Phylogenetic ecology of octocoral - gastropod associations

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Chapter 6

A molecular and morphological exploration of the generic boundaries in the family Melithaeidae (Coelenterata: Octocorallia) and its taxonomic consequences

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Abstract

The validity of the currently recognized melithaeid genera (*Acabaria*, *Clathraria*, *Melithaea*, *Mopsella*, *Wrightella*) with the exception of the recently added genus *Asperaxis*, has puzzled scientists for almost a century. Diagnostic morphological characters are often missing or are obscured by the variation in sclerite forms. Consequently, species are difficult to assign to genera. In this study the current genera and their taxonomic positions are reviewed and reassessed based on material collected from the Indo-Pacific, Red Sea and Indian Ocean as far south as South Africa. Molecular data were obtained for four different loci, both mitochondrial (COI, mtMutS, ND6) and nuclear (28S rDNA). Combining the molecular and morphological data revealed that all former genera, except for the monotypic genus *Asperaxis* and the genus *Wrightella* are paraphyletic. Molecular data for the two subfamilies (Asperaxinae and Melithaeinae) within the Melithaeidae, in comparison with the outgroup, indicated that the family is also paraphyletic. Furthermore we observed that species did not cluster according to their present morphological classification but instead clustered according to a biogeographical pattern. Species from the Red Sea, Indian Ocean and Central Pacific, respectively, grouped into well-supported clades. Consequently, we did not find morphological- or phylogenetic support to maintain the generic names *Acabaria*, *Clathraria*, *Mopsella* and *Wrightella*. Therefore these names are synonymised with the oldest available generic name, *Melithaea*. As a result, five secondary homonyms originated; these junior homonyms are herein renamed, viz. *Melithaea hendersoni* nom. nov, *Melithaea mcqueeni* nom. nov., *Melithaea shanni* nom. nov., *Melithaea thorpeae* nom. nov., and *Melithaea wrighti* nom. nov. Additionally, neotypes are selected for *Melithaea ochracea* to stabilize the genus *Melithaea*, and for *Acabaria rubra*.

Introduction

The Melithaeidae (Cnidaria: Anthozoa) are gorgonians (also commonly known as sea fans), distributed from the Red Sea (Grasshoff, 2000), Indian Ocean (Thomson, 1916, Ofwegen, 1987, Ofwegen, 1989 and Williams, 1992) and Indo-West Pacific (Ofwegen, 1987, Grasshoff, 1999 and Ofwegen *et al*., 2000) to Hawai'i (Bayer, 1956). Based on their internal skeletal elements called sclerites, which are used for genus and species identifications, five genera have traditionally been distinguished. These are *Acabaria* Gray, 1859, *Clathraria* Gray, 1859, *Melithaea* Linnaeus, 1758, *Mopsella* Gray, 1857 and *Wrightella* Gray, 1870. Recently, *Asperaxis* Alderslade (2006) was added. Unfortunately, the sclerites do not always demonstrate clear diagnostic characteristics to assign species to a specific genus. In many cases, species exhibit characters that are consistent with their placement in multiple genera. Therefore the taxonomic position and validity of the genera within the family Melithaeidae have puzzled taxonomists for over a century. Confusion at the generic level is also caused by the many intermediate sclerite forms observed when large numbers of specimens are studied. Often these extensive investigations revealed that specimens may show much variation in morphological characters (Hickson, 1937), obscuring the pre-determined generic borders and keeping taxonomists debating the validity and status of most of the described genera (Hickson, 1937, Broch, 1939 and Fabricius and Alderslade, 2001). Although his overview seemed straightforward, Hickson (1937, p. 89) himself found his proposed classification problematic: "The division of them [Melithaeidae] into definite generic and even specific forms is quite artificial and represents nothing in Nature". Despite these taxonomic uncertainties, species belonging to the Melithaeidae have frequently been used in ecological and chemical studies (Goh *et al*., 1999, Goh and Chou, 1994, Matsumoto, 2004, Oppen *et al*., 2005, Shin and Seo, 1995 and Kobayashi and Kanda, 1991) and in studies of associated fauna such as crustaceans, molluscs, echinoderms and fish (Goh *et al*., 1999 and Kumagai and Aoki, 2003).

As recently as 1999, Grasshoff proposed alteration of Hickson's classification by suggesting synonymising the genera *Melitella*, *Mopsella* and *Wrightella* with the genus *Melithaea*. Consequently, only three genera would have been maintained: *Acabaria*, *Clathraria* and *Melithaea*. Subsequently, Grasshoff (2000) revised his proposed classification and resurrected the genus *Mopsella*. However, Fabricius and Alderslade (2001) maintained the classification as proposed earlier by Hickson (1937) with an additional comment, saying that based on the considerable overlap in sclerite morphology between the alleged genera, they probably represent a single genus.

The latest addition to the family Melithaeidae is the genus *Asperaxis*. This genus was considered to be morphologically so markedly different compared to the other genera, that it was even placed in a new subfamily, Asperaxinae Alderslade, 2006. Only recently molecular data were used to investigate the phylogenetic relationships among the genera and species within the Melithaeidae. Aguilar-Hurtado *et al*. (2012) included the genera *Acabaria*, *Melithaea* and *Mopsella* in their phylogenetic reconstruction based on two genetic markers, COI and 28S rDNA. Their results suggest that the genetic boundaries of these three genera are in concordance with the morphological classification as suggested by Hickson (1937). However, their study includes only specimens collected from subtropical Japanese waters, thereby excluding *Clathraria* Gray, 1859 and *Wrightella* Gray, 1870, genera that are predominately found in the Red Sea and the Indian Ocean. To gain more comprehensive insights into the phylogenetic history of the genera and species within the Melithaeidae, in addition to museum specimens already available, samples for this study were collected from most areas within the known geographic distribution of Melithaeidae, and subsequently used for phylogenetic studies.

Material and methods

Specimen collection

Melithaeidae were collected in Australia, Chagos Archipelago, Eritrea, Indonesia, Israel, Japan, Malaysia, Maldives, New Caledonia, Palau, Seychelles, South Africa, Tanzania and Vietnam (Fig. 1). In total, specimens are from 18 different eco-regions (Marine Ecoregions of the World (MEOW)) (Spalding *et al*., 2007).

All voucher specimens and respective subsamples were stored in either 70% or 96% ethanol except for the Malaysian and some of the Indonesian samples, which were stored in a 20% salt-saturated DMSO-buffer. All specimens are stored in the collections of the Naturalis Biodiversity Center, the Netherlands. An overview of the specimens and their locality and collection data are presented in Table 1.

In addition, 44 type specimens were studied, in an attempt to identify the specimens used in the molecular phylogeny (Table 2; App. 2, Pl. 1-44).

For each (type) specimen microscope slides were made. A small piece (<1 cm) of the distal part of the octocoral was dissolved in a 4% household bleach solution to isolate sclerites. The sclerites were washed with tap water (five times), followed by the same number of wash steps with demineralised water. Sclerites were dried on a hot plate and subsequently embedded in Euparal for visualisation with a Leica DM LB2 light microscope. In addition, sclerites of specimens that represent specific clades or needed further morphological investigations were mounted on SEM stubs and coated with Pd/Au for imaging on a JEOL JSM6490LV scanning electron microscope operated at high vacuum

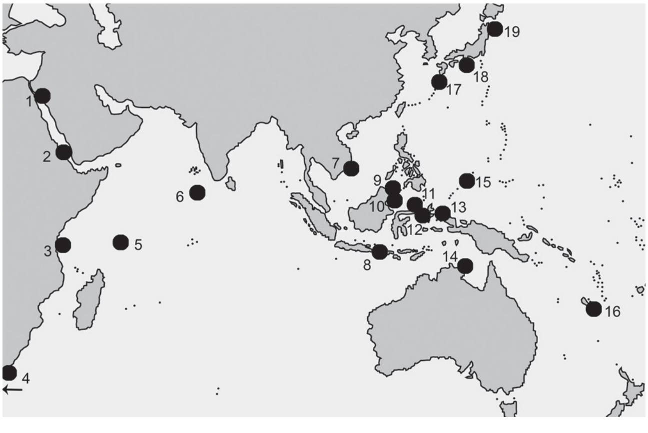

Fig. 1. Overview of the 19 localities where Melithaeidae were collected for this study. See Table 1 for more specific locality data.

Table 1. Overview of all samples including species information, locality data and additional collecting data.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
AKM0615	*Acabaria* sp.	*Melithaea* sp.	Japan, Iwate Prefecture, Otsuchi Bay, entrance of Otsuchi Bay	39.365283 N
AKM0664	*Acabaria tenuis* Kükenthal, 1908	*Melithaea tenuis* (Kükenthal, 1908) comb. nov.	Japan, Wakayama Prefecture, off Tanabe Bay	33.650833 - 33.649333 N
AKM0724	*Acabaria* sp.	*Melithaea* sp.	Japan, Kagoshima prefecture, off Tanegashima Island	30.410333 - 30.415833 N
AKM0743	*Mopsella* sp.	*Melithaea* sp.	Japan, Kagoshima Prefecture, Sata Misaki Cape	30.93333 - 30.938333 N
AKM0980	*Melithaea* sp.	*Melithaea* sp.	Japan, Kagoshima Prefecture, Tsukara-se	31.315833 - 31.308333 N
AKM1148	*Acabaria* sp.	*Melithaea* sp.	Japan, Okinawa Prefecture, off Kerama Island	26.1575 - 26.160833 N
AKM1175	*Acabaria* sp.	*Melithaea* sp.	Japan, Okinawa Prefecture, off Kerama Island	26.0765 - 26.076 N
AKM1200	*Acabaria* sp.	*Melithaea* sp.	Japan, Iwate Prefecture, Otsuchi Bay, off Ohako-zaki	39.35N
AKM1252	*Acabaria* sp.	*Melithaea* sp.	Japan, Iwate Prefecture, Otsuchi Bay, off Ohako-zaki	39.35N
RMNH.Coel.19852	*Melithaea ochracea* (Linnaeus, 1758)	*Melithaea ochracea* (Linnaeus, 1758) comb. nov.	Indonesia, Moluccas, Ambon, Hitu, N coast Mamala	3.537997 S
RMNH.Coel.24373	*Wrightella coccinea* (Ellis & Solander, 1786)	*Melithaea coccinea* (Ellis & Solander, 1786) comb. nov.	Chagos Archipelago, Salomon atoll, off Ile Fouquet	5.339111 S
RMNH.Coel.39452	*Mopsella* sp.	*Melithaea* sp.	Australia, Wigram Island, The English Companys Islands, eastern Arnhem Land, NT	11.7637 S
RMNH.Coel.39455	*Melithaea* sp.	*Melithaea* sp.	Australia, Cotton Island at mouth of the northern bay, The english Companys Islands, eastern Arnhem Land, NT	11.798125 S
RMNH.Coel.39466	*Melithaea* sp.	*Melithaea* sp.	Australia, off Rimbija Island, 3.2 km W of Cape Wessel, Wessel Islands, eastern Arnhem Land, NT	11.006967 S
RMNH.Coel.40034	*Mopsella* sp.	*Melithaea* sp.	Indonesia, Papua, Raja Ampat, S Gam, near mangrove border	0.51895 S
RMNH.Coel.40041	*Wrightella coccinea* (Ellis & Solander, 1786)	*Melithaea coccinea* (Ellis & Solander, 1786) comb. nov.	Seychelles, St. François atoll, W rim	7.25 S
RMNH.Coel.41099	*Acabaria* sp.	*Melithaea* sp.	Indonesia, NE Kalimantan, Berau Islands, Maratua Island, NE side	2.291444 N
RMNH.Coel.41100	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mid Rock Roach Reef	4.1775 N

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	Clade	ND6	COI	mtMutS	28S
142.000517 E	9/12/2005	78	A.K. Matsumoto	A	KC845768	KC802190	KC845667	KC845880
135.164833 - 135.169333 E	11/26/2005	180	A.K. Matsumoto	A	KC845778	KC802203	KC845668	KC845885
131.141 - 131.138667 E	2/23/2007	468-502	A.K. Matsumoto	A	KC845797	-	KC845684	KC845912
130.737167 - 130.723333 E	2/23/2007	116-120	A.K. Matsumoto	A	KC845782	KC802205	KC845669	KC845891
129.769167 - 129.766 E	3/6/2008	154-155	A.K. Matsumoto	A	KC845781	KC802204	KC845670	KC845890
127.448333 - 127.480167 E	12/15/2008	71-85	A.K. Matsumoto	B	KC845780	KC802201	KC845671	KC845889
127.461667 - 127.465833 E	12/16/2008	160-153	A.K. Matsumoto	A	KC845779	KC802202	KC845672	KC845888
142.00E	3/21/2008	ca. 90	K. Morita / A.K. Matsumoto	A	KC845769	KC802188	KC845666	KC845882
142.00E	5/9/2008	ca. 75	K. Morita / A.K. Matsumoto	A	KC845767	KC802189	KC845665	KC845881
128.206414 E	11/21/1990	10-15	J.C. den Hartog	B	KC845766	KC802206	KC845653	KC845879
72.276447 E	3/8/1996	-	G.B. Reinicke	D	KC845761	KC802114	KC845582	KC845868
136.531667 E	3/29/2004	13-16	H. Nguyen	B	KC845698	KC802185	KC845585	KC845878
136.48415 E	3/30/2004	13-16	D. DeMaria	A	KC845699	KC802116	KC845586	KC845824
136.7276 E	4/1/2004	30-40	P. Colin	B	KC845700	KC802117	KC845587	KC845856
130.641117 E	11/30/2007	22	F.R. Stokvis	A	KC845764	KC802145	KC845652	KC845867
52.733333 E	5/6-01-1993	<27	L.P. van Ofwegen	D	KC845759	KC802156	KC845580	KC845869
118.591389 E	10/10/2003	28	L.P. van Ofwegen & M. Slierings	A	KC845799	-	KC845693	KC845923
118.303361 E	11/29/2010	12	B.T. Reijnen	A	KC845701	KC802118	KC845596	KC845827

Table 1. Cont.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
RMNH.Coel.41101	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Alert Patches 3	4.162972 N
RMNH.Coel.41102	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Bohayen Island	4.466917 N
RMNH.Coel.41103	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Bohayen Island	4.466917 N
RMNH.Coel.41104	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Timba timba Island	4.560472 N
RMNH.Coel.41105	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Pandanan Island	4.576667 N
RMNH.Coel.41106	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Pandanan Island	4.576667 N
RMNH.Coel.41107	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Pandanan Island	4.576667 N
RMNH.Coel.41108	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mataking Island	4.582667 N
RMNH.Coel.41109	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Kulapuan Island 2 N	4.536 N
RMNH.Coel.41110	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Kulapuan Island 2 N	4.536 N
RMNH.Coel.41111	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mantabuan Island	4.632222 N
RMNH.Coel.41112	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mantabuan Island	4.632222 N
RMNH.Coel.41113	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Gaya Island 1 SE	4.624722 N
RMNH.Coel.41114	*Acabaria* sp.	*Melithaea* sp.	Indonesia, Sulawesi, Lembeh, Pulau Abadi	1.43354 N
RMNH.Coel.41115	*Melithaea* sp.	*Melithaea* sp.	Indonesia, NE Kalimantan, Berau Islands, Berau delta, Lighthouse 2 reef	2.159417 N
RMNH.Coel.41116	*Melithaea* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Bumbun Island W (channel)	4.461306 N
RMNH.Coel.41117	*Melithaea* sp.	*Melithaea* sp.	Seychelles, St. Joseph atoll, NW rim	5.4 S
RMNH.Coel.41118	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Sulawesi, Lembeh, Tanjung Labuhankompeni	1.43218 N
RMNH.Coel.41119	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Lombok	8.408333 S
RMNH.Coel.41120	*Acabaria* sp.	*Melithaea* sp.	Republic of Palau, Short Drop-Off, outer slope	7.258883 N
RMNH.Coel.41121	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Church Reef 2	4.68625 N
RMNH.Coel.41122	*Acabaria* sp.	*Melithaea* sp.	Indonesia, Sulawesi, Lembeh, Pulau Abadi	1.43354 N
RMNH.Coel.41123	*Mopsella* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Kulapuan Island 1 S	4.511472 N
RMNH.Coel.41124	*Melithaea* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Selakan Island	4.572806 N

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	clade	ND6	COI	mtMutS	28S
118.276611 E	11/30/2010	12	B.T. Reijnen	A	KC845702	KC802119	KC845597	KC845828
118.947667 E	12/8/2010	14	B.T. Reijnen	A	KC845709	KC802120	KC845599	KC845840
118.947667 E	12/8/2010	15	B.T. Reijnen	A	KC845710	KC802121	KC845600	KC845829
118.925111 E	12/8/2010	22	B.T. Reijnen	A	KC845711	KC802122	KC845601	KC845836
118.920583 E	12/8/2010	4	B.T. Reijnen	A	KC845712	KC802123	KC845602	KC845837
118.920583 E	12/8/2010	12	B.T. Reijnen	A	KC845713	KC802124	KC845603	KC845838
118.920583 E	12/8/2010	14	B.T. Reijnen	A	KC845714	KC802125	KC845604	KC845830
118.94625 E	12/8/2010	13	B.T. Reijnen	A	KC845715	KC802126	KC845592	KC845839
118.8385 E	12/9/2010	14	B.T. Reijnen	A	KC845703	KC802127	KC845607	KC845831
118.8385 E	12/9/2010	11	B.T. Reijnen	A	KC845704	KC802128	KC845608	KC845832
118.796833 E	12/10/2010	20	B.T. Reijnen	A	KC845705	KC802129	KC845609	KC845833
118.796833 E	12/10/2010	12	B.T. Reijnen	A	KC845706	KC802130	KC845664	KC845834
118.777472 E	12/10/2010	23	B.T. Reijnen	A	KC845707	KC802131	KC845610	KC845835
125.20628 E	2/3/2012	12	B.T. Reijnen	A	KC845783	KC802199	KC845663	KC845903
118.169833 E	10/5/2003	12	L.P. van Ofwegen & M. Slierings	A	KC845765	KC802158	KC845651	KC845842
118.635861 E	12/5/2010	14	B.T. Reijnen	A	KC845722	KC802187	KC845598	KC845841
53.316667 E	12/26/1992	-	L.P. van Ofwegen	A	KC845720	KC802186	KC845588	KC845825
125.18629 E	2/4/2012	6	B.T. Reijnen	A	KC845770	KC802198	KC845660	KC845904
116.07 E	8/4/2011	5	B.W. Hoeksema	A	KC845721	KC802138	KC845619	KC845826
134.52555 E	5/26/2011	24.4	C.S. McFadden	A	KC845741	KC802179	KC845628	KC845865
118.649028 E	12/13/2010	30	B.T. Reijnen	A	KC845708	KC802132	KC845614	KC845866
125.20628 E	2/3/2012	15	B.T. Reijnen	A	KC845784	KC802200	KC845659	KC845902
118.866222 E	12/9/2010	9	B.T. Reijnen	B	KC845733	KC802146	KC845606	KC845852
118.717861 E	12/12/2010	8	B.T. Reijnen	B	KC845730	KC802152	KC845613	KC845853

Table 1. Cont.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
RMNH.Coel.41125	*Mopsella* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mata Pahi Is.	4.580806 N
RMNH.Coel.41126	*Melithaea* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Mata Pahi Is.	4.580806 N
RMNH.Coel.41127	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Sulawesi, Lembeh, N Sarena Kecil	1.45746 N
RMNH.Coel.41128	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Halmahera, Tidore, Pulau Pilongga N	0.713833 N
RMNH.Coel.41129	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Short Drop-Off, inside wall	7.525383 N
RMNH.Coel.41130	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Siaes Tunnel	7.311433 N
RMNH.Coel.41131	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Wonder Channel	7.18115 N
RMNH.Coel.41132	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Turtle Cove	7.084633 N
RMNH.Coel.41133	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, KB Channel	7.309867 N
RMNH.Coel.41134	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Papua, Raja Ampat, SE Gam, Desa Besir	0.463367 S
RMNH.Coel.41135	*Acabaria* sp.	*Melithaea* sp.	Indonesia, Papua, Raja Ampat, Mayalibit Bay, E Manil Island	0.304133 S
RMNH.Coel.41136	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Wonder Channel	7.18115 N
RMNH.Coel.41137	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Ngerikuul Gap	7.3209 N
RMNH.Coel.41138	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, Turtle Cove	7.084633 N
RMNH.Coel.41139	*Melithaea* sp.	*Melithaea* sp.	Republic of Palau, KB Channel	7.309867 N
RMNH.Coel.41140	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Gaya Island 1 SE	4.624722 N
RMNH.Coel.41141	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Selakan Island	4.572806 N
RMNH.Coel.41142	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Timbun Mata Island	4.633222 N
RMNH.Coel.41143	*Acabaria* sp.	*Melithaea* sp.	Malaysia, Borneo, Semporna, Balusuan Island	4.685528 N
RMNH.Coel.41144	*Melithaea* sp.	*Melithaea* sp.	Vietnam, Con dao, Bong Lan Island, S side of Bong Lan	8.650667 N
RMNH.Coel.41145	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Halmahera, Ternate, Sulamadaha Beach	0.863222 N
RMNH.Coel.41146	*Melithaea* sp.	*Melithaea* sp.	Indonesia, Sulawesi, Lembeh, Lobangbatu	1.43407 N
RMNH.Coel.41147	*Mopsella* sp.	*Melithaea* sp.	Indonesia, Bali, Tanjung Benoa, Loloan Benoa	8.762778 N
RMNH.Coel.41148	*Mopsella* sp.	*Melithaea* sp.	Republic of Palau, Neco Channel	7.205267 N
RMNH.Coel.41149	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Table Top	33.982 S
RMNH.Coel.41150	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41151	*Melithaea* sp.	*Melithaea* sp.	South Africa, Cape Peninsula, Vulcan Rock	34.066167 S

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	clade	ND6	COI	mtMutS	28S
118.547056 E	12/17/2010	10	B.T. Reijnen	B	KC845731	KC802153	KC845617	KC845854
118.547056 E	12/17/2010	10	B.T. Reijnen	B	KC845732	KC802154	KC845618	KC845855
125.22711 E	2/16/2012	28	B.T. Reijnen	B	KC845774	KC802194	KC845661	KC845908
127.479278 E	11/12/2009	16	B.T. Reijnen	B	KC845727	KC802140	KC845595	KC845862
134.525383 E	5/19/2011	-	C.S. McFadden	B	KC845734	KC802172	KC845620	KC845863
134.2266 E	5/21/2011	24.4	C.S. McFadden	B	KC845744	KC802173	KC845621	KC845859
134.3602 E	5/21/2011	16.2	M. Janes	B	KC845735	KC802174	KC845622	KC845860
134.262167 E	5/24/2011	15.2	M. Janes	B	KC845739	KC802177	KC845626	KC845861
134.52475 E	5/26/2011	6.0	C.S. McFadden	B	KC845742	KC802180	KC845593	KC845864
130.687383 E	12/2/2007	13	J. van Egmond	B	KC845728	KC802136	KC845605	KC845857
130.904333 E	12/12/2007	20	B.T. Reijnen	B	KC845729	KC802137	KC845632	KC845858
134.3602 E	5/21/2011	13.6	C.S. McFadden	B	KC845736	KC802175	KC845623	KC845844
134.487567 E	5/22/2011	14.0	M. Janes	B	KC845737	KC802182	KC845624	KC845850
134.262167 E	5/24/2011	4.3	C.S. McFadden	B	KC845738	KC802176	KC845625	KC845849
134.52475 E	5/26/2011	6.5	C.S. McFadden	B	KC845743	KC802181	KC845633	KC845883
118.777472 E	12/10/2010	2	B.T. Reijnen	B	KC845719	KC802141	KC845611	KC845845
118.717861 E	12/12/2010	5	B.T. Reijnen	B	KC845723	KC802142	KC845612	KC845846
118.589333 E	12/15/2010	17	B.T. Reijnen	B	KC845724	KC802143	KC845615	KC845847
118.541556 E	12/15/2010	12	B.T. Reijnen	B	KC845725	KC802144	KC845616	KC845848
106.676333 E	7/25/2008	18.3	CRRF	B	KC845694	KC802113	KC845579	KC845843
127.334472 E	10/26/2009	7	B.T. Reijnen	B	KC845726	KC802139	KC845594	KC845851
125.2027 E	2/6/2012	28	B.T. Reijnen	B	KC845771	KC802197	KC845662	KC845905
115.233611 E	4/7/2001	<20	L.P. van Ofwegen & M. Slierings	B	KC845798	-	KC845689	KC845921
134.377433 E	5/21/2011	11.9	C.S. McFadden	B	KC845740	KC802178	KC845627	KC845884
25.693167 E	3/13/2008	10-12	C.S. McFadden	E	KC845752	KC802162	KC845649	KC845813
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845754	KC802164	KC845640	KC845809
18.31045 E	3/22/2004	15-27	C.S. McFadden	E	KC845808	-	KC845687	KC845922

Table 1. Cont.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
RMNH.Coel.41152	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 2	33.984483 S
RMNH.Coel.41153	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41154	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41155	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41156	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41157	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.984483 S
RMNH.Coel.41158	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Bell Buoy 2	33.980717 S
RMNH.Coel.41159	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Bell Buoy 2	33.980717 S
RMNH.Coel.41160	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Bell Buoy 2	33.980717 S
RMNH.Coel.41161	*Melithaea* sp.	*Melithaea* sp.	South Africa, Port Elizabeth, Algoa Bay, Bell Buoy 3	33.980267 S
RMNH.Coel.41162	*Melithaea* sp.	*Melithaea* sp.	South Africa, Kwazulu-Natal, Park Rynie, Lander's Reef	30.332883 S
RMNH.Coel.41163	*Melithaea* sp.	*Melithaea* sp.	Seychelles, NW of Alphonse Atoll	7.000 S
RMNH.Coel.41164	*Melithaea* sp.	*Melithaea* sp.	Seychelles, NW of Praslin Island	4.266667 S
RMNH.Coel.41165	*Acabaria* sp.	*Melithaea* sp.	Indonesia, Papua, Raja Ampat, S Gam, shoal near mangrove border	0.51895 S
RMNH.Coel.41166	*Acabaria* sp.	*Melithaea* sp.	Indonesia, Papua, Raja Ampat, S Friwin Island	0.481817 S
RMNH.Coel.41167	*Melithaea caledonica* Grasshoff, 1999	*Melithaea caledonica* Grasshoff, 1999	New Caledonia, Baie de Nakety	21.509467 S
RMNH.Coel.41168	*Melithaea caledonica* Grasshoff, 1999	*Melithaea caledonica* Grasshoff, 1999	New Caledonia, Passe de Goyeta	21.249117 S
RMNH.Coel.41169	*Wrightella coccinea* (Ellis & Solander, 1786)	*Melithaea coccinea* (Ellis & Solander, 1786) comb. nov.	Seychelles, E of Mahé, N of Moyenne Island	4.616667 S
RMNH.Coel.41170	*Acabaria formosa* Nutting, 1911	*Melithaea formosa* (Nutting, 1911) comb. nov.	Indonesia, Sulawesi, Lembeh, SW Sarena Kecil	1.45551 N
RMNH.Coel.41171	*Acabaria formosa* Nutting, 1911	*Melithaea formosa* (Nutting, 1911) comb. nov.	Indonesia, Sulawesi, Lembeh, Tanjung Kungkungan	1.46622 N
RMNH.Coel.41172	*Acabaria formosa* Nutting, 1911	*Melithaea formosa* (Nutting, 1911) comb. nov.	Indonesia, Sulawesi, Lembeh, Tanjung Nanas I	1.46097 N
RMNH.Coel.41173	*Clathraria maldivensis* van Ofwegen, 1987	*Melithaea maldivensis* (van Ofwegen, 1987) comb. nov.	Maldives, Lankanfinolhu	4.285753 N

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	clade	ND6	COI	mtMutS	28S
25.864017 E	3/11/2008	14-17	S. Parker-Nance	E	KC845745	KC802163	KC845639	KC845823
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845746	KC802167	KC845642	KC845816
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845757	KC802168	KC845643	KC845817
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845750	KC802169	KC845644	KC845818
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845749	KC802165	KC845645	KC845819
25.864017 E	3/11/2008	15-20	C.S. McFadden	E	KC845751	KC802170	KC845646	KC845820
25.6601 E	3/13/2008	18-22	C.S. McFadden	E	KC845753	KC802166	KC845648	KC845821
25.6601 E	3/12/2004	18-22	C. McFadden	E	KC845804	-	KC845685	KC845920
25.6601 E	3/12/2004	18-22	S. Parker-Nance	E	KC845805	-	KC845690	KC845919
25.69295 E	3/14/2008	12-15	C.S. McFadden	E	KC845756	KC802171	KC845650	KC845822
30.79205 E	3/17/2004	22-28	C.S. McFadden	E	KC845806	-	KC845686	KC845918
52.716667 E	1/3/1993	-	L.P. van Ofwegen	A	KC845802	-	KC845682	KC845913
55.666667 E	12/17/1992	25	L.P. van Ofwegen	A	KC845800	-	KC845683	KC845914
130.641117 E	11/30/2007	23	F.R. Stokvis	A	KC845803	-	KC845691	KC845917
130.69835 E	12/7/2007	21	F.R. Stokvis	A	KC845801	-	KC845692	KC845916
166.097083 E	4/22/2012	<20	B.W. Hoeksema	B	KC845776	KC802192	KC845655	KC845900
164.775917 E	4/7/2012	20	B.W. Hoeksema	B	KC845777	KC802191	KC845654	KC845899
55.516667 E	12/25/1992	7	L.P. van Ofwegen	D	KC845760	KC802157	KC845581	KC845870
125.22362 E	12/7/2012	10	B.T. Reijnen	A	KC845772	KC802196	KC845657	KC845906
125.23396 E	2/10/2012	6	B.T. Reijnen	A	KC845773	KC802195	KC845656	KC845907
125.22661 E	2/17/2012	10	B.T. Reijnen	A	KC845775	KC802193	KC845658	KC845901
73.546742 E	5/3/2011	<20	B.T. Reijnen	D	KC845716	KC802133	KC845589	KC845875

Table 1. Cont.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
RMNH.Coel.41174	*Clathraria maldivensis* van Ofwegen, 1987	*Melithaea maldivensis* (van Ofwegen, 1987) comb. nov.	Maldives, Lankanfinolhu	4.285753 N
RMNH.Coel.41175	*Clathraria maldivensis* van Ofwegen, 1987	*Melithaea maldivensis* (van Ofwegen, 1987) comb. nov.	Maldives, Lankanfinolhu	4.285753 N
RMNH.Coel.41176	*Acabaria rubra* Esper, 1798	*Melithaea rubra* (Esper, 1798) comb. nov.	South Africa, Port Elizabeth, Algoa Bay, Riy Banks 1	33.98495 S
RMNH.Coel.41177	*Acabaria rubra* Esper, 1798	*Melithaea rubra* (Esper, 1798) comb. nov.	South Africa, Port Elizabeth, Algoa Bay, White Sands 15	33.998333 S
RMNH.Coel.41178	*Acabaria rubra* Esper, 1798	*Melithaea rubra* (Esper, 1798) comb. nov.	South Africa, Port Elizabeth, Algoa Bay, Bell Buoy 2	33.980717 S
RMNH.Coel.41179	*Acabaria rubra* Esper, 1798	*Melithaea rubra* (Esper, 1798) comb. nov.	South Africa, Cape Peninsula, Oudekraal, Justin's Caves	33.98165 S
RMNH.Coel.41180	*Acabaria rubra* Esper, 1798	*Melithaea rubra* (Esper, 1798) comb. nov.	South Africa, Cape Peninsula, Oudekraal, Justin's Cave	33.98165 S
RMNH.Coel.41181	*Chironephthya* sp.	n/a	Malaysia, Borneo, Semporna, Sebangkat Island	4.55525 N
RMNH.Coel.41182	*Siphonogorgia* sp.	n/a	Malaysia, Borneo, Semporna, Sebangkat Island	4.55525 N
RMNH.Coel.41183	*Annella* sp.	n/a	Indonesia, Halmahera, Hiri, Tanjung Ngafauda	0.910639 N
RMNH.Coel.41184	*Chironephthya* sp.	n/a	Malaysia, Borneo, Semporna, Timba timba Island	4.560883 N
RMNH.Coel.41185	*Solenocaulon* sp.	n/a	Malaysia, Borneo, Semporna, Pandanan Island	4.577967 N
RMNH.Coel.41186	*Chironephthya* sp.	n/a	Malaysia, Borneo, Semporna, 2 N Kulapuan Island	4.5354 N
RMNH.Coel.41187	*Siphonogorgia* sp.	n/a	Malaysia, Borneo, Semporna, Church Reef 1	4.681683 N
RMNH.Coel.41188	*Chironephthya* sp.	n/a	Malaysia, Borneo, Sipadan Island, Barracuda Point	4.12035 N
RMNH.Coel.41189	*Euplexaura* sp.	n/a	Indonesia, Sulawesi, Lembeh, N Sarena Kecil	1.45746 N
ZMTAU.CO.32749	*Acabaria* sp.	*Melithaea* sp.	Tanzania, Pemba Island, Nijao Gap, mega wall	4.961111 S
ZMTAU.CO.32939	*Clathraria rubrinodes* Gray, 1859	*Melithaea rubrinodes* (Gray, 1859) comb. nov.	Eritraea, Dahlak Archipelago, between Nocra Island and Dhlak Island, southern entrance channel	15.689333 N
ZMTAU.CO.34054	*Clathraria rubrinodes* Gray, 1859	*Melithaea rubrinodes* (Gray, 1859) comb. nov.	Israel, Gulf of Aqaba, Eilat, Interuniversity Institute for Marine Sciences	29.502333 N

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	clade	ND6	COI	mtMutS	28S
73.546742 E	5/3/2011	<20	B.T. Reijnen	D	KC845717	KC802134	KC845590	KC845876
73.546742 E	5/3/2011	<20	B.T. Reijnen	D	KC845718	KC802135	KC845591	KC845877
25.8629 E	3/11/2008	15-20	C.S. McFadden	E	KC845755	KC802159	KC845641	KC845810
25.7087 E	3/19/2008	14-16	C.S. McFadden	E	KC845748	KC802160	KC845634	KC845812
25.6601 E	3/13/2008	18-22	C.S. McFadden	E	KC845758	KC802161	KC845647	KC845814
18.359833 E	3/23/2004	7-11	C.S. McFadden	E	KC845807	-	KC845688	KC845915
18.359833 E	3/24/2008	7-11	C.S. McFadden	E	KC845747	KC802155	KC845635	KC845811
118.655217 E	12/12/2010	15	B.T. Reijnen	Out-group	KC845790	KC802212	KC845676	KC845896
118.655217 E	12/12/2010	15	B.T. Reijnen	Out-group	KC845791	KC802213	KC845677	KC845894
127.317417 E	10/31/2009	16	B.T. Reijnen	Out-group	KC845788	KC802214	KC845678	KC845892
118.924817 E	12/8/2010	17	B.T. Reijnen	Out-group	KC845794	KC802215	KC845679	KC845893
118.9204 E	12/8/2010	22	B.T. Reijnen	Out-group	KC845796	KC802207	KC845680	KC845887
118.838383 E	12/9/2010	20	B.T. Reijnen	Out-group	KC845795	KC802208	KC845681	KC845895
118.658017 E	12/13/2010	23	B.T. Reijnen	Out-group	KC845792	KC802209	KC845673	KC845898
118.629433 E	12/18/2010	24	B.T. Reijnen	Out-group	KC845793	KC802210	KC845674	KC845897
125.22711 E	2/16/2012	28	B.T. Reijnen	Out-group	KC845789	KC802211	KC845675	KC845886
39.664167 E	29-112004	10-20	Y. Benayahu	C	KC845762	KC802183	KC845583	KC845815
39.934667 E	2/14/2005	0-5	Y. Benayahu	C	KC845763	KC802184	KC845584	KC845871
34.917917 E	7/23/2007	23.2	Y. Benayahu	C	KC845695	KC802115	KC845636	KC845872

Table 1. Cont.

Catalogue number	Species (author)	New species name (author)	Locality	Latitude (decimal)
ZMTAU.CO.34200	*Acabaria sinaica* Grasshoff, 2000	*Melithaea sinaica* (Grasshoff, 2000) comb. nov.	Israel, Gulf of Aqaba, Eilat, North Oil jetty	29.5235 N
ZMTAU.CO.34216	*Acabaria erythraea* (Ehrenberg, 1834)	*Melithaea erythraea* (Ehrenberg, 1834) comb. nov.	Israel, Gulf of Aqaba, Eilat, Speed boat jetty	29.548 N
ZMTAU.CO.35497	*Acabaria sinaica* Grasshoff, 2000	*Melithaea sinaica* (Grasshoff, 2000) comb. nov.	Israel, Gulf of Aqaba, Eilat, North Oil jetty	29.5235 N
ZMTAU.CO.35499	*Acabaria sinaica* Grasshoff, 2000	*Melithaea sinaica* (Grasshoff, 2000) comb. nov.	Israel, Gulf of Aqaba, Eilat, North Oil jetty	29.5235 N
ZMTAU.CO.35500	*Acabaria sinaica* Grasshoff, 2000	*Melithaea sinaica* (Grasshoff, 2000) comb. nov.	Israel, Gulf of Aqaba, Eilat, North Oil jetty	29.5235 N

at 10 kV. Consequently, microscope slides and SEM images were used to assign specimens to the nominal genera according to the following key based on van Ofwegen (1987).

(1) Sclerites at coenenchymal surface predominantly spindles and occasionally a few thorn-clubs in some species. (*Acabaria*/*Asperaxis*).
(2) Sclerites at coenenchymal surface consisting of predominantly double discs. Leaf-clubs and thorn-clubs might be present but in few number. (*Melithaea*).
(3) Predominantly leaf-clubs and thorn-clubs in the coenenchymal surface. (*Mopsella*).
(4) Coenenchymal surface is formed by large foliate spheroids forming a complete pavement-like protection. (*Wrightella*).
(5) Coenenchymal surface without any predominant sclerite type, and includes spindles, clubs and small leafy spheroids. (*Clathraria*).

Species were identified, where possible, based on the traditional characters used for Octocorallia identification such as: overall shape and size of sclerites, absence and presence of projections and/or tuberculation and the occurrence of certain sclerite types.

Molecular analysis
DNA was extracted using the DNEasy Kit (QIAGEN) with the corresponding protocol for animal tissue (v. 07/2006). Approximately 1 cm of the gorgonian was cut into small pieces before the tissue was added to the extraction buffers. The digestion was performed overnight (c. 16 h). In some cases the extract had to be diluted before DNA amplification. The PCR mixture contained: 2.5 µl PCR CoralLoad Buffer (containing 15 mM $MgCl_2$) (QIAGEN), 0.5 µl dNTP's (2.5 mM), 1.0 µl per primer (10 pmol), 0.3 µl

					accession numbers			
Longitude (decimal)	Date	Depth (m)	Collector	clade	ND6	COI	mtMutS	28S
34.935667 E	7/26/2007	15.2	Y. Benayahu	C	KC845696	KC802148	KC845638	KC845874
34.953667 E	7/27/2007	0.3-0.6	Y. Benayahu	C	KC845697	KC802147	KC845637	KC845873
34.935667 E	2/15/2012	16	Y. Benayahu	C	KC845785	KC802151	KC845630	KC845909
34.935667 E	2/15/2012	16	Y. Benayahu	C	KC845787	KC802150	KC845631	KC845910
34.935667 E	2/15/2012	16	Y. Benayahu	C	KC845786	KC802149	KC845629	KC845911

Taq polymerase (15 units/µl) (QIAGEN) and 18.7 µl of extra pure PCR water and 1.0 µl (diluted) DNA extract. The primer pairs and PCR amplification settings used are presented in Table 3. Apart from the different annealing temperatures, all PCR cycles consisted of an initial denaturing step of 95°C for 1 min. followed by 39 cycles of 95°C for 10 s, preferred annealing temperature (see Table 3) for 1 min. and an extension step of 72°C for 1 min. The final PCR cycle was followed by an elongated extension step of 72°C for 5 min.

PCR products were analysed on a 1% agarose gel and stained with ethidium bromide, and visualized on a Cell Biosciences Red. Amplified samples were sent to Macrogen Europe for PCR cleaning and sequencing on an ABI Automated Sequencer 3730xl or were purified by PEG-precipitation (Sánchez *et al.*, 2003) and sent to htSeq (University of Washington, Seattle) for sequencing. Sequences were assembled with Sequencher 4.10.1. The resulting consensus sequences were aligned in BioEdit (Hall, 1999), except for the 28S data. The 28S data contained insertions and/or deletions, therefore nucleotides were aligned with the help of the GUIDANCE server (Penn *et al.*, 2010) using the MAFFT algorithm. All consensus sequences were also blasted against GenBank to check for nonspecific amplification or contamination. All novel sequences have been submitted to GenBank (accession numbers: KC802113 – KC802215 (COI); KC845579 – KC845693 (mtMutS); KC845694 – KC845808 (ND6); KC845809 – KC845923 (28S)). The outgroup species for our analyses were selected based on the phylogenetic tree of McFadden *et al*. (2006, 521, Fig. 3) from which direct sister species (*Siphonogorgia* spp. and *Chironephthya* spp.) and other less related species (*Annella* sp., *Solenocaulon* sp., and *Euplexaura* sp.) were selected for inclusion in our phylogenetic analyses.

Table 2. List of type specimens of Melithaeidae studied. BMNH = British Museum of Natural History, London, Un
de Strasbourg, France; NTM = Museum and Art Gallery of the Northern Territory, Australia; ZMA = Naturalis Biod
für Naturkunde, Berlin, Germany.

Original species (author)	Collection code	Type	Depth
Acabaria amboinensis Hentschel, 1903	MZS.Cni.105	Holotype	n/a
Acabaria baladea Grasshoff, 1999	MNHN.HG.198	Paratype	33m
Acabaria biserialis Kükenthal, 1908	ZMB.5810	Syntype	litoral
Acabaria cinquemiglia Grasshoff, 1999	MNHN.HG.172	Paratype	30m
Acabaria divaricata Gray, 1859	BMNH 1846.7.30.59	Holotype	n/a
Acabaria formosa Nutting, 1911	ZMA.Coel.2100	Holotype	9-45m
Acabaria haddoni Hickson, 1937	BMNH 1937.7.14.8	Paratype	18-31m
Acabaria hicksoni Nutting, 1911	ZMA.Coel.2102	Holotype	23m
Acabaria kuea Grasshoff, 1999	MNHN.HG.164	Paratype	10-15m
Acabaria laevis Wright & Studer, 1889	BMNH.1947-03-22-007	Holotype	27-37m
Acabaria nuttingi Hickson, 1937	ZMA.Coel.2103	Holotype	937m
Acabaria ouvea Grasshoff, 1999	MNHN.HG.165	Paratype	n/a
Acabaria planoregularis Kükenthal, 1910	ZMB.5818	Syntype	8-10m
Acabaria ramulosa Kükenthal, 1910	ZMB.5804	Holotype	18m
Acabaria serrata Ridley, 1884	BMNH 1882.2.23.129-132	Paratype	12-22m
Acabaria squarrosa Kükenthal, 1910	ZMB.5806	Syntype	15m
Acabaria triangulata Nutting, 1911	ZMB.5802	Syntype	57m
Acabaria valdiviae Kükenthal, 1908	ZMB.5802	Syntype	318m
Asperaxis karenae Alderslade, 2006	NTM.C.13575	Paratype	4-6m
Birotulata splendens (Nutting, 1911)	ZMA.Coel.1684	Holotype	n/a
Clathraria robusta Kükenthal, 1919	ZMB.5106	Holotype	litoral
Clathraria roemeri Kükenthal, 1908	ZMB.5800	Holotype	n/a
Melitella elongata Gray, 1859	BMNH 1983.3.2.11	Holotype	n/a
Melithaea caledonica Grasshoff, 1999	MNHN.HG.163	Paratype	33m
Melitodes africana (Kükenthal, 1908)	ZMB.5819	Syntype	70m
Melitodes albitincta Ridley, 1884	BMNH 1881.10.21.189	Holotype	22-37m
Melitodes contorta Dean, 1932	BMNH 1937.7.14.2	Holotype	n/a
Melitodes esperi (Wright & Studer, 1889)	BMNH.1889-05-27-148	Holotype	n/a
Melitodes flabellum Thomson & Mackinnon, 1910	BMNH 1912.2.24.59	Holotype	11m
Melitodes mertoni (Kükenthal, 1910)	ZMB.5808	Syntype	18m
Melitodes modesta (Nutting, 1911)	ZMA.Coel.2860	Holotype	13m
Melitodes philippinensis Wright & Studer, 1889	BMNH 1889.5.27.145	Syntype	n/a
Melitodes rugosa Wright & Studer, 1889	BMNH 1889.5.27.114	Holotype	70-73m
Melitodes sinuata Wright & Studer, 1889	BMNH 1889.5.27.144	Holotype	n/a
Melitodes squamata (Nutting, 1911)	ZMA.Coel.2867	Holotype	34m
Melitodes stormii (Studer, 1895)	ZMB.3762	Syntype	4-10m
Melitodes sulphurea (Studer, 1895)	ZMB.3766	Holotype	4-10m
Mopsella clavigera Ridley, 1884	BMNH 1881.10.21.116-125	Syntype	7-26m
Mopsella spinosa Kükenthal, 1910	ZMB.5811	Syntype	10m
Mopsella spongiosa Nutting, 1911	ZMA.Coel.2902	Holotype	13m
Mopsella studeri Nutting, 1911	ZMA.Coel.2904	Syntype	13m
Mopsella zimmeri Kükenthal, 1908	ZMB.5812	Syntype	n/a
Wrightella robusta Shann, 1912	BMNH 1937.7.14.12	Holotype	shallow water
Wrightella tongaensis Kükenthal, 1908	ZMB.5813	Holotype	n/a

nited Kingdom; MNHN = Muséum national d'Histoire naturelle, Paris, France; MZS = Musée zoologique de la ville
diversity Center, Leiden, The Netherlands (former collection of Zoological Museum of Amsterdam); ZMB = Museum

Locality	Remarks
Ambon	is synonym of *Acabaria laevis*
New Caledonia, Banc Gail stn. 114	
Red Sea, sta.95, 34°47.7 E; 29°12.7 N (nowadays Gulf of Aqaba)	
New Caledonia, S lagon, Canal Woodin stn. 252	
n/a	
Indonesia, Sta. 240 Banda anchorage	
N Australia, Saibai Channel	
Indonesia, Samau Island near Timor, sta. 60 Haingsisi	
New Caledonia, S Grecife/rext Recife Kue stn. 445	
Indonesia, Ambon	
Indonesia, near Ternate, Siboga expedition sta. 139	
New Caledonia, no precise locality data	
Indonesia, Aru Islands, SW of Lola	
Indonesia, Aru Islands, Sungi Barkai	
N coast of Australia, Port Darwin	
Indonesia, Aru Islands, Sungi Barkai	
Indonesia, Siboga expedition Sta. 274, 5°28.2 S 134°53.9 E	
South Africa, Cape of Good Hope	
Australia, Tasmania , Port Davey, Mundy Island, Bathurst Channel	
Indonesia, Siboga expedition Sta. 213, anchorage in Saleyer (South Celebes)	is *Melithaea mcqueeni* nom. nov.
Neu Pommern (=New Britain), Tallasia	is *Melithaea shanni* nom.nov.
Indonesia, Ambon	is synonym of *Melithaea ochracea*
n/a	
New Caledonia, S lagon, Chenal des cinq milles, stn 242	
South Africa, Simons Bay	
Australia, Queensland, Port Molle	
Indonesia, Papua, Sorong	
Australia, Torres Strait	
Seychelles, Providence	
Indonesia, Aru Islands, Sungi Barkai	
Indonesia, East Coast of the Aru Islands, Siboga expedition sta. 273, anchorage off Pulau Jedan	is synonym of *Acabaria planoregularis*
N Philippines, Saboangan, Reefs,	
Australia, Tasmania, Bass strait, East moncouer Isl., Sta. 162	
N Philippines, Saboangan, Reefs,	
Indonesia, Siboga expedition Sta. 299, 10°52.4 S 123°01.1 E	
Singapore, Bintang Isls.	
Singapore, Bintang Isls.	is synonym of *Melithaea stormii*
Australia, Queensland, Port Curtis/Port Molle/Thursday Isl	
Indonesia, Aru Islands	
Indonesia, East Coast of the Aru Islands (Pearl Banks), Siboga expedition Sta. 273, anchorage off Pulau Jedan	
Indonesia, East Coast of the Aru Islands (Pearl Banks), Siboga expedition Sta. 273, anchorage off Pulau Jedan	
Australia, Sydney	
Near Singapore	
Tonga Isl.	

Table 3. Overview of primer pairs, targeted area, fragment size, annealing temperatures and references.

Name	Targeted gene area	Fragment size	Forward primer (length)
Alc_715_Fw & Alc_1303_RV	ND6	~600	GGG CCA ATC CAG TAG AGG (18)
COII8068xF & COIOCTr	COI	~900	CCA TAA CAG GAC TAG CAG CAT C (22)
ND42599F & MUT3458R	mtMutS	~800	GCC ATT ATG GTT AAC TAT TAC (21)
msh-2761F & msh-3270R	mtMutS	~530	TAT GAA CTT TGG CAT GAG CC (20)
MSH_mod_F & MSH_mod_R	mtMutS	~800	TTA CCG TTT ACG TGG CAC AA (20)
28S-F252 & 28S-R1283	28S	~900	CAC GAG ACC GAT AGC GAA CAA GTA (24)

Phylogenetic analyses
Molecular datasets were analysed in MEGA 5.0.5 (Tamura *et al*., 2011) and jModeltest 2.1.1 (Darriba *et al*., 2012) to test for the most optimal evolutionary model based on the Akaike Information Criterion (AIC) (Yang, 2005). Phylogeny reconstructions were estimated based on the maximum parsimony (MP) method and Maximum Likelihood (ML) algorithm implemented in MEGA 5.0.5. For the ML and MP analyses 1,000 bootstrap replicates for which the heuristic search method Nearest-Neighbor-Interchange and Close-Neighbor-Interchange were used respectively. Gaps and missing data were treated as complete deletion. Additionally, datasets were also subjected to MrBayes 3.2.0 to check for congruency with the MP and ML analyses. MrBayes was run for 5,000,000 generations, with six chains (four cold and two heated ones). Data were sampled every 100 generations and the burnin was set to 12,500. For *Asperaxis karenae* Alderslade, 2006, mtMutS data was already available in GenBank (accession number DQ302847.1) (McFadden *et al*., 2006). To investigate the position of this subfamily compared to our Melithaeinae specimens, we have included the sequence in our mtMutS dataset. As outgroup we have used the same specimens as in the previous analyses.

Results

Sampling and molecular datasets
In total 103 specimens, including the outgroup selection, were sequenced for four molecular markers, three mitochondrial (ND6, COI, mtMutS) and one nuclear (28S rDNA). Among these specimens ten species could be identified with the help of type specimens and original species descriptions. The total length of the concatenated sequences ranged from 2,294-2,579 bp due to insertions, deletions or missing data. In particular the 28S sequences proved to be variable in length. Unfortunately not all samples amplified well with the standard mtMutS primers, therefore new nested primers were developed (Table 3). As a result approx. 270 bp less were amplified for four specimens included in this study. Some double peaks were observed while editing the 28S data, which were coded according to the IUPAC ambiguity codes. The most difficult marker to amplify proved to be COI. Therefore a second dataset was prepared from which the COI marker was removed. Consequently it was possible to include sequence data for 12 additional spec-

Reverse primer (length)	Annealing temp.	Reference
AGG TGA ATT TGG YTG CTT RG (20)	50°C	This publication
ATC ATA GCA TAG ACC ATA CC (20)	58°C	McFadden *et al*. (2011)
TSG AGC AAA AGC CAC TCC (18)	48°C	France and Hoover (2002); Sánchez *et al*. (2003)
TGC CCA AAT TAC TAT TTC TCT AAT ACG (27)	48°C	This publication
ATT GGG CGA TGT TTC CAT AA (20)	48°C	This publication
TCA TTT CGA CCC TAA GAC CTC (21)	50°C	McFadden and Ofwegen (2013)

imens. This resulted in a dataset containing 115 sequences. The dataset containing four molecular markers had 1,861 constant characters, 206 parsimony uninformative, variable characters and 533 parsimony informative characters while the dataset based on three molecular markers had 1,226 constant characters, 162 parsimony uninformative, variable characters and 397 parsimony informative characters. The alignment scores for both datasets calculated by GUIDANCE under the MAFFT algorithm were respectively 0.998603 and 0.999201. Alignments can be requested from the corresponding author.

The model searching analysis in both MEGA 5.0.5 and jModelTest 2.1.1 resulted in GTR + I + Γ being the most general model for the concatenated datasets as well as some of the single-gene datasets. On two occasions, jModeltest 2.1.1 selected different models for the COI and ND6 datasets respectively viz. TVM + I + Γ and TrN + I + Γ. Neither of these evolutionary models are implemented in MrBayes therefore the best approximation of the model in MrBayes was selected (GTR + I + Γ) (Hofman *et al*., 2007). Based on the results of both model testing programs, and the congruent topology for the single-gene trees we did not partition the dataset for the phylogenetic analyses. Molecular datasets including the parameters for the best evolutionary model were subjected to (ML)- (dataset with four markers, best log likelihood was –9051.4462, three markers –7200.6663), Maximum Parsimony (MP)- (dataset with four markers, 93 most parsimonious trees; length 1,055, three markers 103 most parsimonious trees; length 872) and Bayesian analyses. For the Bayesian analyses the final average split frequency after 5.000.000 runs for the datasets was, respectively, 0.0038 and 0.0049. Unidentified specimens or species groups that formed well-supported clades are illustrated with SEM images (App. 1, Pl. 1-39). At least one representative per species group is illustrated, except for the deep water Melithaeidae which are published separately (Matsumoto & van Ofwegen, 2015).

Phylogenetic analyses

The results from the ML, MP and Bayesian analyses revealed highly congruent phylograms. In Fig. 2 and Fig. 3 the ML trees of the datasets with and without the COI marker are shown. Both phylograms have a very similar tree topology representing five basal clades based on the biogeographic origin of the specimens viz. clade A-E which will be discussed below.

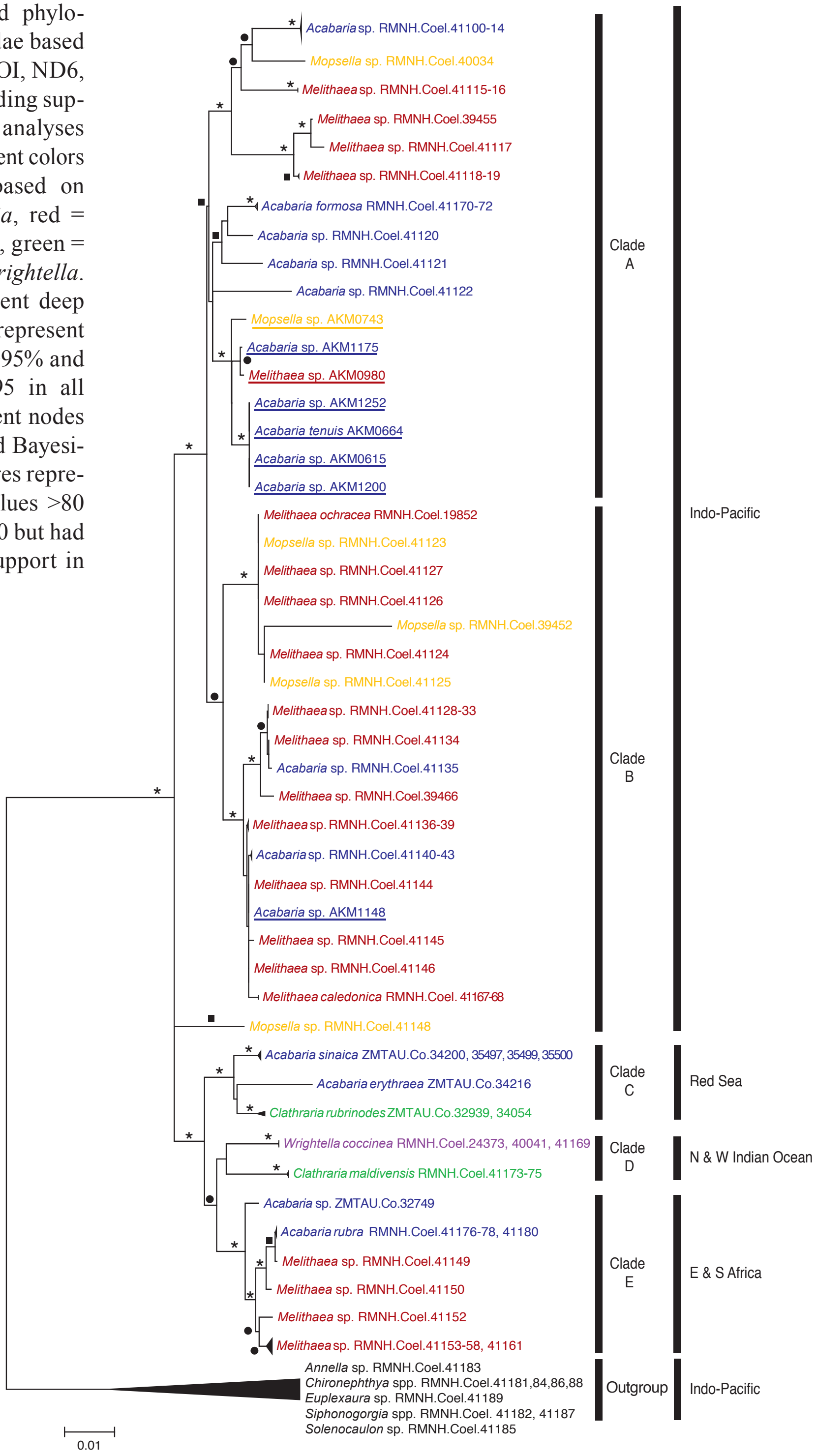

Fig. 2. Maximum likelihood phylogram of the family Melithaeidae based on four molecular markers (COI, ND6, mtMutS and 28S rDNA) including support values of three different analyses (ML/MP/MrBayes). The different colors represent different genera based on morphology: blue = *Acabaria*, red = *Melithaea*, yellow = *Mopsella*, green = *Clathraria* and purple = *Wrightella*. Underlined specimens represent deep water specimens. Asterisks represent nodes with bootstrap values >95% and Bayesian probabilities of >95 in all three analyses, circles represent nodes with bootstrap values >80 and Bayesian probabilities >90 and squares represent nodes with bootstrap values >80 and Bayesian probabilities >90 but had a considerable lower or no support in the MP analysis.

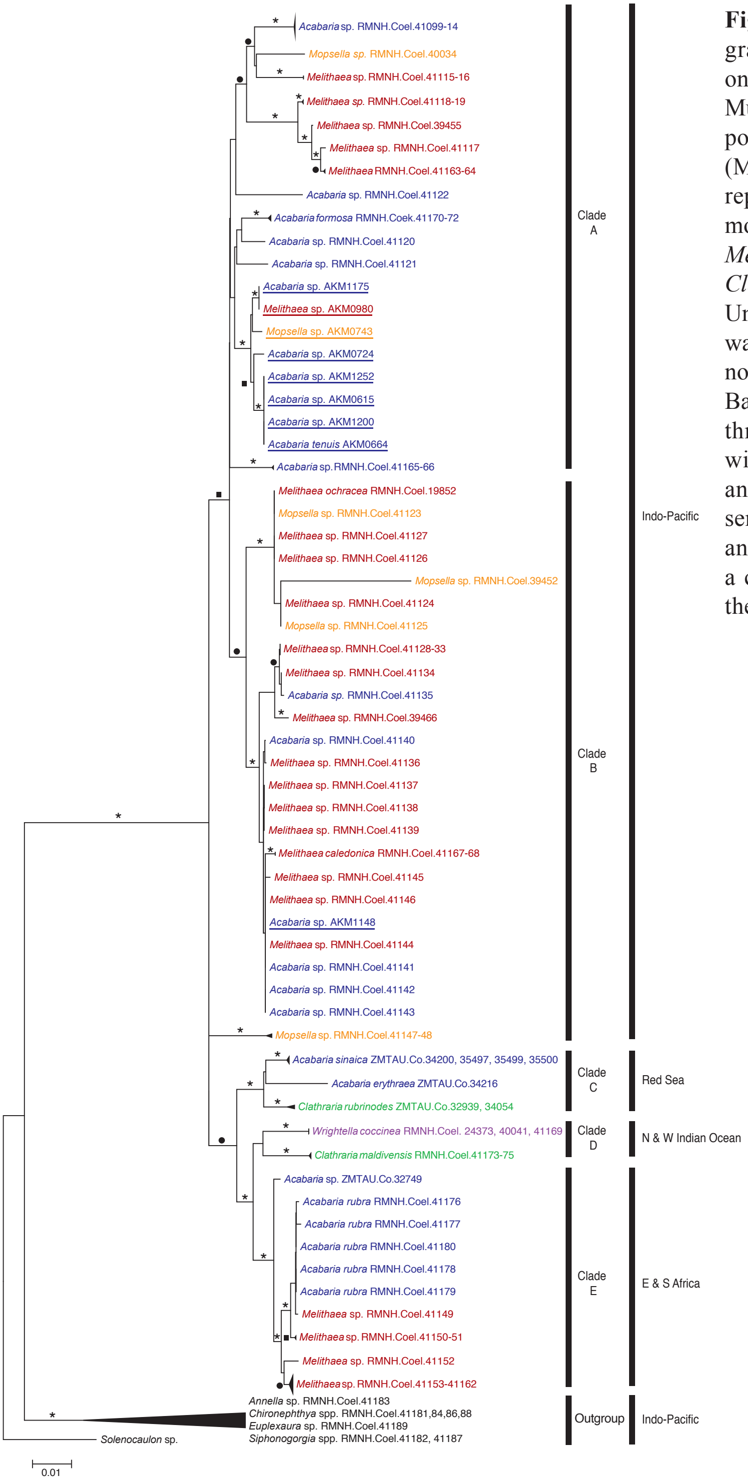

Fig. 3. Maximum likelihood phylogram of the family Melithaeidae based on three molecular markers (ND6, mtMutS and 28S rDNA) including support values of three different analyses (ML/MP/MrBayes). The different colors represent different genera based on morphology: blue = *Acabaria*, red = *Melithaea*, yellow = *Mopsella*, green = *Clathraria* and purple = *Wrightella*. Underlined specimens represent deep water specimens. Asterisks represent nodes with bootstrap values >95% and Bayesian probabilities of >95 in all three analyses, circles represent nodes with bootstrap values >80 and Bayesian probabilities >90 and squares represent nodes with bootstrap values >80 and Bayesian probabilities >90 but had a considerable lower or no support in the MP analysis.

Clade A

In both trees, clade A consists of central Pacific specimens except for one specimen from the Seychelles (*Melithaea* sp. RMNH.Coel.41117). In the phylogenetic tree based on three markers two additional specimens cluster with this specimen *Melithaea* sp. (RMNH.Coel.41163–64), also from the Seychelles. These three specimens are the only ones that do not cluster according to the biogeographic pattern found for all other specimens in Fig. 2 and Fig. 3. Other species or genera represented in Clade A (based on the phylogeny consisting of four genetic markers) are *Acabaria formosa* Nutting, 1911, nine unidentified species of *Acabaria*, five of *Melithaea* and two *Mopsella* specimens. Both shallow as well as deep water species are represented in this clade. The deep water samples were collected at 71-502 m and are nested within the shallow water clade. One of the deep water specimens does not cluster with the other deep water specimens and is not retrieved in this clade (see section Clade B). The specific localities represented in clade A are: Northern Territory (Australia); Lombok, East Kalimantan, North Sulawesi, Papua (Indonesia); North Honshu, Ryukyu Archipelago (Japan); Semporna (Malaysia); Seychelles and Palau.

Clade B

Clade B, like clade A, also consists of central Pacific specimens but has a different species and generic composition than clade A. Clade A predominantly consists of species belonging to the genus *Acabaria* whereas this genus is only represented by three species in clade B. More specifically, clade B consists of: *Melithaea caledonica* Grasshoff, 1999, *M. ochracea* (Linnaeus, 1758), and an additional ten unidentified species of *Melithaea*, three of *Mopsella* and three of *Acabaria*. The only deep water specimen that was not retrieved in clade A (*Acabaria* sp. AKM1148) falls among all other representatives in this clade. Some species e.g. *Melithaea* sp. (RMNH.Coel.41128-34, 41136-39, 41144-45) appear morphologically very similar to the type specimen of *Melithaea squamata* (Nutting, 1911) but are genetically different. Additional morphological investigation of these specimens did not provide any strong characters to assign one or more of these specimens as representatives of *M. squamata*. Noticeable in our phylogenies is the relatively long branch length for *Melithaea* sp. (RMNH.Coel.39452). By investigating the concatenated alignment it clearly shows that only within the 28S marker this individual sequence has a consecutive region of approximately 100 bp that is unique in comparison to all other sequences. Since the 28S marker is known for having pseudogenes, the elongated branch is most probably an effect of these genes and does not represent actual species differences. The specific localities represented in clade B are: Northern Territory (Australia); West Halmahera, Moluccas, North Sulawesi, Papua (Indonesia); Okinawa Prefecture (Japan); Semporna (Malaysia); New Caledonia; Palau and South Vietnam.

Clade C

Clade C is represented by three species (in total seven specimens) all from the Red Sea viz. *Acabaria erythraea* (Ehrenberg, 1834), *Acabaria sinaica* Grasshoff, 2000 and *Clathraria rubrinodes* Gray, 1859. Together they form a well-supported clade (all support values >95%) and are considered a highly supported sister group (all support values >95%) to the representatives from the NW Indian Ocean and S and E Africa (clades D and E). Support for the phylogenetic position of *A. erythraea* is low (bootstrap and parsimony support <80%; Bayesian support <90%) and this species alternates between being a sister species of *C. rubrinodes* and *A. sinaica* in the two phylograms. Localities represented are: Dahlak Archipelago (Eritrea) and Eilat (Israel).

Clade D

Clade D is the smallest clade and is not well supported (bootstrap and parsimony support below 80%; Bayesian support below 90%). This clade consists of two species: *Clathraria maldivensis* van Ofwegen, 1987 and *Wrightella coccinea* (Ellis & Sollander, 1786) both found in the North West Indian Ocean. *Clathraria maldivensis* is represented by three specimens from the Maldives. *Wrightella coccinea* is also represented by three specimens, one from the Chagos Archipelago and two from the Seychelles. The individual specimens cluster together with high support values (all support values >95%), but the relationship between both species is not very well supported (bootstrap and parsimony support <80%; Bayesian support <90%). As a result the support for this clade is low, but the split between clades D and E is rather well supported (bootstrap and parsimony support >80%; Bayesian support >90%). Therefore we decided to maintain the specimens as representatives of a separate clade and did not include the specimens in clade E. Localities represented are: Lankanfinolhu (Maldives); Salomon Atoll (Chagos Archipelago), and the Seychelles.

Clade E

The final clade, clade E, consists of an East African species and many South African species. One of the South African specimen groups could be identified as *Acabaria rubra* (Esper, 1798) (see also the neotype designation). The other species are considered to be an unidentified *Acabaria* and four *Melithaea* species. The East African species (*Acabaria* sp. (ZMTAU.Co.32749)) is sister to the South African specimens, a relationship that is very well supported (bootstrap and parsimony support >80%; Bayesian support >90%) in both phylogenies. Within the South African specimens, excluding *A. rubra*, four additional clades are identified possibly representing different species. Unfortunately there are more names available for African melithaeid species (Williams, 1992) than there are species in our phylogeny. Without a revision of the African melithaeids and investigations of the type species we are unable to identify the other species represented in this clade. Remarkably, all specimens in the South African clade have sclerites that often exceed 0.2 mm in length, which is large in comparison to melithaeid species from Indonesia and Malaysia. Localities represented in clade E are: Tanzania (East Africa); Cape of Good Hope, Natal (South Africa).

Relationships among clades

In contrast to the expectations that species representing the different nominal genera would cluster together, all genera except for *Wrightella* were found to be paraphyletic. For example, the genus *Acabaria* is represented in all clades except for clade D and species which morphologically belong to the genus *Melithaea* are represented in clades A, B and E. To further investigate the relationships among clades we added the COI data from Aguilar-Hurtado *et al*. (2012), to our dataset. The intrageneric genetic variability in COI is relatively low, and therefore does not provide enough resolution to satisfactorily resolve clades. But although large polytomies are present, all sequences from Aguilar-Hurtado *et al*. (2012) cluster within our Indo-Pacific group viz. clades A and B. We were unable to include their 28S rDNA data in our phylogeny because they sequenced a different region of that gene.

Status of the subfamilies Melithaeinae and Asperaxinae

The results of the phylogenetic analyses revealed that the sequence of *Asperaxis karenae* does not cluster within the subfamily Melithaeinae, but although the coenenchymal sclerites are morphologically similar to the other members of the Melithaeidae, it is positioned in between the outgroup specimens, *Solenocaulon* sp. and the sister group containing *Annella* sp., *Euplexaura* sp., *Chironephthya* spp. and *Siphonogorgia* spp. (Fig. 4). These results indicate that based on the position of the mtMutS sequence of *Asperaxis karenae*, the family Melithaeidae is paraphyletic. Since this result was unexpected we obtained additional material of the type specimen sequenced by McFadden *et al*. (2006), to check the validity of their sequence. Although several attempts were made, and different methods used, we were not able to re-amplify DNA from the type material.

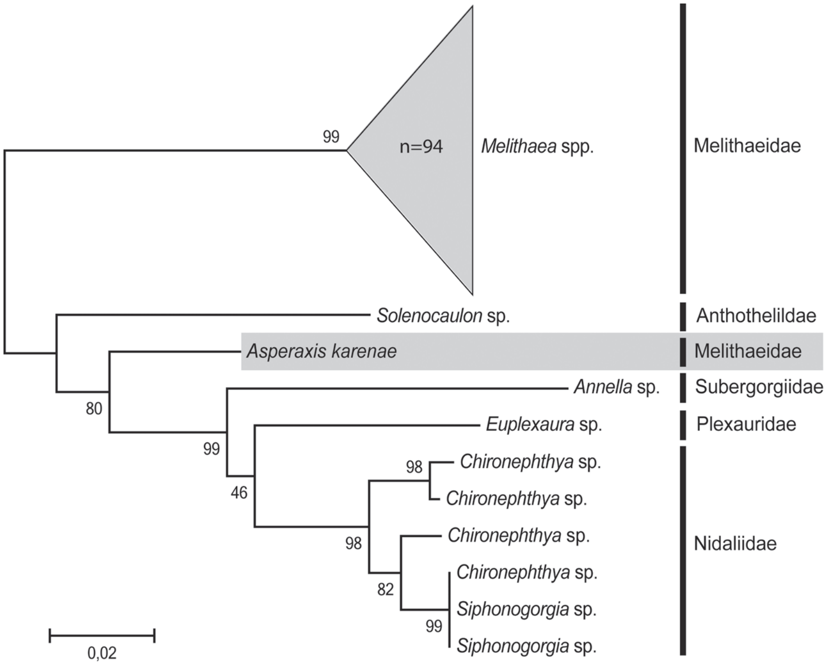

Fig. 4. ML analysis with bootstrap values showing the paraphyly of the Melithaeidae based on the mtMutS marker.

Systematic consequences within the family Melithaeidae

In this phylogenetic study we did not find molecular support to maintain the traditional morphologically defined genera. Therefore we synonymise the genera *Acabaria*, *Clathraria*, *Mopsella* and *Wrightella* with the earliest established genus in the Melithaeidae, *Melithaea*. As a result of synonymising the former genera only the genera *Asperaxis* and *Melithaea* will remain in their respective subfamilies Asperaxinae and Melithaeinae within the family Melithaeidae. According to the World Register of Marine Species (WoRMS) database (accessed on 22-01-2013) there are currently 114 names accepted for Melithaeidae. Yet, for five of these species identical species names are already in use and have become secondary homonyms, for which substitute names are given (Table 4).

Neotype designations for some Melithaeidae species

Besides renaming species due to secondary homonyms, neotype specimens are designated for the type species of *Melithaea*, namely *Melithaea ochracea* (Linnaeus, 1758), and for the South African species *Melithaea rubra* (Esper, 1798), neotypes are designated hereafter. Numerous type specimens in the family Melithaeidae are in very poor condition or lost.

Neotype designation of Melithaea ochracea (Linnaeus, 1758)

The original description of *Melithaea ochracea* is by Linnaeus *as Isis oc*[*h*]*racea* and is as follows: '*Stirpe coralline, articulis decorticates, geniculus nodosis*'. The type locality mentioned is: M.[Mare] indico. Linnaeus used the habitus drawing of *Accarbarium rubrum* from Rumphius (1750) for his description, of which the actual specimen is now considered lost. Additionally, in their descriptions Rumphius and Linnaeus only described

Table 4. Secondary homonyms and new substitute names for six melithaeid species after synonymising the genera *Acabaria*, *Clathraria*, *Mopsella* and *Wrightella* with *Melithaea.*

Secondary homonyms	New senior homonym combinations and new substitute names
Acabaria fragilis Wright & Studer, 1889	*Melithaea fragilis* (Wright & Studer, 1889) comb. nov.
Wrightella fragilis Thomson, 1917	*Melithaea wrighti* nom. nov.
Acabaria hicksoni Nutting, 1911	*Melithaea hicksoni* (Nutting, 1911) comb. nov.
Mopsella hicksoni Thorpe, 1928	*Melithaea thorpeae* nom. nov.
Acabaria modesta Kükenthal, 1908	*Melithaea modesta* (Kükenthal, 1908) comb. nov.
Melitodes modesta Nutting, 1911	*Melithaea kukenthali* nom. nov.
Wrightella robusta Shann, 1912	*Melithaea robusta* (Shann, 1912) comb. nov.
Clathraria robusta Kükenthal, 1919	*Melithaea shanni* nom. nov.
Melitodes splendens Thomson & McQueen, 1908	*Melithaea splendens* (Thomson & McQueen, 1908) comb. nov.
Birotulata splendens Nutting, 1911	*Melithaea mcqueeni* nom. nov.
Melitodes variabilis Hickson, 1905	*Melithaea variabilis* (Hickson, 1905) comb. nov.
Wrightella variabilis Thomson & Henderson, 1906	*Melithaea hendersoni* nom. nov.

the colony form. Therefore we designate RMNH.Coel.19852 as neotype for *M. ochracea* which was collected at the type locality viz. Ambon, Moluccas, Indonesia.

Melithaea ochracea (Linnaeus, 1758) (Neotype)
(Fig. 5 (habitus); Fig. 6 (sclerites))

Isis ocracea Linnaeus, 1758: 799
Isis ochracea Linnaeus, 1766: 1287
Clathraria roemeri Kükenthal, 1908: 201; 1919: 195 pl. 37 fig. 39; 1924: 87 fig. 60.; Hickson, 1937: 188.
Melithaea ochracea van Ofwegen, 1987: 7 Fig. 1 and Fig. 2

Locality: Sta.21 of the Rumphius Biohistorical Expedition 1990, Indonesia, Moluccas, Ambon, Hitu, N coast, Mamala, 3.537997° S; 128.206414° E, depth: 10-15 m, date: 21-09-1990. coll. L.P. van Ofwegen. RMNH.Coel.19852; Clade B, Fig. 2 and Fig. 3; GenBank accession numbers: KC845766 (ND6), KC802206 (COI), KC845653 (mtMutS), KC845879 (28S).

Description: The colony is approximately 25 cm long and dichotomously branched. Both the nodes and internodes are red, the calyces are yellow and the polyps are white.

Fig. 5. Habitus overview of the neotype specimen for *Melithaea ochracea* (Linnaeus, 1758). Scale bar 5.0 cm.

The polyps are irregularly situated around the branches, contracted, and relatively small (<1.0 mm). Calyces project slightly above the coenenchyme. Sclerites of the coenenchyme include capstans, double discs (both 0.05-0.10 mm long) (Fig. 6g), leaf clubs

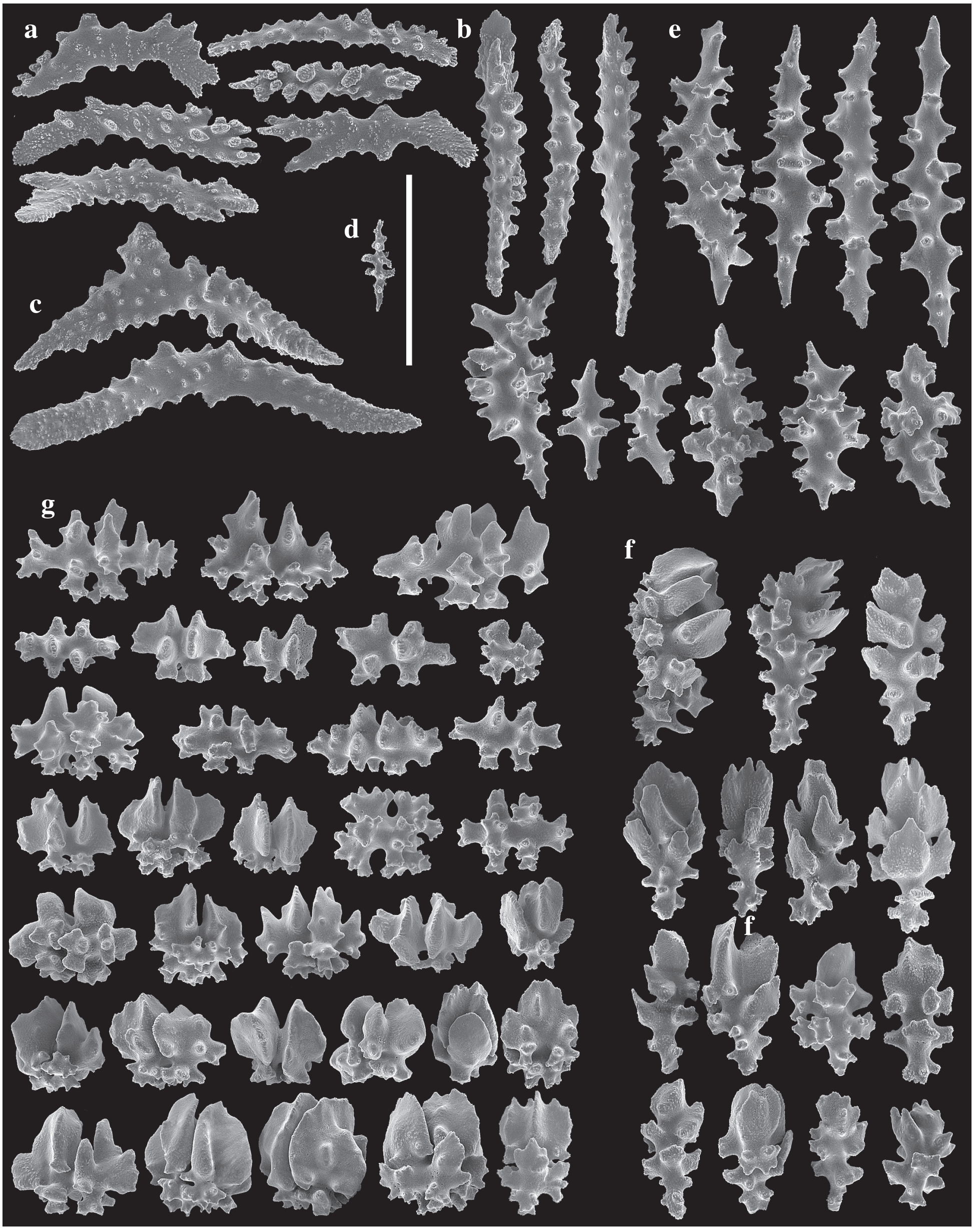

Fig. 6. Sclerite diversity in the neotype specimen of *Melithaea ochracea* (Linnaeus, 1758) (RMNH. Coel.19852); a) tentacle sclerites; b) point sclerites; c) collaret sclerites; d) pharynx sclerite; e) spindles; f) club sclerites; g) double discs and capstans. Scale bar 0.1 mm.

(0.06-0.12 mm long, longer ones are from the calyces) (Fig. 6f) and spindles (0.06-0.20 mm long) (Fig. 6e). The point sclerites are tuberculate, slightly curved spindles, and have spines or leaf-like projections appearing at one of the tips. These sclerites are 0.11-0.19 mm long (Fig. 6b). Collaret has flattened spindles with an ornamentation that is tuberculate in the middle and becomes less tuberculate and more granular at the distal end. Some collaret sclerites have an additional projection at the central bend, approaching a triradiate shape (Fig. 6c). The tentacles have flat, tuberculate, slightly crescent shaped platelets which are 0.07-0.12 mm long (Fig. 6a). The surface of the tentacle platelets appears somewhat granular. The pharynx sclerites are straight rods with spines on the middle area. They are on average 0.06 mm long (Fig. 6d).

Remarks: We examined and compared many melithaeid type specimens. While comparing them with the specimens included in the phylogeny we also compared the sclerites of *Clathraria roemeri* (App. 2, Pl. 18) with those of the neotype specimen of *Melithaea ochracea*. Based on this comparison, *C. roemeri* proved to be a synonym of *M. ochracea*. Both species were collected from Ambon (Moluccas, Indonesia) and are very similar in sclerite morphology. Morphologically the species *Melithaea* sp. (RMNH. Coel.39452, 41124-27; App. 1, Pl. 13-17) and *M. ochracea* have the same sclerite composition (double wheels and small clubs) but the shapes of the sclerites vary, ranging from small and pointed to large and very rounded (App. 1; Pl. 12-17). The taxonomic value of this type of variation in sclerites has to be studied before this variety can be positively identified as *M. ochracea*.

We were also able to check identifications of *M. ochracea* specimens from Singapore (van Ofwegen *et al*., 2000) which proved to be *M. stormii* (Studer, 1895), and of *M. ochracea* from New Caledonia (Grasshoff, 1999) which proved to be *M. caledonica*. So far the only other specimens we consider to truly belong to the species *M. ochracea* are those from Seram (van Ofwegen, 1987). Therefore the distribution of *M. ochracea* is limited to the Moluccas, Indonesia.

Neotype designation of Melithaea rubra Esper, 1798

This species was originally described by Esper (1798) as *Isis dichotoma cortice rubro*. Therein a piece of the octocoral is figured and the type locality ("das Vorgebürg der Guten Hoffnung" [Cape of Good Hope]) is provided. Details on the sclerite morphology are lacking. Grasshoff and Scheer (1990) provided an extensive overview of Esper's work and noted that the type material is lost and that *M. africana* (Kükenthal, 1908) is a possible synonym of *M. rubra*. The current status of the taxonomy and systematics of South African Melithaeidae is not considered satisfactory (Williams, 1992). Fortunately Williams (1992), who reassigned the species to the genus *Acabaria* mentions this is the commonest species around Cape of Good Hope. Therefore we designate a neotype for *M. rubra*, of which the habitus matches the description of Esper (1798) and was collected from the type locality.

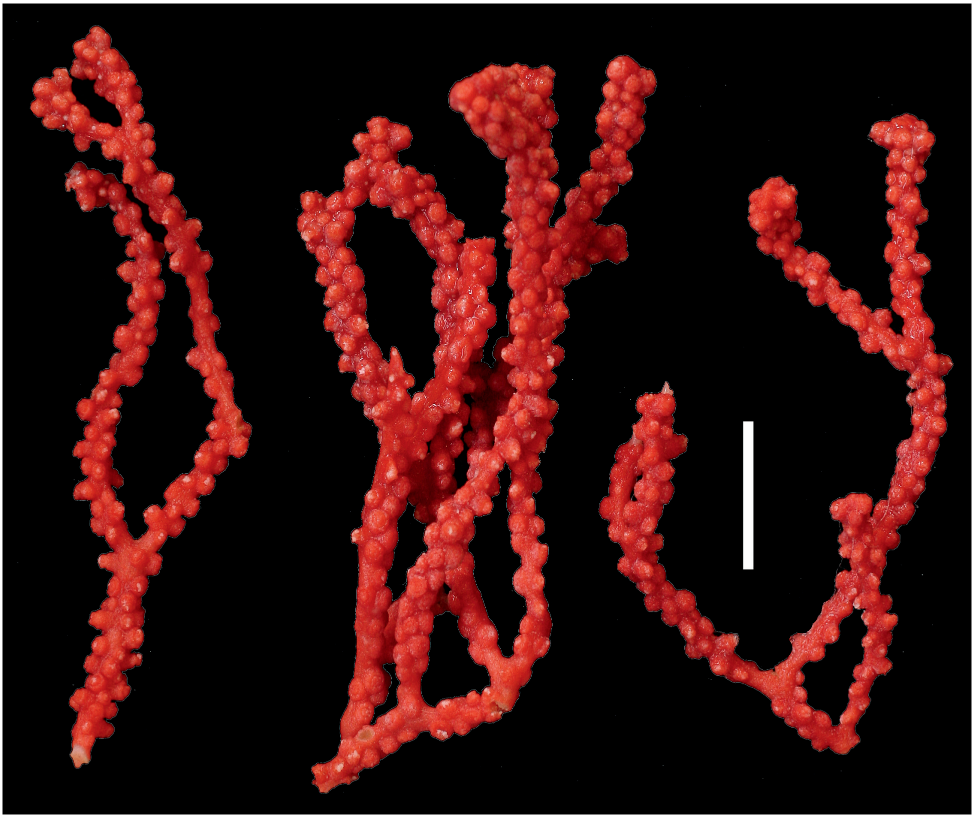

Fig. 7. Habitus overview of the neotype fragments for *Melithaea rubra* (Esper, 1798). Scale bar: 1.0 cm.

Melithaea rubra comb. nov. (Esper, 1798) (Neotype) (Fig. 7 (habitus); Fig. 8 and Fig. 9 (sclerites))

Isis dichotoma cortice rubro Esper, 1798: 6 pl. 1 Fig. 4 and Fig. 5.
Acabaria rubra Williams (1992): 197 Figs. 1A-B, 10–13 (in part).

Locality: South Africa, Cape Peninsula, Oudekraal, Justin's Cave, 33.98165° S; 18.359833° E, depth: 7-11 m, date: 24-3-2008. coll. C.S. McFadden. RMNH.Coel.41180; Clade E, Fig. 2 and Fig. 3; GenBank accession numbers: KC845747 (ND6), KC802155 (COI), KC845635 (mtMutS), KC845811 (28S).

Description: The colony consists of 3 fragments, which are all dichotomously branched, 4.0–5.5 cm long and a light red to pinkish colour. The calyces are of the same colour but the polyps are white. Nodes are not visible. The polyps are large (1.0-1.6 mm in diameter), irregularly situated around the branches giving them a thick and rugged appearance. Calyces project prominently above the coenenchyme. Sclerites of the

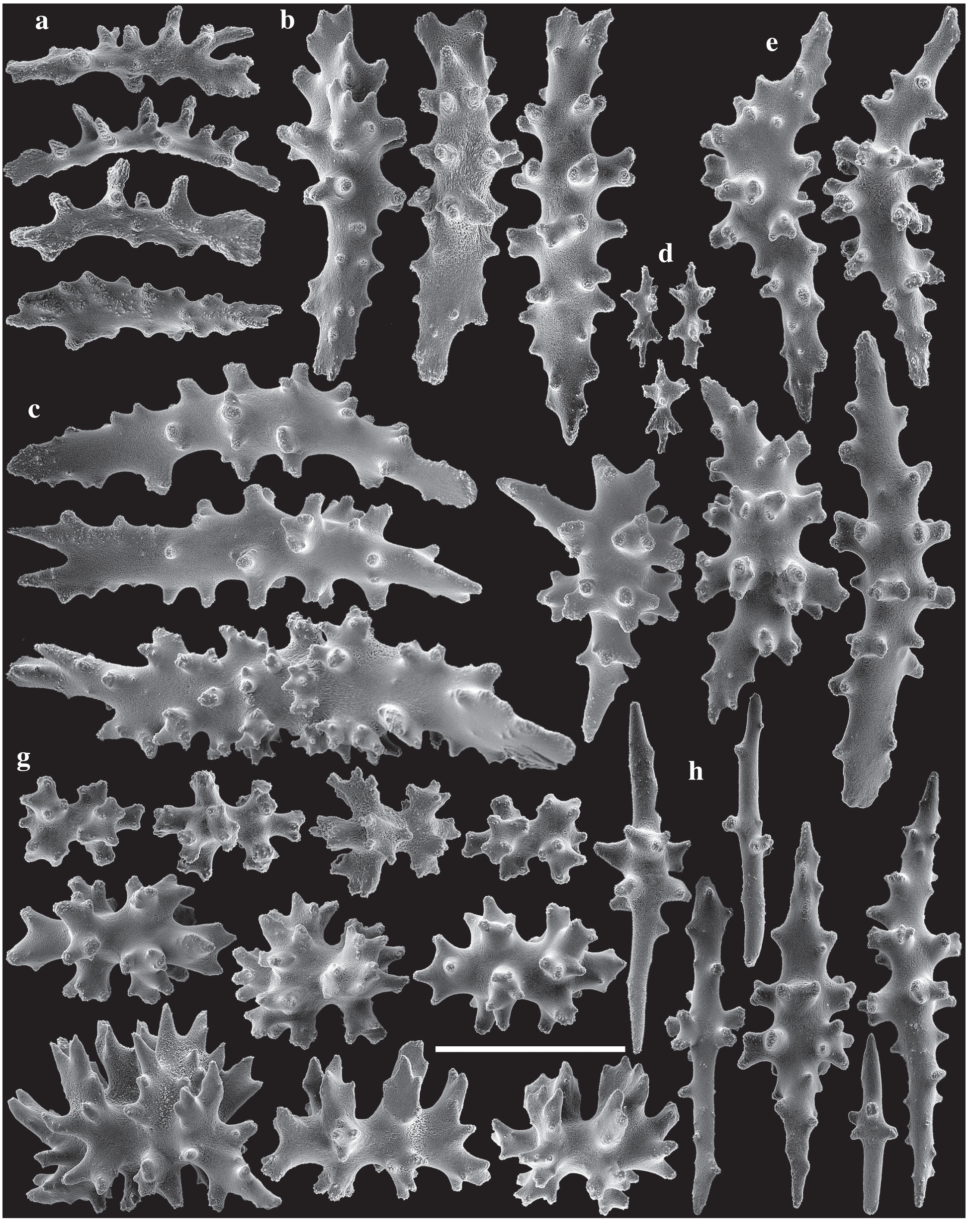

Fig. 8. Overview of the sclerite diversity in the neotype specimen for *Melithaea rubra* (Esper, 1798) (RMNH.Coel.41180); a) tentacle sclerites; b) point sclerites; c) collaret sclerites; d) pharynx sclerites; e) spindles; g) unilaterally spinose spindles and capstans; h) sclerites from nodes and internodes. Scale bar: 0.1 mm.

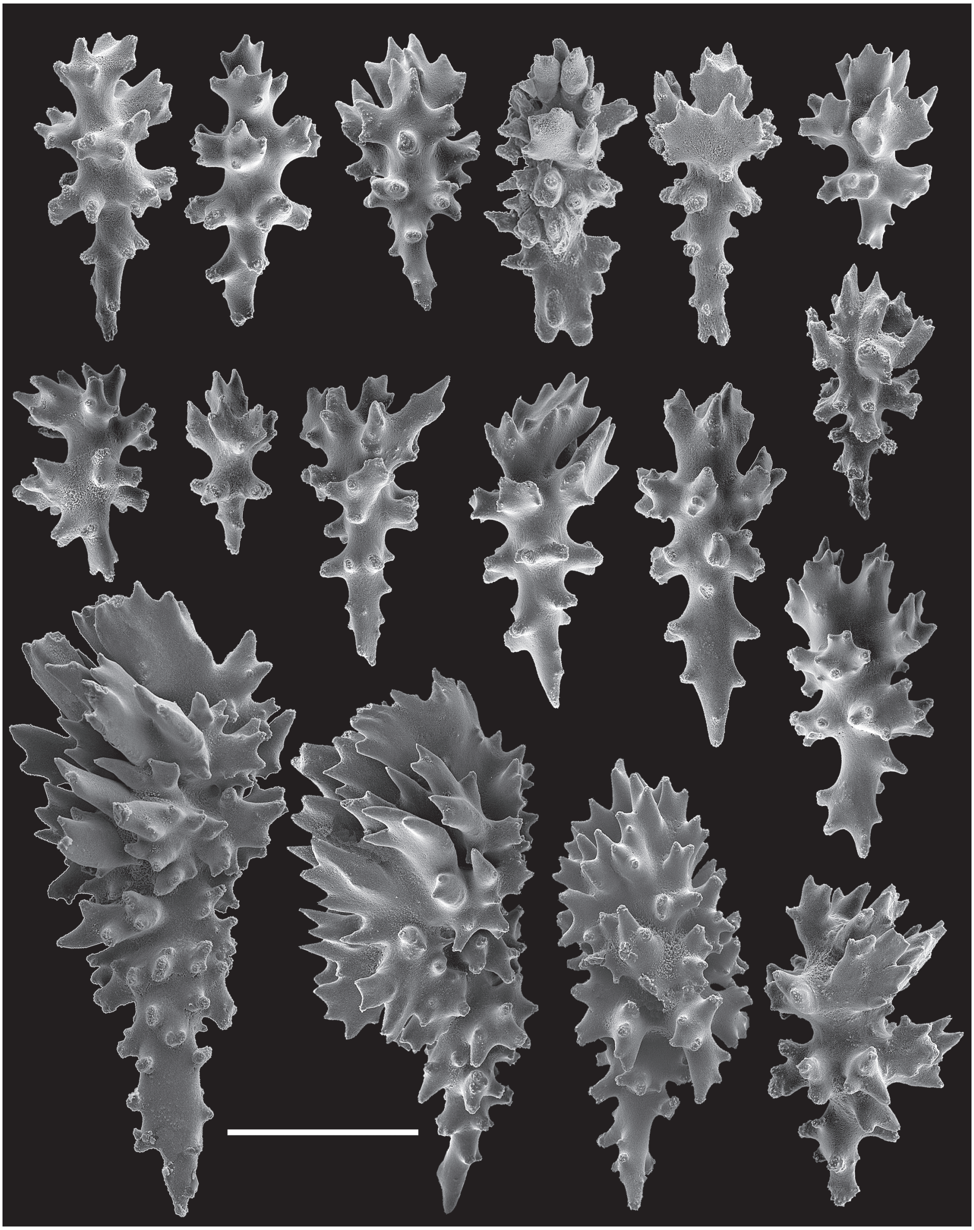

Fig. 9. Overview of the club sclerites in the neotype specimen for *Melithaea rubra* (Esper, 1798) (RMNH.Coel.41180). Scale bar: 0.1 mm.

coenenchyme include capstans (0.08-0.15 mm long) (Fig. 8g), leaf clubs (0.10-0.32 mm long) (Fig. 9) and spindles (0.09-0.28 mm long) (Fig. 8e). Capstans can also have leafy or spinose projections almost giving them a double disk appearance. The leaves on the clubs are very spinose and may take up ⅔ of the total length of the club (Fig. 9). Most spindles in the coenenchyme are slightly crescent shaped and are tuberculate to spiny in the middle area. The point sclerites (Fig. 8b) are relatively thick and blunt on one side and have large projecting tubercles. Point spindles are 0.24-0.27 mm long. The collaret and point spindles can be very similar in appearance, but in general the collaret spindles have more tapered endings, projecting tubercles in the middle and become less tuberculate at the distal end (Fig. 8c). The tentacles contain flat, branched platelets (Fig. 8a), 0.09-0.17 mm long. Spindles or rods from the nodes and internodes (Fig. 8h) often have large median projections resembling some of those of *Asperaxis karenae*. The pharynx sclerites (Fig. 8d) are 0.05-0.06 mm long, straight, and have a waist situated between two girdles of spines and large tubercles.

Discussion

Phylogenetic results

The phylogenetic results obtained in this study differ from those presented earlier by Aguilar-Hurtado *et al*. (2012). Based on their molecular and morphological data at least three melithaeid genera could be validated: *Acabaria*, *Melithaea* and *Mopsella*. Based on our molecular phylogenies these results are not supported. In our case, the phylogenetic results indicate that four genera are paraphyletic, and have been reorganized into the single genus *Melithaea*. The difference in results between our study and Aguilar-Hurtado *et al*. (2012) are most probably the result of a sampling bias. Although the sampling was limited to Japan, comparison between the phylogenetic tree by Aguilar-Hurtado *et al*. (2012) and the phylogenies presented herein show similar, basal topologies. For example, clade A which predominantly consists of *Acabaria* spp. resembles the clade containing *Acabaria* sp. A-D in Aguilar-Hurtado *et al*. (2012). Consequently, Clade B is comparable to the clades containing *Mopsella* and *Melithaea* spp. in Aguilar-Hurtado *et al*. (2012). Clade B in our phylogenies is also predominantly composed of *Mopsella* and *Melithaea* spp. Aguilar-Hurtado *et al*. (2012) sampled solely from tropical Japanese reefs, which automatically excludes species and genera primarily occurring in the Indian Ocean and Red Sea. It therefore appears that intensive sampling of Melithaeidae in a relatively small biogeographic area biases the subsequent molecular phylogenies and as a result different conclusions are reached. The four different markers that were used in this phylogenetic study provided enough information to support decisions on the generic level and in most cases also on species level for the species that could be identified. Unfortunately in some species groups, genetic resolution at the species level was lacking. In these specific cases morphological features and molecular data also contradict each other, which provokes the discussion on species variation and sequence diversity related to species identifications. To fully resolve these taxonomic issues within the Melithaeidae, new approaches such as next generation sequencing are needed because species-specific molecular markers are still lacking. However, barcoding efforts can still help in the identification of species. Case studies on the genus *Alcyonium* and species collected during a biodiversity assay in Eilat, Israel (McFadden *et al*., 2011) revealed that approximately 70% of the morphospecies can be recognized by means of DNA barcoding with multiple markers. These rapid advancements in sequencing techniques and genomic research on Octocorallia might therefore help to identify gene regions useful for species level identifications in the near future which will provide more insight into the evolution and species numbers in the family Melithaeidae.

Distributional patterns within the Melithaeidae

Both phylogenies (Fig. 2 and Fig. 3) presented in this paper reveal that species do not cluster according to their original morphological classification. Instead a pattern based on their larger scale biogeographic distribution was observed. Specimens from the Indo-Pacific and Red Sea that were formerly classified as *Acabaria* do not form a well-defined group, but are divided among several different clades. Additionally the well-supported sister clade relationship between the Indo-Pacific (clade A and B) and the other three clades (C-E) suggests an ancient divergence with independent diversification in each region. Within the Indian Ocean, the monophyly of these three clades suggests that those most probably originated from ancient one-time events.

To our knowledge this is the first time that such a distributional pattern has been observed within a family of octocorals. Historically the distribution of most melithaeid species such as *M. ochracea* was considered widespread. For example Hickson (1937) stated that *M. ochracea* occurs from Singapore to Fiji. Our findings contradict these historical opinions and indicate that species seem to be distributed according to regional endemism based on oceanic basins. Investigation of type specimens has also shown that species formerly identified as *Wrightella tongaensis* Kükenthal, 1908 collected at Tonga Island stretched the distribution of this species from its sister species in the Indian Ocean towards the East Pacific. Recent investigation of the type specimen showed that this species does not concur with the description of the genus *Wrightella* but represents the original concept of the genus *Melithaea*. In many cases these incorrect identifications have obscured the distribution patterns of species and genera.

Studies on other marine organisms that involve both molecular phylogenetic research and distributional patterns are limited. Cowman and Bellwood (2013) studied three marine fish families occurring circum-globally and found that Atlantic and East-Pacific lineages have been largely independent and isolated from the Indo-Pacific since the Oligocene. Therefore, there was no influx on the Indo-Pacific biota, which in our case can explain why the different clades retrieved from our phylogenetic analyses each represent the different biogeographic areas. For Scleractinia, Fukami *et al*. (2004) found that Atlantic representatives of a specific genus were according to the phylogenetic analyses more closely related to other Atlantic genera than to their Indo-Pacific congeners (Fukami *et al*., 2004). In that specific case the morphological convergence has probably obscured the evolutionary distinctiveness of these corals. Consequently the scleractinian taxonomy is currently being revised based on these results. Close examination of Melithaeidae specimens has not revealed such morphological convergence, but species of e.g. *Acabaria* from the Indo-Pacific versus the Red Sea resemble one another more closely than they resemble other former genera from the same biogeographic region. Instead the phylogenetic history shows resemblance to the biogeographical patterns found by Cowman and Bellwood (2013).

Deep phylogenetic divergence between western Atlantic and Indo-Pacific fauna is most often explained by lack of genetic connectivity following the formation of the Panama isthmus (Knowlton *et al*., 1993, Williams *et al*., 2001 and Reimer *et al*., 2012). In contrast, the distribution of melithaeids is primarily limited to sub-tropical and tropical waters and ranges from the Red Sea, Indian Ocean and Central Pacific to New Caledonia, east to Hawai'i. Additionally species also occur in deeper or colder waters e.g. South Africa and northern Japan. One species (*Acabaria erythraea* (Ehrenberg, 1834)) has also invaded the Mediterranean Sea (Fine *et al*., 2005). Accordingly species distributions cannot be explained by the formation of physical barriers. Therefore, the regulatory factors in melithaeid distribution are most probably oceanic currents. Within the Central Pacific, the North Equatorial Current feeds e.g. the Mindanao- and the Indonesian Through-flow current which via various ways connects the water bodies around southern Japan as far south as North East Australia. These currents primarily explain the distribution of species within the Central Pacific but do not seem to directly influence the distribution outside this area. However, the average sea level is higher in the Central Pacific than in the East Indian Ocean, and Pacific water can therefore permeate through the Indo-Malayan region into the Indian Ocean (Hoeksema, 2007). This one directional route enables some exchange between the Central Pacific and Indian Ocean. In our case this might be expressed by the occurrence of three specimens with their origin in the Seychelles clustering within clade A, for which all other specimens are from the Central Pacific. If this is a recent dispersal event the larvae have probably come from the central Indo-Pacific into the Indian Ocean. In favourable conditions the larvae of other Octocorallia (e.g. *Dendronephthya hemprichi* Klunzinger, 1877) can survive up to 59 days (Dahan and Benayahu, 1998), which could be long enough to reach coral reefs in the Indian Ocean via these oceanic currents.

Taxonomic implications

Most of the genera (*Acabaria*, *Clathraria*, *Melithaea* and *Mopsella*) as defined in the identification key of Hickson, 1937 and Ofwegen, 1987 were found to be paraphyletic in our phylogeny. The findings were supported by each of the single locus analyses as well as in the analyses of the concatenated sequence datasets with and without the COI marker. Several solutions can reconcile the taxonomy with the phylogeny e.g.: (1) The paraphyly of several genera can be maintained as it is. In addition the assumption has to be made that identical morphological characters have evolved in different regions over time by convergent evolution; (2) All former genera (except for *Asperaxis*) can be synonymized within the genus *Melithaea*; or (3) The existing genera can be maintained but split based on biogeographic affinity. If we were to adopt either 1 or 3, the taxonomy of the Melithaeidae would become more confused by unclear characters that will not help to differentiate between genera or species. In particular, morphological features to clearly describe these (new) genera are lacking.

By adopting the second solution the genus *Melithaea* will contain almost all species (n = 114; WoRMS database, accessed 22-01-2013) described in the Melithaeinae. *Asperaxis karenae* is the only exception and will remain in its separate subfamily (Asperaxinae). Although this solution creates a genus representing over one hundred species, future studies of the Melithaeidae will likely show that there are more species names than valid species. The type specimens we examined suggest that several species morphologically resemble each other and should be synonymized such as suggested for *M. roemeri* with *M. ochracea*. *Melithaea amboinensis* (Hentschel, 1903) (App. 2; Pl. 1) (formerly *Acabaria amboinensis*) can be synonymised with *Melithaea laevis* (Wright and Studer, 1889) (App. 2; Pl. 10) (formerly *Acabaria laevis*); *Melithaea sulphurea* (Studer, 1895) (App. 2; Pl. 37) (formerly *Melitodes sulphurea*) which is a synonym of *Melithaea stormii* (Studer, 1895) (App. 2; Pl. 36) (formerly *Melitodes stormii*) [Hickson (1935) already mentioned that *M. amboinensis*, *M. fragilis* and *M. laevis* are very similar but he never formerly synonymised these species, and we did not investigate the type specimen of *M. fragilis* so the status of this species remains tentative]; and *Melithaea modesta* (Nutting, 1911) (App. 2; Pl. 31) (formerly *Melitodes modesta*) is a synonym of *Melithaea planoregularis* (App. 2; Pl. 13) (formerly *Acabaria planoregularis* Kükenthal, 1910). Another species that closely resembles the former two species is *Melithaea esperi* (Wright and Studer, 1889) (App. 2; Pl. 28) (formerly *Melitodes esperi*), but based on the differences in tuberculation of the sclerites we refrain from synonymising this species with *M. planoregularis* until more is known about the sclerital variety within species.

Likely there are more species that should be synonymised, but studying type specimens is a time-consuming and meticulous process. With the figures of the sclerites of type specimens added as Appendix 2 we provide a baseline for future taxonomic and phylogenetic research on the family Melithaeidae.

Validity of Asperaxinae

The mtMutS phylogeny (Fig. 4), which includes the single representative of the subfamily Asperaxinae (*Asperaxis karenae*), shows a well-supported distinction between the Melithaeinae and Asperaxinae. As a result the molecular data suggest that the family Melithaeidae should be considered paraphyletic. However, when the morphological features of *A. karenae* are taken into account a different conclusion is reached. Based on the morphological features described for *Asperaxis karenae* by Alderslade (2006) (App. 2, Pl. 19A-B), this species would formerly be referred to as a “true” Melithaeidae species which resembles the characteristics of the genus formerly recognised as *Acabaria*. According to Hickson’s classification (1937) the main feature of the genus *Acabaria* is the dominance of spindles in the coenenchyme and the absence of clubs and capstans. Likewise, *A. karenae* is also dominated by spindles and lacks double discs, clubs or foliate capstans. The characters Alderslade (2006) used to separate the Asperaxinae from the Melithaeinae are axial sclerites in the form of rods and sticks that are often sinuous and branched and possess simple, sparse, tubercles. Morphological examinations of our material revealed that these characteristics are also found in other species that are placed in the Melithaeinae. For example in specimens of *Melithaea rubra* similar (branched) rods with tubercles can be found and therefore these characters do not clearly differentiate between the two subfamilies. The phylogenetic research on the position of Asperaxinae, as performed herein, is only based on a single mtMutS sequence from GenBank. Since additional data could not be obtained its position remains inconclusive, but with the current morphological and phylogenetic data it is doubtful whether *A. karenae* deserves its own subfamily and should most probably be included in the Melithaeinae pending new specimens for molecular studies.

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Appendices are available in the online version of Molecular Phylogenetics and Evolution.