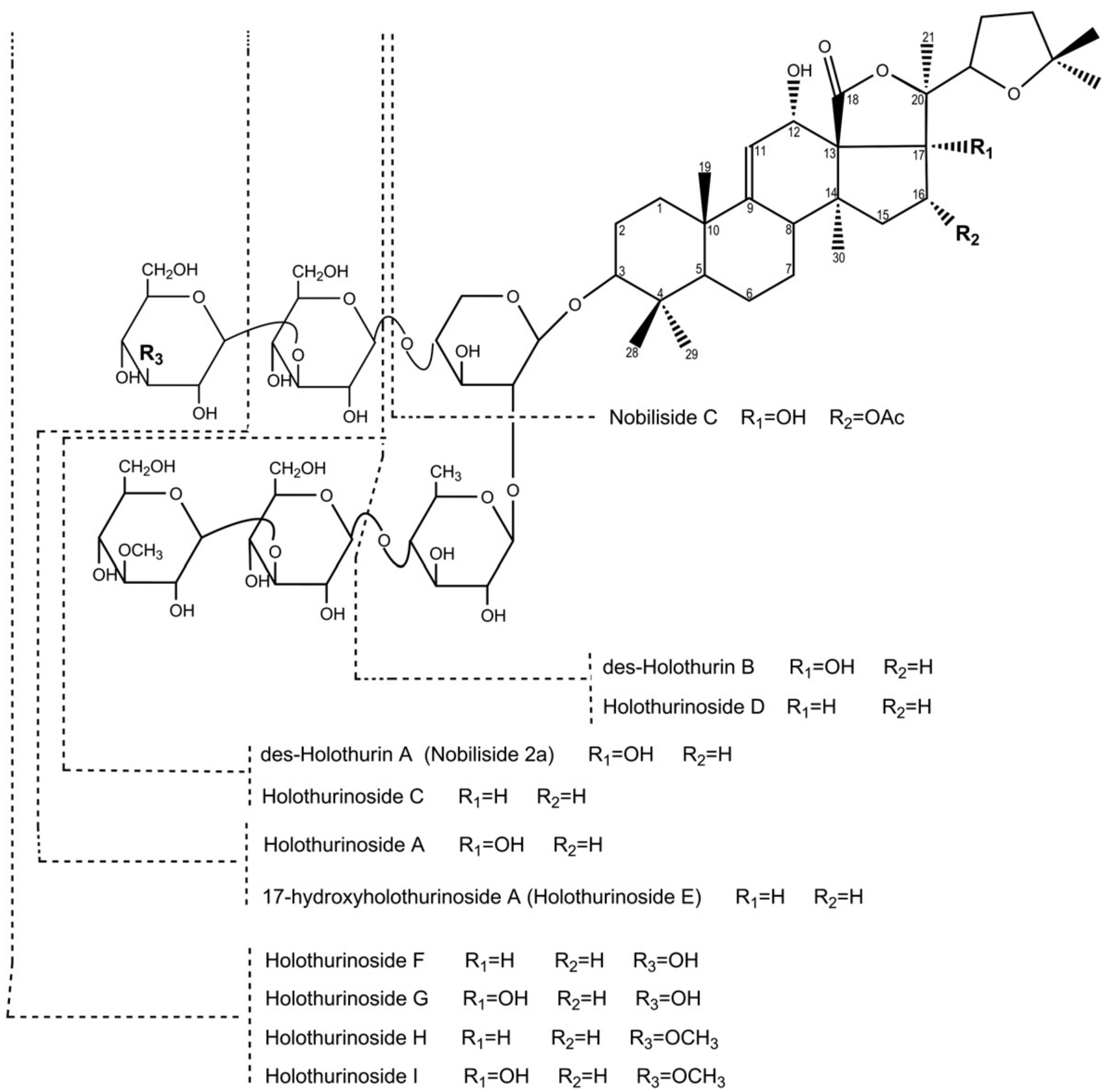

Fig. 11. Non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae. The suffix des- refers to desulfated compounds.

in the side chain are above 65%, making the compounds present in the genus *Holothuria* more oxidized than the ones present in *Bohadschia*, which both values are below 50%.

In the present review, we could find species with different number of described triterpene glycosides, from only one compound per species [*Holothuria* (*Cystipus*) *cubana*, *H.* (*Platyperona*) *difficilis*, *H.* (*Semperothuria*) *cinerascens*, and *H.* (*S.*) *surinamensis*] up to 34 known and 39 nonidentified compounds in a single species (*Bohadschia cousteaui*). This great difference is due to an unequal effort in the study of the species, as we can tell by the number of manuscripts found in the literature regarding each species. The sensibility and resolution of the equipment used to characterize the glycosides is another reason for the different number of compounds. Only in few recent papers the use of different techniques of characterization allowed the identification of isomers, of which many still remain unidentified.

The number of triterpene glycosides of sea cucumbers of family Holothuriidae as listed in this review is what currently can be found in literature. This count will rise abruptly as more species are studied, the unidentified compounds described and more sensitive equipment used. Hence, the number of saponins is still underestimated.

4. Biological considerations

If we consider the presence of triterpene glycosides as a chemical defense against predators, the unique distribution of sulfated, non-sulfated, and oxidized compounds in Holothuriidae may relate to the presence, expellability, and stickiness of the Cuvierian tubules, a defensive organ present only in this family.

Actinopyga species generally live hidden at night or during the day (Purcell et al., 2012). In many species Cuvierian tubules have not been detected, and when they have been documented, they possessed a different ultrastructure and appeared to be nonfunctional, at least not in the usual way, i.e. they are expellable and sticky (VandenSpiegel and Jangoux, 1993). Yamanouchi (1955) nevertheless documented considerable amounts of triterpene glycosides in Cuvierian tubules, suggesting their defensive function. Recently it has been demonstrated that sea cucumber while being under stress may release the saponins into the seawater from the skin or from interior organs (Van Dyck et al., 2011). This phenomenon may explain why the genus *Actinopyga* contains only sulfated triterpene glycosides in both the skin and the Cuvierian organs, and in different number and concentration in each body part. *Actinopyga* contains a few highly concentrated triterpene glycosides in

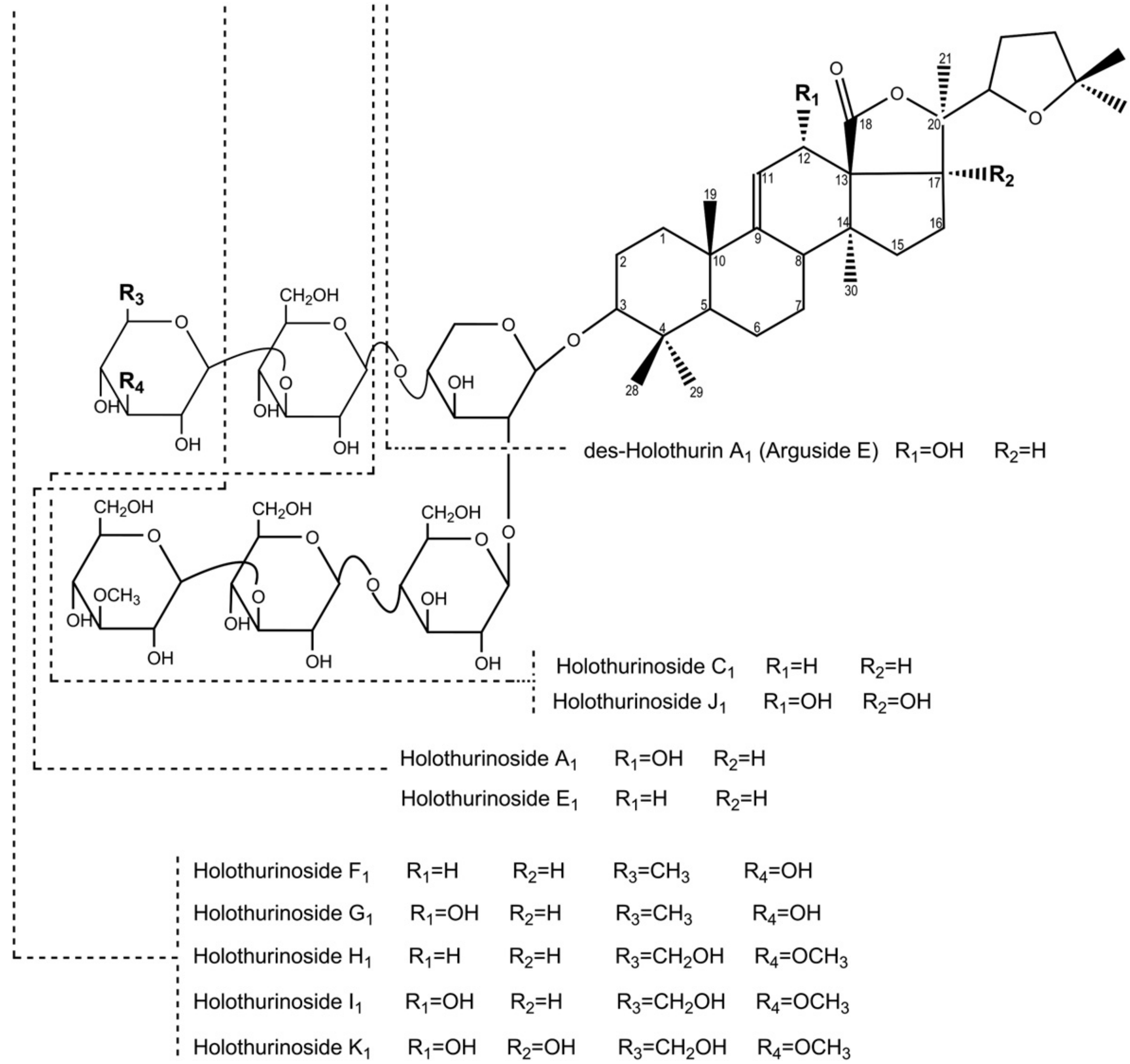

Fig. 12. Non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae. The suffix des- refers to desulfated compounds.

the Cuvierian tubules (like Van Dyck et al., 2010 found for *Actinopyga echinites*) and a greater diversity but in lower concentration in the skin. In the absence of a physical defense mechanism (expelling the Cuvierian tubules), the species of this genus may release these compounds from the skin and/or the Cuvierian tubules through the cloacum into the water. Since these saponins have a sulfate group and most of them are oxidized, they are more soluble in water and more toxic (Kalinin, 2000) to the skin or gills of the predator than are non-sulfated saponins. They may act as a warning signal of the bad taste of the animal (Van Dyck et al., 2011).

Bohadschia species have on the other hand a well-developed Cuvierian organ that is fully expellable and sticky. *Bohadschia* species have non-sulfated mainly non-oxidized compounds in their body wall and defensive organ. Non-sulfated and non-oxidized saponins are less soluble in water and less toxic, so it is possible that the main defense of *Bohadschia* species has evolved more towards full functionality of Cuvierian organs rather than to fast toxicity of sulfated saponins. Since the saponins slowly diffuse into the water because of the absence of sulfate and hydroxyl groups, it takes longer for them to reach the predator. As some of these sea cucumbers live partially or fully uncovered during day or at night, it is a plausible explanation that a quicker defense should be the expulsion of the Cuvierian tubules, while releasing the saponins from the skin into the water. If the predator is confronted with the defensive organ, the higher number and more concentrated saponins in this structure than in the body wall, will affect it due to the membranolytic properties of these compounds. Van Dyck et al. (2010) explain that the non-sulfated saponins may stay longer in the defensive organs creating in the predator a conditioned response to these compounds, so when it senses them in lower amounts and number (like when secreted only from the body wall) they will act as deterrent to the attack.

Most of the species of the genus *Holothuria* live under rocks, covered with sand, live uncovered, or only come out at night. Some species do not have Cuvierian tubules, others have the structures but they are never expelled, and some have Cuvierian tubules and readily expel them [e.g. *Holothuria* (*Mertensiothuria*) *leucospilota*, *H.* (*Platyperona*) *difficilis*, *H.* (*Stauropora*) *pervicax*]. *Holothuria* species differ in their content of sulfated and non-sulfated compounds, most of them oxidized, which make them a little more hydrophilic than the non-sulfated compounds of *Bohadschia*. For several species for which only holothurin A and/or holothurin B have been described (Tables 3, 4, 5, and 6), the relationship between the Cuvierian organs and the presence of sulfated compounds is difficult to establish. Apparently, since most of these species do not have Cuvierian tubules [*Holothuria* (*Acanthotrapeza*) *coluber*, *H.* (*Cystipus*) *cubana*, *H.* (*Halodeima*) *mexicana*, *H.* (*Holothuria*) *tubulosa*, *H.* (*Metriatyla*) *martensii*, *H.* (*Microthele*) *nobilis*, *H.* (*Semperothuria*) *cinerascens*, *H.* (*S.*) *surinamensis*, *H.* (*Theelothuria*) *squamifera* and *H.* (*Thymiosycia*) *gracilis*] the relationship between

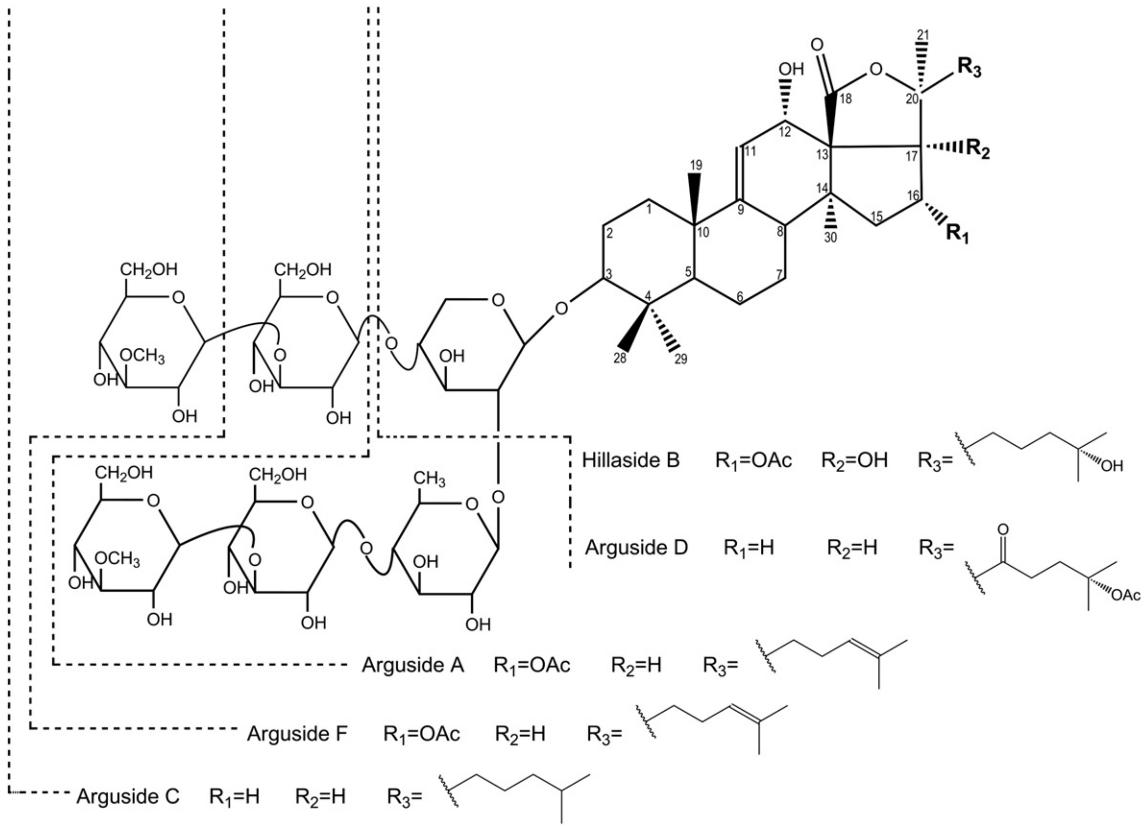

Fig. 13. Non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae.

sulfated glycosides and absence of these organs is maintained. Such a conclusion is, however, premature given the few species that have been investigated for triterpene glycoside content, especially since we know of three species that have or expel their defensive organ [*Holothuria* (*Halodeima*) *pulla*, *H.* (*Metriatyla*) *martensii*, *H.* (*Platyperona*) *difficilis*] but contain only sulfated glycosides.

For species with a higher number of triterpene glycosides, the pattern is more evident in the ones that lack or do not expel the Cuvierian tubules. *Holothuria* (*Halodeima*) *atra*, *H.* (*H.*) *edulis*, *H.* (*H.*) *floridana*, *H.* (*H.*) *grisea*, *H.* (*Microthele*) *whitmaei*, *H.* (*Metriatyla*) *scabra*, and *H.* (*Thymiosycia*) *arenicola* do not have defensive organs, and most or all of their components are sulfated ones. Most of these species live exposed almost all the time and have a higher number of saponins than the ones that live always concealed. For the species *H.* (*Microthele*) *fuscopunctata* and *H.* (*Metriatyla*) *lessoni* which have almost the same number of sulfated and non-sulfated compounds, lack defensive organs and live exposed, the pattern is not clear. Another defense that sea cucumbers have is evisceration of the entire digestive track through the cloacum. Perhaps this system of defense occurs more commonly in the species lacking Cuvierian tubules, so maybe this is the reason why a really high number of glycosides was discovered in the viscera of *H.* (*Metriatyla*) *lessoni*.

Following the pattern found for the species with expellable Cuvierian tubules, all or most of their triterpene glycosides should lack a sulfate group. Nevertheless, only in *Holothuria* (*Panningothuria*) *forskali* is evident, because it contains non-sulfated compounds in a great number. *H.* (*Mertensiothuria*) *hilla*, *H.* (*M.*) *leucospilota*, *H.* (*Stauropora*) *fuscocinerea*, *H.* (*S.*) *pervicax*, and *H.* (*Thymiosycia*) *impatiens* have more sulfated than non-sulfated compounds (Tables 3 and 4). Since most of these species live always concealed or buried during the day, this may be a factor in the presence of sulfated compounds. If they were found and attacked, the presence of these saponins would act quickly together with the Cuvierian tubules to deter the predator.

Pearsonothuria graeffei has three times more sulfated saponins than non-sulfated ones, for a total of twelve compounds, most of which are oxidized. The Cuvierian organs of this species are seldom expelled and they are not sticky. Since this species always lives exposed on the coral reef, it is probable that it uses a mixed defensive system, combining both powerful chemical protection (sulfated and oxidized saponins) and physical protection (semi-functional Cuvierian tubules).

Seemingly, the defense system of species of sea cucumbers of family Holothuriidae is complex, with different combinations of the presence or absence of Cuvierian organs and sulfated, non-sulfated, and oxidized saponins for buried or exposure habits.

5. Well done taxonomy will always be progressive

Taxonomy delivers end products such as Linnean names and predictive classifications that are based on phylogenetic relationships. These end products of taxonomy are important not only to protect and study biodiversity (Samyn and De Clerck, 2012), but also to frame observations on biodiversity components in an evolutionary framework.

The present study on the variation of saponin signatures across different taxa in the Holothuriidae is in this way not different in that only correct identifications of the investigated species will reveal natural patterns in the observed distribution of saponins. Taking this into account, it is quite a problem that of the examined 83 papers, only 22 reported that a sea cucumber taxonomist did the identification of the investigated ns) and/or that voucher specimens were deposited in a reference collection (e.g. a natural history museum). This makes understanding and interpreting saponin signatures in a phylogenetic context difficult and even unrepeatable. For future research on saponin distribution we therefore recommend that, (i) a competent taxonomist identifies the species, preferentially through an integrative approach (Padial et al., 2010; Kim et al., 2013); (ii) vouchers be deposited in recognized natural history depositories to allow cross-checking of the

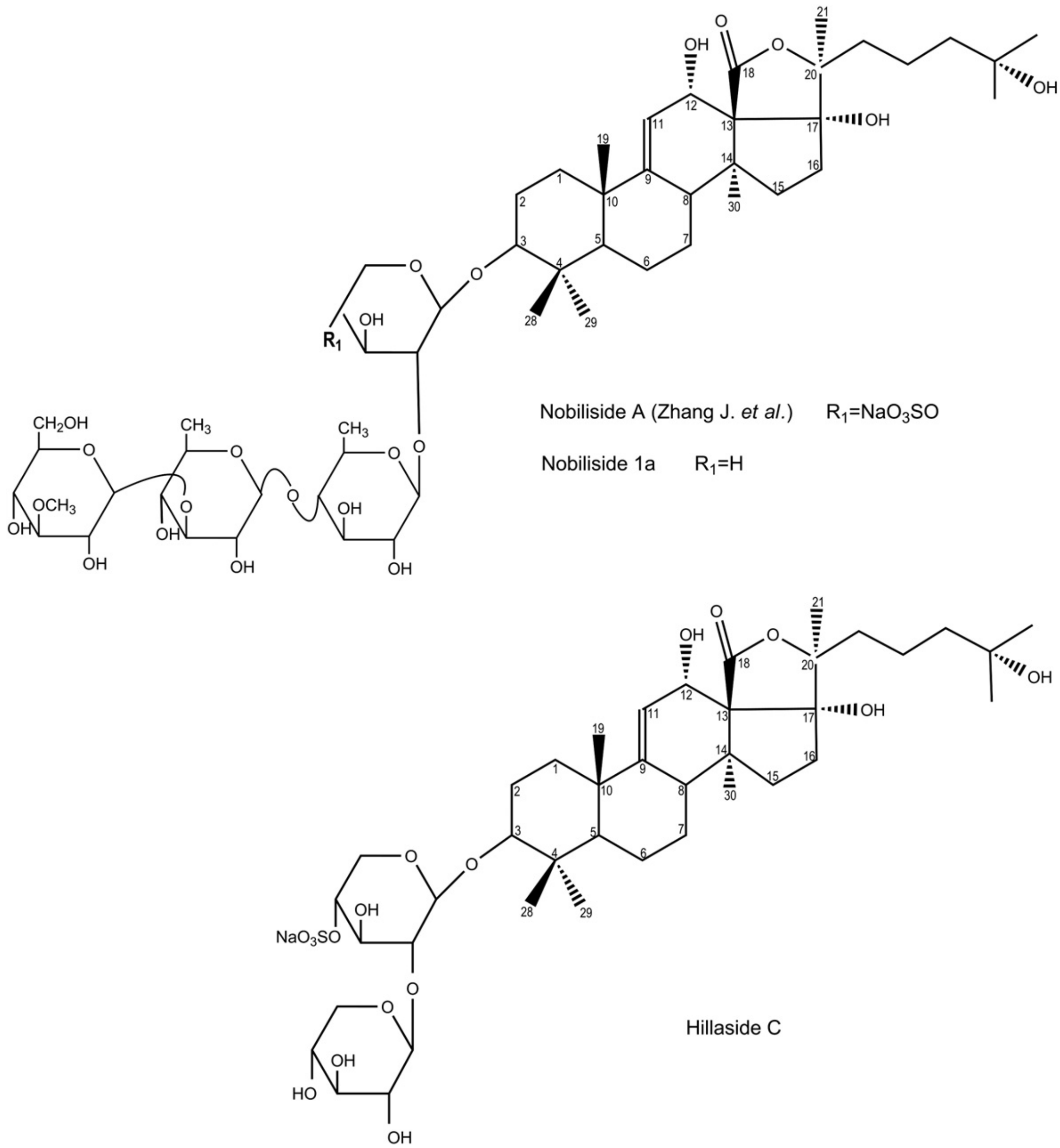

Fig. 14. Sulfated and non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae. The structure of nobiliside A is the one described by Zhang J. et al. (2008) (see text).

name given by taxonomists; (iii) specimens are adequately labeled in terms of locality and season to allow interpretation of geographical, seasonal, and/or reproductive variations (Matsuno and Ishida, 1969), and (iv) identifications are backed up as much as possible with genetic data to account for cryptic species.

The problem of correct identification seems trivial, but in a taxon as sea cucumbers where the last decennium has embraced the principles and methodologies of phylogenetic and integrative taxonomy it is crucial. O'Loughlin et al. (2012) for instance gives adequate practice in how species can be recognized through an integrative taxonomy that respects the rules of zoological nomenclature.

Most of the species covered in this review should have been identified through analysis of the types and distribution of ossicles in at least the body wall and the podia. In general, the species of the genus *Actinopyga* can be identified by color and a certain combination of ossicles. This is important to do, because some species have marked varieties in coloration for the Pacific and Indian Ocean (*Actinopyga echinites*, *A. mauritiana*) or a variation in color throughout their range of distribution (*A. agassizii*, *A. lecanora*, *A. miliaris*). The species named in the references as *Actinopyga flammea* is probably the commercially exploited species that has recently been identified genetically as *A.* aff. *flammea* (Purcell et al., 2012).

In *Bohadschia* species on the other hand, their simple ossicles are of limited use in identification at this taxonomic level. Species are more easily defined by skin coloration and geographic distribution, and such groups can be confirmed genetically (Kim et al., 2013). Nevertheless, there is still some variation in coloration, and expert advice should be sought. *Bohadschia argus*, for example, is distributed only in the Indo-West Pacific Ocean and its color pattern and variation are similar to *B. ocellata*; *B. marmorata* is now recognized as a single body form and pattern of coloration, whereas the species *B. subrubra* and *B. vitiensis* have a lot of variation in color (Kim et al., 2013).

For *Holothuria*, a genus with many cryptic species, identification by color and habitat should always be confirmed by the characteristics of ossicles from various body parts as well as by DNA data whenever possible. Among the species included in this review, *Holothuria* (*Metriatyla*)

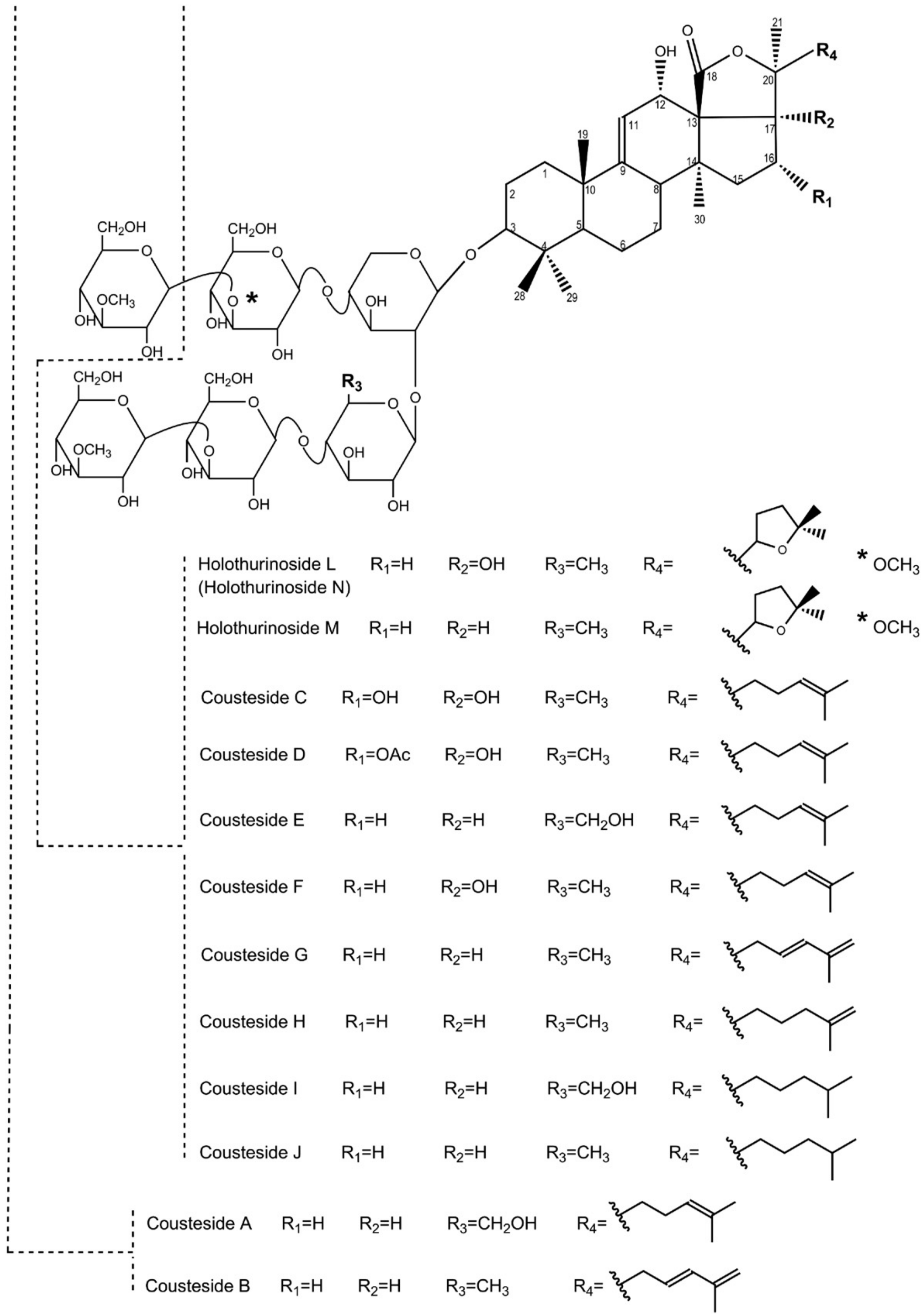

Fig. 15. Non-sulfated triterpene glycosides documented for sea cucumbers of the family Holothuriidae.

scabra, have different patterns of coloration in the Pacific and the Indian Ocean, each with its respective variation. *H.* (*M.*) *scabra* must be distinguished by ossicle characteristics from the new species *H. (M.) lessoni* (Massin et al., 2009), the golden sandfish, which has three main patterns of coloration. Other species with both variation in color and similar color patterns is *H.* (*M.*) *fuscogilva*, which has an Indo-Pacific distribution and different color morphs in each ocean. Possibly, the Indian Ocean form is a new species that has been mistaken for *H.* (*M.*) *fuscogilva*. For the species *H.* (*Microthele*) *nobilis* and *H.* (*M.*) *whitmaei* the morphology would be sufficient to identify them but it is important to remember that the former is present only in the Indian Ocean, and the latter in the Western Pacific Ocean (Uthicke et al., 2004). Ossicles must also aid the identification of *H.* (*Stauropora*) *fuscocinerea* and *H.* (*S.*) *pervicax* since these are so similar morphologically (Purcell et al., 2012). Several species in this review have an Indo-Pacific distribution, *Holothuria* (*Halodeima*) *edulis*, *H.* (*H.*) *atra*, *H.* (*Platyperona*) *difficilis*, *H.* (*Semperothuria*) *cinerascens*, *H.* (*Stauropora*) *fuscocinerea*, *H.* (*Mertensiothuria*) *leucospilota*, *H.* (*M.*) *hilla*, *H.* (*Thymiosycia*) *arenicola*, and *H.* (*T.*) *impatiens*. Of these, at least the last three are a species complex that can be differentiated genetically in a more reliable way than morphologically.

The genus *Pearsonothuria* has a unique pattern of coloration and dermal ossicles, so field identification is not an issue.

Due to the mis-identification or use of an incorrect method of identification, some species and their triterpenoid glycosides have been excluded from this review because of concerns regarding their identification, and some others, although included, must be treated cautiously. Yasumoto et al. (1967) refer to *Holothuria lubrica* from the coasts of Japan, yet the species is distributed only on the Pacific coast of the American continent; species resembling *H. lubrica* morphologically and occurring in Japan are either *Holothuria moebii* or *Holothuria erinaceus*.

The papers that used non-valid or misspelled names may not have identified by a taxonomist and must be viewed circumspectively. This is the case with Kitagawa et al. (1981a), Kobayashi et al. (1991), Lakshmi et al. (2008, 2011), Melek et al. (2012), Radhika et al. (2002), Wu et al. (2006a,b,c), Zhang J. et al. (2008), Yuan et al. (2008b, 2009), Li et al. (2010) and Zhang (2011). In this review and in Tables 3, 4, 5, and 6, the valid name has been used, assuming that the identification was correct, *Actinopyga* aff. *flammea* instead of *A. flammea*, *Bohadschia vitiensis* instead of *B. bivittata* or *B. tenuissima*, *Holothuria* (*Mertensiothuria*) *leucospilota* instead of *H. vagabunda*, *H.* (*Microthele*) *fuscopunctata* instead of *H. axiloga* and *H.* (*M.*) *whitmaei* instead of *H. nobilis* for species distributed in the Pacific Ocean. Other papers not included here reported compounds for specimens of sea cucumbers identified only at the genus level.

6. Chemical nomenclature

During this review we frequently found triterpene glycosides with the same structure but different names (synonyms). Since the publications are in a range of languages and are published in national journals, the exchange of information is sometimes difficult, and this contributes to duplicity of names or compounds. In this review, at least thirteen triterpene glycosides have identifiable synonyms (same structure, different name), which hindered the identification of compounds and distribution by genus.

A serious problem of homonyms (same name, different structures) was found for the nobiliside compounds. Mainly two groups of authors (Zhang J. et al. and Wu et al.) have isolated and described these glycosides. Wu et al. (2006b) isolated nobilisides A, B, and C and Zhang J. et al. (2008) isolated nobilisides A, B, C, D, E, F, G, H, I, and J. The structure of nobiliside A differs between the two publications; nobiliside A (Wu et al., 2006b) has a sulfate group, whereas nobiliside A (Zhang J. et al., 2008) is a non-sulfated glycoside with only one monosaccharide and two double bonds in the holostane. The structures of two different compounds, nobilisides B and C *sensu* Wu et al. (2006c) are the same as nobiliside B and C *sensu* Zhang J. et al. (2008). Wu et al. (2006c) also isolated nobilisides 1a and 2a, the latter having the same structure as desulfated holothurin A. Later, Zhang (2011) described two novel compounds, nobilisides I (roman number one instead of capital i) and II, both with the same formula as previously described compounds, holothurin A and ananaside C, respectively (Wu, 2007). These are examples of encountered difficulties in the present review, and they demonstrate the lack of information at local and international level; it seems that more importance is given in publication or patenting a product than to the verification of its novelty.

Besides the taxonomic effort to correctly identify the species used, enhanced communication is needed between specialists that study triterpenoid glycosides on different species of sea cucumbers; this is to avoid problems giving different names or multiple formulas to these compounds.

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