Diversity of Zoanthids (Anthozoa: Hexacorallia) on Hawaiian Seamounts: Description of the Hawaiian Gold Coral and Additional Zoanthids

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Abstract

The Hawaiian gold coral has a history of exploitation from the deep slopes and seamounts of the Hawaiian Islands as one of the precious corals commercialised in the jewellery industry. Due to its peculiar characteristic of building a scleroproteic skeleton, this zoanthid has been referred as Gerardia sp. (a junior synonym of Savalia Nardo, 1844) but never formally described or examined by taxonomists despite its commercial interest. While collection of Hawaiian gold coral is now regulated, globally seamount habitats are increasingly threatened by a variety of anthropogenic impacts. However, impact assessment studies and conservation measures cannot be taken without consistent knowledge of the biodiversity of such environments. Recently, multiple samples of octocoral-associated zoanthids were collected from the deep slopes of the islands and seamounts of the Hawaiian Archipelago. The molecular and morphological examination of these zoanthids revealed the presence of at least five different species including the gold coral. Among these only the gold coral appeared to create its own skeleton, two other species are simply using the octocoral as substrate, and the situation is not clear for the final two species. Phylogenetically, all these species appear related to zoanthids of the genus Savalia as well as to the octocoral-associated zoanthid Corallizoanthus tsukaharai, suggesting a common ancestor to all octocoral-associated zoanthids. The diversity of zoanthids described or observed during this study is comparable to levels of diversity found in shallow water tropical coral reefs. Such unexpected species diversity is symptomatic of the lack of biological exploration and taxonomic studies of the diversity of seamount hexacorals.

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Introduction

Deep-sea corals, particularly on seamounts, have received significant attention over the last decade [1]. These corals have been shown to play a role as ecosystem biobuilders, acting as habitats for diverse invertebrate and fish communities [2]. In these studies, significant attention is given to scleractinians and more recently to octocorals. However, as in shallow water, the hexacorallian order Zoantharia (also sometimes called Zoanthidea) may also make up a considerable component of some deep-sea coral communities [3,4,5] but have received little attention.

The order Zoantharia is characterised by two rows of tentacles and, most often, a colonial mode of life. This order is commonly referred to as ‘zoanthids’ in English despite the possible confusion with the family Zoanthidae, a group within this order, restricted to shallow tropical and subtropical environments. In this paper, we follow the most used standards in past and present specialised taxonomic literature, using Zoantharia as the scientific order name and “zoanthids” as the common name for the order. Zoanthids can be found in most marine environments although very little is known of their ecological role in the ecosystem. Deep-sea zoanthids remain largely unknown with the exception of a few species regularly dredged or trawled on deep-sea soft sediments. Most of these zoanthids belong to the genus Epizoanthus Gray, 1867, in the family Epizoanthidae Delage and Hérouard, 1901, with a few species associated with pagurids [6] and some associated with hexactinellid sponge stalks. Isozoanthus Carlgren in Chun, 1903 within the family Parazoanthidae Delage and Hérouard, 1901 is the second most speciose genus of deep-sea zoanthid species, (however recent taxonomic work casts doubt on the status of this genus [7]). Another group of deep-sea zoanthids was recently described from over 3000 m depth in a cold seep environment, the family Abyssozoanthidae Reimer and Fujiwara in Reimer et al., 2007. Recently specimens belonging to this group have been found in other bathyal and abyssal environments around the world [8,9]. Between 100 m and 2000 m depth, numerous epizoanths, usually belonging to the family Parazoanthidae, can be found. However, most of them remain undescribed despite some species such as the Hawaiian gold coral (often referred as Gerardia) having been known for decades. Table 1
reviews the currently recognised zoanthid genera and families with an indication of the genera found in the deep-sea.

**Hawaiian gold coral**

The Hawaiian gold coral (fig. 1A) is a zoanthid found in the Hawaiian Archipelago at depths ranging from 343 m to 575 m. This species was discovered in 1970 following investigations of *Corallium secundum* Dana, 1846 [10]. It is one of the few zoanthids able to secrete a scleroproteic skeleton (fig. 1D). Its name “gold coral” originates in the golden colour of this secretion. This peculiarity has been used in jewellery and led to the commercial exploitation of the Hawaiian gold coral from the 1970’s until 2001 with an interruption between 1979 and 1999 [11]. The gold coral is not currently exploited in Hawaii due to a restriction on the fishery which requires selective harvest making collection of this species cost prohibitive. Besides its role in the fishery, the Hawaiian gold coral, have been observed and collected in Hawaii, but far less is known of their biology and ecology and they have not been described taxonomically.

**Zoanthid taxonomy**

In 1913 Carglren, who was probably the most active researcher on this order, said “Among the Anthozoa (…) there is hardly a group which is so uniform in its morphological characteristics as the Zoantharia …” [23]. Nearly a hundred years later, despite numerous taxonomical investigations of the morphological characteristics of this order, Carlgren’s statement is still accurate. The sphincter position has been traditionally used to identify zoanthid genera, although Lwowsky [24] illustrated the risks of misidentification using this character. This is exemplified in the *Parazoanthidae*, in which recent taxonomic work casts doubts on the significance of the sphincter muscle position (the main distinguishing feature of *Isozoanthus*) as a valid character [7] and recent studies based on morphology assigned various unrelated zoanthids to the genus *Isozoanthus* [25–28], none of which matched with the ecological characteristics of previously described *Isozoanthus* species. Similarly, Swain [29] showed clearly that the sphincter position did not allow proper identification at the genus level and does not represent the evolutionary history of this group.

At the species level, nematocysts appear to be the most promising morphological character for use within the order [28,30,31]. However, Ryland et al. [32] suggests using very large sample size to obtain reliable information, although such size are too large for most descriptions of new species (which are often represented by only a few polyps). Additionally, until now no studies have been made on the intraspecific and intra-individual variation of nematocysts sizes and assemblages in zoanthids through their life cycle and through changes in biological activity. For these reasons, nematocysts as a diagnostic character are to be
interpreted with caution until more research is being made both on intra- and interspecific cnidome variations.

Recently, DNA information has shed light on the phylogenetic relationships among zoanthids and has also helped revise the taxonomy of several groups of zoanthids as well as describe new taxa [7,8,21,33–35]. However, the sole use of DNA to identify zoanthid species might not be sufficient to distinguish closely related species [36] and it is therefore necessary to integrate not only DNA and morphology but also ecological parameters to identify specimens.

Epizoism

Within the suborder Macrocnemina (comprising all the specimens examined here) most species are epizoic. Recent molecular phylogenetic studies suggest a possible relation between the group of organisms used as substrate and the evolution of the suborder [7,21]. This hypothesis concerns essentially the family Parazoanthidae. Species within the other commonly epizoic family, Epizoanthidae, are often associated with gastropod shells (regularly inhabited by pagurid crustaceans) or tube worms, and do not appear to form monophyletic groups according to the type of substrate used [37]. Parazoanthid species on the other hand, do appear to form monophyletic groups correlated to the organisms colonized (such as sponges, antipatharians or octocorals) and a recent revision of the family used this character to help redefine genera [7]. Most species associated with hydrozoans were recently separated from Parazoanthidae to be placed in their own family Hydrozoanthidae (only 18 genera were recognised).

Materials and Methods

Sampling

Samples were collected using Hawaiian Undersea Research Laboratory (HURL) submersibles *Pisces IV and V*, during cruises in 1998–2004. All necessary permits were obtained for the described field studies. Zoanthids collected in the Northwestern Hawaiian Islands were collected when it was the NWHI Coral Reef Ecosystem Reserve under permit # NWHICRER-2003-003 and -004. Collections were made prior to establishment of the Papahanaumokuakea Monument. CITES does not apply as zoanthids are not listed in CITES. Where possible, but consistently from 2003 on, each colony was filmed in situ before being sampled. Samples were fixed with 8–10% formaldehyde and later stored in 70% alcohol in the Baco-Taylor lab. A subsample of each associated with octocorals have been described, all in the family Parazoanthidae: *Savalia savagia* [38] from the Mediterranean Sea and East Atlantic, *S. lucifica* [39] from California (originally described as *Parazoanthus lucificus*), *Corallizoanthus tsukaharai* [35] from Japan and *Isozoanthus primnoidus* [28] from the Azores. The Hawaiian zoanthids and a gold-coral related species from the Bahamas represent additional known, yet undescribed, zoanthids presumably associated with octocorals.

The purpose of this study is to re-examine specimens of the Hawaiian gold coral and four other species of arborescent zoanthids from the Hawaiian Archipelago to evaluate the biodiversity of these ecologically important zoanthids and to provide taxonomic placement of these species in consideration of their evolutionary history. This aim was achieved and results in the description of several new zoanthid genera, a significant advance in the understanding of zoanthid biodiversity considering that until this study only 18 genera were recognised.
et al. examined. The classification and terminology of nematocysts for each species and all the colonies available were differential interference contrast optic system. A minimum of ten examined with a light microscope equipped with a Nomarski of nematocysts are included.

Results

Taxonomic section

Abbreviation used: 16S = mitochondrial large ribosomal subunit; ARB = Amy Baco; BPBM = Bernice Pauahi Bishop Museum; MMC = Museo del Mar, Ceuta (Spain); MNHG = Natural History Museum of Geneva (Switzerland); NIIWA = National Institute of Water and Atmospheric Research (Wellington, New Zealand); NSMT = National Museum of Nature and Science (Tokyo, Japan); USNM = Smithsonian Institution (Washington DC, USA); RMNH = Naturalis (Leiden, The Netherlands).

Macronemata zoanthids characterised by a complete fifth pair of mesenteries. Family Parazoanthidae Delage & Hérouraud, 1901 Macrocnemic zoanthids with an endodermal sphincter (according to the original description).

Kulamanamana gen. n.

urn:lsid:zoobank.org:act:DE4B202A-3564-4111-8885-81B5AC0DBCE7

Type species: Kulamanamana haumeaae sp. n.

Etymology: the feminine name of this genus is derived from the Hawaiian terms Kula (= gold) and manamana (= branch) referring to the particular skeleton of the type species of this genus.

Diagnostic: Macronemic genus associated with octocorals secreting a golden to dark brown scleroprotein skeleton. Absence of mineral inclusions in the ectoderm, well developed coenenchyme completely covering the host. Characteristic insertion/deletion pattern in the 16S V5 region sensu Sinniger et al. [21] (fig. 2).

Kulamanamana haumeaae sp. n. (Hawaiian gold coral)

Figs. 1 A, D, E, 3 (top)

Etymology: this species name is dedicated to Haumea, Hawaiian goddess of fertility.

Material examined:

Holotype: P5 593, Keahole Point (19°48.203′-19°47.943′ N, 156°08.047′-156°07.478′ W), Hawaii, 15.10.2004, 396.5 m, coll. ARB, more than 200 polyps on fragments of different sizes, presence of some polyps of the parasitic zoanthid Kulamanzoauskerbyi, USNM 1190187 (formalin-fixed), fragment of 20–25 small polyps, USNM 1190188 (ethanol-fixed).

Paratypes: P5-582, Makapuu, Hawaii, 02.10.2004, 395 m, two fragments of 30–35 and 20 polyps of small size, BPBM-D2250 (ethanol-fixed); P5-582, Makapuu, Hawaii, 02.10.2004, 401 m, three fragments of 18–20, 15 and 25–30 polyps medium to large size, MNHG-INVE-82279 (ethanol-fixed); P5-583, Makapuu, Hawaii, 03.10.2004, 427 m, three fragments of 24, 30–35 and 10–15 polyps of different sizes, NIWA-84101 (ethanol-fixed); P5-583, Makapuu, Hawaii, 03.10.2004, 432 m, two fragments of 90–95 and 30–35 polyps of different sizes, MMC-T3 (ethanol-fixed); P5-583, Makapuu, Hawaii, 03.10.2004, 406 m, two fragments
Table 2. Distribution, type, relative abundances and size ranges of cnidae in the new zoanthids species described here.

| Tissue                | Nematocyst types          | Ku. haumeae gen. n. sp. n. | Z. ammophilus gen. n. sp. n. | H. parrishii gen. n. sp. n. | Ka. kerbyii gen. n. sp. n. | B. emilyacadiaarum gen. n. sp. n. |
|-----------------------|---------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------------|
| Tentacles             | Spirocysts                | vc; 15–30 × 3–4 (A)         | vc; 16–30 × 2.5–4 (A)        | vc; 15–25 × 2.5–4 (A)       | vc; 20–35 × 3–5 (A)         | vc; 20–35 × 3–5 (A)             |
|                       | Spirulae                  | rc; 20–27 × 3.5–4.5 (B)     | rc; 17–21 × 4–5 (B)          | rc; 18–21 × 3.5–4 (B)       | c; 16–23 × 2.5–3 (B)        | vc; 20–30 × 3–5 (B)             |
|                       | Special Spirulae          | r; 12–16 × 3–4 (C)          |                             |                             |                             |                                  |
|                       | Penicillii                | r; 14–20 × 5–6              |                             |                             |                             |                                  |
|                       | Penicillii A              | u; 25–35 × 6–7 (C)          |                             |                             |                             |                                  |
|                       | Penicillii E              | c; 18–25 × 8–11 (D)         |                             |                             |                             |                                  |
|                       | Homotrichs 1*             | c-vc; 11–15 × 4–5 (E)       | rc; 9–12 × 3–4 (C)          |                             |                             |                                  |
|                       | Homotrichs 2*             | r; 8–12 × 2.5–3 (E)         |                             |                             |                             |                                  |
| Pharynx               | Spirulae                  | rc; 20–25 × 3–5 (F)         | u; 16–18 × 4 (D)            | vc; 18–24 × 2.5–4 (F)       | c; 20–24 × 4–4 (F)          |                                  |
|                       | Penicillii E              | rc; 20–25 × 5–7 (G)         | rc; 18–21 × 5–7 (E)         | r; 17–22 × 4–6 (E)          | u-rc; 19–21 × 5–6 (G)       |                                  |
|                       | Penicillii E Special      | c-vc; 18–23 × 8–10 (H)      |                             |                             |                             |                                  |
|                       | Homotrich 1*              | r; 32 × 5                   |                             |                             |                             |                                  |
|                       | Homotrich 2*              | r; 16–12 × 1.5–4 (G)        |                             |                             |                             |                                  |
| Filaments             | Spirulae                  | r; 20–23 × 4 (H)            |                             |                             |                             |                                  |
|                       | Special Spirulae          | r; 12–13 × 6–7 (H)          |                             |                             |                             |                                  |
|                       | Penicillii                | r; 15–17 × 8–10 (I)         |                             |                             |                             |                                  |
|                       | Penicillii A              | c; 20–26 × 6–7 (J)          | rc-rc; 16–20 × 5–6 (J)      | c; 16–25 × 5–6 (G)          | c-vc; 17–20 × 5–7 (R)       |                                  |
|                       | Penicillii E              | c-vc; 20–23 × 8.5–10 (K)    | rc; 37–30 × 11–16 (J)       | u-rc; 27–35 × 12–16 (S)     | c-vc; 30–50 × 5–7 (T)       |                                  |
|                       | Penicillii E Special      | c; 30–50 × 5–7 (T)          |                             |                             |                             |                                  |
|                       | Homotrichs 1*             | u-rc-rc; 10–15 × 3–4 (K)    | rc; 12–19 × 2–3 (H)         | c-vc; 11–14 × 4–6           | c-vc; 10–15 × 4–5           |                                  |
|                       | Homotrichs 2*             | r; 15 × 3                   |                             |                             |                             |                                  |
|                       | Homotrichs 3*             | r; 15 × 3                   |                             |                             |                             |                                  |
| Body wall             | Spirulae                  | r; 22–27 × 3.5–4 (M)        |                             |                             |                             |                                  |
|                       | Special Spirulae          | c-vc; 18–25 × 13–19 (L)     |                             |                             |                             |                                  |
|                       | Penicillii A              | u-rc; 29–40 × 6–8 (N)       |                             |                             |                             |                                  |
|                       | Penicillii E              | c-vc; 21–25 × 8–10 (O)      | rc-rc; 32–40 × 18–25 (K)    | u; 43–55 × 18–23 (L)        | u-rc; 35–38 × 15–20 (O)     | vc; 21–30 × 11–16 (M)          |
|                       | Homotrichs 1*             | rc; 12–16 × 3.5–4 (P)       | u; 8–9 × 2.5–3 (L)          | c; 10–15 × 3–4 (M)          | c; 10–12 × 1.5–2 (J)        | rc-rc; 10–12 × 3.5–4.5 (N)      |
|                       | Homotrichs 2*             | r-rc-rc; 11–15 × 4–5 (O)    |                             |                             |                             |                                  |

Abbreviations: c; rare, u; uncommon, rc; relatively common, c; common, vc; very common. Capital letters refer to figure 3. Number ranges indicate cnidae dimensions (length x width) in μm.

*Several types of small homotrichs are distinguished; however they might be extremes of a wide morphological variation range of a single type.

Cnidae nomenclature differs according to different authors. Here is a list of correspondant terms: Spirulae (= b-mastigophore; Basitrichs), Penicilli (= P-mastigophore), Homotrichs (Holotrichs), Penicilli A (= p-mastigophore A), Penicilli E (= Holotrichs; Homotrichs; p-mastigophores E), Penicilli E Special (= p-mastigophore E special; Homotrichs, Holotrichs), Homotrichs (Holotrichs).

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from the same colony of 45–50 and 60–65 polyps of different sizes, MMC-T4 (formalin-fixed); P5-585, Lanikai, Hawaii, 05. 10. 2004, 400 m, fragment of 10 small polyps and also a fragment of 85–90 polyps of small size, USNM 1190189 (formalin-fixed); P5-586, Lanikai, Hawaii, 05.10.2004, 410 m, three fragments of, 10–15, 10–15 and 30–35 polyps of different sizes, NIWA-84102 (ethanol-fixed); P5-588, Cross Seamount, Hawaii, 09.10.2004, 394 m, three fragments of 15–20 and 20–25 polyps of medium to big sizes, NSMT-Co 1547 (ethanol-fixed), two fragments of 8–10 and 20–25 polyps of medium size, MNHG-INVE-82280 (ethanol-fixed); P5-

Figure 2. Genetic signatures of Savalia and related zoanthid genera. This figure shows the V5 region sensu Sinniger et al. [21] with characteristic insertion/deletion pattern specific to each genus. This region is located in the second half of the mt16S rDNA gene. Sequences are represented from 5’ to 3’ and the sequences used are AY995925, EU035623, KC218431, KC218438, KC218434, KC218433, KC218435, KC218436, KC218435. doi:10.1371/journal.pone.0052607.g002
Kulamanana haumeeae gen. n. sp. n.

| Tentacles | Pharynx | Filaments | Body wall |
|-----------|---------|-----------|-----------|
| A B C D E F G H I J K L M O P Q R S T U V W |

Zibrowius ammonphilus gen. n. sp. n.

| Tentacles | Pharynx | Filaments | Body wall |
|-----------|---------|-----------|-----------|
| A B C D E F G H I J K L M O P Q R S T U V W |

Hurlizoanthus Parrishii gen. n. sp. n.

| Tentacles | Pharynx | Filaments | Body wall |
|-----------|---------|-----------|-----------|
| A B C D E F G H I J K L M O P Q R S T U V W |

Kauluozanthus kerbyii gen. n. sp. n.

| Tentacles | Pharynx | Filaments | Body wall |
|-----------|---------|-----------|-----------|
| A B C D E F G H I J K L M O P Q R S T U V W |

Bullagummozoanthus emiliyacadiaearum gen. n. sp. n.

| Tentacles | Pharynx | Body wall |
|-----------|---------|-----------|
| A B C D E F G H I J K L M O P Q R S T U V W |

Figure 3. Cnidae of the different species. Cnidae in the different tissues of the zoanthids described here. Letters correspond to the cnidae listed in table 2.
doi:10.1371/journal.pone.0052607.g003

589, Cross Seamount, Hawaii, 09.10.2004, 388 m, two fragments from the same colony of 80–90 polyps of different sizes and 25–30 polyps of small size, BPBM-D2251 (ethanol-fixed); GP5 P5-523, Bank 8, Hawaii, 06.10.2003, 526 m, USNM 1190190 (ethanol-fixed); GP6 P5-522, Bank 8, Hawaii, 05.10.2003, two fragments of 75–80 and 35–40 polyps of different sizes, 544 m, NSMT-Co 1548 (ethanol-fixed); P4-19, Keahole point, Hawaii, 22.11.2000, 385 m, fragment of 55 polyps of equal size, RMNH Coel. 40076 (ethanol-fixed). All samples collected by ARB.

Sequences: 18S: KC218399, KC218400, KC218401; 16S: KC218431, KC218432; COI: KC218386, KC218387, KC218388.

Diagnosis: Golden axis, tissue colour ranging from pale yellow to medium orange, secretion of excessive mucus when collected and absence of mineral incrustations are distinctive characters of this species. In terms of cnidome, the main diagnostic characters appear to be the presence of enlarged penicilli A (p-mastigophores A) in tentacles and body wall and penicilli E in all the tissues.

External morphology: In preserved specimens, size of contract-ed and semi-contracted polyps from 1–8 mm length and 2–7 mm diameter. 28–31 pointed tentacles (usually 28). In expanded living polyps, length of the tentacles equivalent to up to three quarters of the diameter of the oral disc. 13–16 bractea on the edge of the oral disc. Apex of closed polyps flat (but might appear rounded in small or extremely contracted polyps). Column cylindrical, with no incrusted particles. Well developed coenenchyme completely covering the host. Polyp density variable within the colony with coenenchyme barely visible in the most dense regions (usually the smallest branches) and polyps separated by over 1 cm in the less dense area (such as the main stem). In situ characterised by bioluminescence when disturbed. Size of the fan-shaped colonies up to over 2 m diameter and over 1 m height. Golden scleroprotein skeleton, distinct from the black skeleton found in the genus Savalia Nardo 1844 (= Gerardia L.-D. 1864). In most major parts of the skeleton, host axis can be observed in the centre. Anastomosed branches are frequently observed and the complex branching pattern and larger size of the colonies is clearly distinct from that of the isidids or other octocorals found in the area, suggesting the ability of this zoanthid to build its own branches (fig. 1a). As observed in other epizoic genera (Savalia and Antipathozoanthus particularly) the colony can spread on other substrata at the base of the original host.

Colour: In vivo, polyps, tentacles and coenenchyme bright yellow to dark orange. Upon formalin fixation tissues turns red as observed in several other zoanthid species. Bioluminescence has been observed in situ.

Microanatomy: 25 to 30 mesenteries following macrocnemic arrangement. Most mesenteries fertile, including incomplete mesenteries. Retractor muscle absent, parietobasilar muscle developed and forming visible pennons. Single siphonoglyph prominent. Sphincter endodermal (0.1–0.3 mm), concentrated in the upper part of the column, forming sinus and processes.

Cnidome: spirocysts, spirulae (b-mastigophores, basitrichs), small homotrichs (holotrichs); penicilli A (p-mastigophores A); penicilli E (Homotrichs; Holotrichs, p-mastigophores E) see table 2 and fig. 3.

Biological interactions: This genus appears to colonise primarily isidid octocorals (bamboo coral), [3]. Previous studies observed this species on the skeletons of several living isidids [16] as well as on several primnoids. More investigations are necessary to determine if this species has a host preference. It is also not clear if this species is colonizing branches free of tissue, or if it parasitises or competes with the host tissue as it spreads along the branches. Comparison
with the closely related genus Savalia suggests a parasitic/competitive relationship, but further studies are necessary to understand the ecological relations between this zoanthid and the organisms used as host. Occasionally, colonies of Clavularia grandiflora can colonise Ku. haumeaae [3]. As found in the genus Savalia, a parasitic ascothoracic cirriped was found in several polyps. However, the exact nature of the interactions between this zoanthid and the crustacean are not known. Due to the depths at which this species occurs the absence of observation of zooxanthellae in the histological analyses was not surprising, although the recent findings of Symbiodinium in Hawaiian black corals down to 396 m depth [60] indicate we can not exclude the possibility of an extremely low density of symbionts. Attempts to PCR-amplify zooxanthellae using dinoflagellate specific primers and to grow dinoflagellate cultures using fresh material of this species following collections in 2004 were also unsuccessful (A. Baco and R. Gast unpubl data).

Distribution: Throughout the Hawaiian Archipelago seamount and island slopes between 343–575 m on hard substrata in low-sediment areas with high relief. Usually one of the most abundant taxa in this depth range and habitat type, often with other corals present. Also observed in Line and Jarvis Islands, Palmaya Atoll and Kingman Reef however at lower densities [3]. Similar specimens with golden or dark brown, almost black axis, also referred as “Gerardia”, were collected in the West Atlantic [16,17], and in New Zealand [61]. If those zoanthids appear to be closely related enough to be congeneric, species-level relationships require further morphological and molecular analyses.

Zibrowius gen. n.
urn:lsid:zoobank.org:act:B139CFB3-E2A3-48D2-A753-84D25645BFA2

Type species: Zibrowius ammophilus sp. n.

Etymology: The masculine genus name is dedicated to Dr. Helmut Zibrowius for his precious contribution to deep-sea zoanthid science through the collection of several important samples. He also introduced the first author to deep-sea zoanthid research and especially to octocoral-associated zoanthids such as Savalia through his accurate naturalistic observations and critical comments. This genus should not be confused with the coral-parasitic crustacean genus Zibrowia Gryger, 1985.

Diagnosis: sand incrust ed, arborescent fan shaped colonies, golden skeleton, well developed coenenchyme completely covering the host, can be confused with Kulamanavanana, but easily distinguished by the presence of sand incrustation in the ectoderm, characteristic insertion/deletion pattern in the 16S V5 region sensu Sinniger et al. [21] (fig. 2).

Zibrowius ammophilus sp. n.
Figs. 1 B, 3 (second from top)
urn:lsid:zoobank.org:act:0839CA17-871D-41CA-BFE2-9E17E2318F4B

Etymology: comes from the greek word for sand: ammos, and the greek word for fondness: phileo.

Material examined:
Holotype: P5-389, Cross Seamount (18'43.995'-18'43.759' N, 158'15.743'-158'15.413' W), Hawaii, 10.10.2004, 389 m, coll. ARB, colony of 150 polyps of different sizes, USNM 1190191 (formalin-fixed), several fragments, all of them bearing 30 polyps, USNM 1190192 (ethanol-fixed), fragment of 50 polyps MNHG-INVE-82281 (formalin-fixed)
Paratypes: P5-587, Cross Seamount, Hawaii, 08.10.2004, 409 m, coll. ARB, three fragments, all of them bearing 120 polyps of different sizes, BPBM-D2252 (formalin-fixed), twelve fragments, all of them bearing 100 polyps of different sizes, MMC-T3 (formalin-fixed).

Sequences: 18S: KC218407, KC218408; 16S: KC218438, KC218439; COI: KC218394, KC218395.

Diagnosis: Golden axis, orange/brownish colour and presence of mineral incrustations are distinctive characters of this species. In terms of cnidome, the presence of medium to big penicilli E in filaments and body wall appears to be characteristic of this species.

External morphology: In preserved specimens, size of contract ed and semi-contracted polyps from 1–3 mm length and 1–3 mm diameter. 20–33 pointed tentacles. In expanded living polyps, length of the tentacles smaller than the diameter of the oral disc. 13–17 bractea (corresponding to capitular ridges on closed polyps) on the edge of the oral disc. Apex of closed polyps flat or slightly rounded. Column cylindrical, with incrusted particles (mainly basalt and foraminifera tests). Well developed coenenchyme completely covering the host. Polyp density variable within the colony with coenenchyme barely visible in the most dense regions (usually the smallest branches) and polyps separated by over 1 cm in the less dense area (such as the main stem). Size of the fan-shaped colonies up to about 50 cm diameter and 70 cm height. Golden scleroprotic skeleton, distinct from the black skeleton found in the genus Savalia Nardo 1844 (=Gerardia L.-D. 1864). However, due to the rarity of the species, we could not determine if this zoanthid secretes its own skeleton. As observed in other epizoic genera (Savalia and Anthopathozoa particularly) the colony can spread on other substrata at the base of the original host.

Colour: In vivo, polyps, tentacles and coenenchyme are brownish-yellow, similar to the Hawaiian gold coral Ku. haumeaae, but distinct enough in colour to be recognised as a different species from the submersible, (fig. 1b).

Microanatomy: 22–32 poorly developed mesenteries following macrocennom arrangement, Mesogloea in the column very well developed (0.3–0.5 mm), with presence of numerous lacunae with organic content. No retractor or parieto-basal muscles were noticed. All specimens analysed were fertile. Small siphonoglyph, not specially developed. Endodermal sipheon, short and concentrated in the upper part of the column. No zooxanthellae (see previous species for consideration on potential rare symbiosis with zooxanthellae in deep-sea anthozoans).

Cnidome: spirocysts; spirulae (b-mastigophore); penicilli A (p-mastigophores); penicilli E (p-mastigophores or even holotrichs or homotrichs for other authors); homotrich (holotrich for other authors), see table 2 and fig. 3.

Biological interactions: No information could be obtained on organisms (such as octocoral) potentially used as host. Based on external similarities with Ku. haumeaae and Savalia spp, this species might colonise octocorals.

Distribution: To date, only observed or collected on Cross Seamount.

Hurizoanthus gen. n.
urn:lsid:zoobank.org:act:C0034FA0-804C-476C-B67A-F79DF0753DD0

Type species: Hurizoanthus parishii sp. n.

Etymology: The genus name refers to HURL (the Hawaiian Undersea Research Laboratory) which has provided significant funding and the use of their submersible Pisces for deep-sea coral research in the Hawaiian Archipelago. Work with agency has provided many deep-sea coral species new to science. Due to budget restrictions HURL was closed in the year of the description of these zoanthid species and this genus name is to acknowledge the important contribution of HURL in the discovery of deep-sea diversity around the Hawaiian archipelago. The ending -zoanthus, is a common ending of genera names in the order, historically referring to the flower-like appearance of the animal polyps.
Hawaiian Seamount Zoanthid Diversity

Diagnosis: Macrocnemic genus associated with primitoids. Characteristic insertion/deletion pattern in the 16S V5 region sensu Sinniger et al. [21] (fig. 2).

*Hurlzoanthus* parrishi sp. n.

Figs. 1 C, 3 (third from top)

urn:lsid:zoobank.org:act:F3CF4097-6439-4E8B-A154-083CD36 F937B

Etymology: This species name is dedicated to Dr. Frank Parrish for his contribution to the knowledge of the biology of *Ku. haumeae* and other precious corals in the Hawaiian Archipelago as well as his support of the research of ARB and FS.

Material examined:

Holotype: P5-593, Keahole Point (19°47.907’-19°48.418’ N, 156°07.786’-156°07.631’ W), Hawaii, 17.10.2004, 390 m, coll. ARB, ramified colony, more than 300 polyps, USNM 1190193 (formalin-fixed), branches of the main colony, 70–80 polyps, USNM 1190194 (ethanol-fixed).

Paratypes: P5-533, NW Laysan Island, Hawaii, 19.10.2003, 496 m, coll. ARB, well developed colony, more than 400 polyps, NMST-Co 1549 (ethanol-fixed), small branches with about 70 polyps, NIWA-84100 (ethanol-fixed), several branches bearing 150 polyps, BPBM-D2253 (ethanol-fixed).

Sequences: 18S: KC218402; 16S: KC218433; COI: KC218438.

Diagnosis: White colour and few ramifications are distinctive characters of this species in the studied area. In the cnidome, the presence of big penicilli E (= Holotrichs; Homotrichs; p-mastigophores E) in the body wall appears to be characteristic of this species.

External morphology: In preserved specimens, size of contracted and semi-contracted polyps from 1–5 mm length and 1–3 mm diameter. 30–35 pointed tentacles. In expanded living polyps, length of the tentacles shorter than the diameter of the oral disc. 15–17 bractea (corresponding to capitular ridges on closed polyps) on the edge of the oral disc. Apex of closed polyps flat. Column cylindrical, with incrusted particles (foraminifer tests and basalt). Polyps arranged around the axis, usually in an orthogonal arrangement. Thin coenenchyme completely covering the host. Coenenchyme always visible between the polyps. Colony size reaching 30 cm height and 15 cm diameter, with no additional branches apparent beyond the host octocoral skeleton.

Colour: In vivo, polyps, tentacles and coenenchyme are white (fig. 1c). Upon formalin fixation tissues turn light red as observed in several other zoanthid species.

Microanatomy: 20 to 30 mesenteries following the macrocnemic arrangement. Most mesenteries fertile, including incomplete mesenteries. Mesoglea of the column 0.1–0.2 mm thick, showing sometimes a line of lacunae with organic content. No retractor muscle or parietobasilar muscle features were noticed. Small single siphonoglyph. Short endodermal sphincter, concentrated in the upper part of the column, forming processes (as can be observed in *I. primodus* Carreiro-Silva et al. 2010 but see discussion). No zooxanthellae.

Cnidome: spirocysts; spiralae (b-mastigophore); penicilli A (p-mastigophore); penicilli C (p-mastigophore); penicilli E (p-mastigophore or even holotrich or homotrich for other authors); homotrich (holotrich for other authors); see table 2 and fig. 3.

Biological interactions: Based on the observation of the axis branching and aspect, this genus colonises unidentified octocorals (Primoidae).

Distribution: Besides collection locations, also observed in submersible and ROV videos at the Makapuu’u coral bed off Oahu, Hawaii.

*Kauluzoanthus* gen. n.

**Kauluzoanthus** kerbyi sp. n.

Type species: *Kaulozanthus kerbyi* sp. n.

Etymology: This genus is named after Kaulu, a trickster god that killed Haumea (see etymology of *Kalamanamana haumeae*), and -zoanthus, a common ending of genera names in the order, historically referring to the flower-like appearance of the animal polyps.

Diagnosis: Characteristic insertion/deletion pattern in the 16S V5 region sensu Sinniger et al. [21] (fig. 2). Polyps not contracting when fixed (fig. 1d).

*Kauluzoanthus* kerbyi sp. n.

Figs. 1 E, 3 (second from bottom)

urn:lsid:zoobank.org:act:6090DA23-F323-45A0-A253-19B81C 2965DE

Etymology: This species is named after Terry Kerby, who has been a HURL submersible pilot through most of the time period HURL has been in operation and was pilot during the collection of most of the specimens in this paper. His knowledge of the Hawaiian deep-sea fauna considerably facilitated the sampling of the zoanthids described here.

Material examined:

Holotype: P5-593, Keahole Point (19°48.203’-19°47.943’ N, 156°08.047’-156°07.478’ W), Hawaii, 15.10.2004, 397 m, coll. ARB, 30 polyps of different sizes, USNM 1190195 (formalin-fixed), 20–25 polyps of different sizes, USNM 1190196 (ethanol-fixed).

Paratypes: P5-590, Cross Seamount, Hawaii, 11.10.2004, 343 m, coll. ARB, 35 polyps with specially developed column, some polyps of *Ku. haumeae* also present in the sample, NMST-Co 1550 (formalin-fixed), 5 polyps, NMST-Co 1551 (ethanol-fixed); P5-523, Bank 8, Hawaii, 06.10.2003, 536 m, 10 polyps, BPBM-D2254 (ethanol-fixed). All samples collected by ARB.

Sequences: 18S: KC218404, KC218405, KC218406; 16S: KC218439; COI: KC218391, KC218392, KC218393.

Diagnosis: Light beige polyps, large polyps, associated to *Ku. haumeae*. In the cnidome, the presence of medium to big penicilli E in the tentacles and body wall and special spiralae in the tentacles appears to be characteristic of this species.

External morphology: In preserved specimens, size of polyps from 1–5 mm length and 1–5 mm diameter (although some colonies may have very short polyps); 23–28 pointed tentacles. In fixed polyps, length of the tentacles up to the diameter of the oral disc. No bractea or marginal teeth observed may be present but inconspicuous to be observed under dissecting microscope. Column cylindrical, no incrusted particles. Thin developed coenenchyme partially covering *Kalamanamana haumeae* colonies as well as several octocorals, forming patches up to several centimetres diameter. So far, no host colony has been observed that was fully covered by this zoanthid.

Colour: In vivo, polyps, tentacles and coenenchyme light beige.

Microanatomy: 20–23 mesenteries following the macrocnemic arrangement. No gonads observed. Due to the short length of the column, mesenteries are very difficult to observe. Mesenterial filaments well developed. Deep and narrow siphonoglyph. No sphincter observed. Retractor muscle and parietobasilar muscles with very low development. Tentacle musculature with ectodermal process. No zooxanthellae.

Cnidome: spirocysts, spiralae (b-mastigophore); special spiralae (special b-mastigophores for other authors), penicilli A (p-mastigophore); penicilli C (p-mastigophore or even holotrich or homotrich for other authors); homotrich (holotrich for other authors)
Authors), see table 2 and fig. 3. Cellular structures containing potential micronematocysts were observed in the body wall.

Biological interactions: This species was found on *Kulamanana haumeae* colonies. Further investigations are necessary to study the range of hosts used by this species.

Distribution: So far found in the Hawaiian archipelago with a distribution similar to the Hawaiian gold coral, *Ku. haumeae*. Similar and potentially identical zoanthid was observed in other Pacific locations such as Line, Jarvis, Palmyra and Kingman [3].

**Bullagummizoanthus** gen. n.

urn:lsid:zoobank.org:act:8CDA7F91-217A-48CA-BB55-90B6B4BD1E3D

Type species: *B. emilyacadiaarum* n. sp.

**Etymology:** The name refers to the affinity of this zoanthid genus to bubble gum corals (family Paragorgiidae) with “bulla” and “gummi” being the Latin words for “bubble” and “gum” respectively, and -zoanthus, a common ending of genera names to bubble gum corals (family Paragorgiidae) with “bulla” and “gummi” being the Latin words for “bubble” and “gum” respectively, and -zoanthus, a common ending of genera names in the order, historically referring to the flower-like appearance of the animal polyps.

**Diagnosis:** Characteristic insertion/deletion pattern in the 16S V5 region sensu Sinniger et al. [21] (fig. 2).

**Bullagummizoanthus emilyacadiaarum** sp. n.

Figs. 1 F, 3 (bottom)

urn:lsid:zoobank.org:act:35C895F6-CA66-4189-BD77-487749

**DA25A3**

**Etymology:** This species is dedicated to Emily and Acadia Baco-Taylor, both born during the taxonomical investigations of these Hawaiian species and presenting positive taxis towards bubble gum.

**Material examined:**

Holotype: P5-595, Keahole Point (19°47.907′-19°48.418′ N, 156°07.786′-156°07.631′ W), Hawaii, 17.10.2004, 558 m, coll. ARB, fragment of 70 polyps, USNM 1190197 (formalin-fixed), fragment of 32 polyps, USNM 1190198 (ethanol-fixed), fragment of 25 polyps, BPRM-D2255 (formalin-fixed), fragment of 35 polyps, MMC-T6 (formalin-fixed).

**Sequences:** 18S: KC218403; 16S: KC218434; COI: KC218390.

**Diagnosis:** colony distributed across entire host colony, but not entirely covering its host, low density of polyps, associated with a paragorgiid. In the cnidome, the presence of special spirulae in the body wall and special penicilli E in the filaments appears to be characteristic of this species.

**External morphology:** In preserved specimens, size of contracted and semi-contracted polyps from 1–7 mm length and 2–7 mm diameter. 30–39 pointed tentacles. 15–20 bractea (corresponding to capitular ridges on closed polyps) on the edge of the oral disc. Apex of closed polyps flat. Column cylindrical, with incrusted mineral particles (white sand grains and gorgonian sclerites). Poorly developed coenenchyme not completely covering the host. Polyp sparsely distributed on the host, in groups of few joined polyps issued of asexual reproduction (fig. 1c).

**Size of the colony observed at least 30–40 cm diameter.**

**Colour:** In situ, polyps bright yellow, in strong contrast to bright red of host. In vivo, polyps, tentacles and coenenchyme beige, colour influenced by incrustation, especially pink sclerites. No significant change of colour in formalin.

**Microanatomy:** 30 to 40 mesenteries following the macrocnemic arrangement. Most mesenteries fertile. No retractor muscle or parietobasilar muscle features were noticed. Single conspicuous siphoglyph. Weak endodermal sphincter, difficult to observe in small polyps, extending over the first half of the column. No sinus but small processes were observed. No zooxanthellae.

Cnidome: spirocysts; spirulac (b-mastigophore); special spirulac (special b-mastigophores for other authors); penicilli A (p-mastigophore); penicilli E (p-mastigophore or even holotrich or homotrich for other authors); special penicilli E (new category of nematocysts, enlarged and incurved capsule with a thick filament full of conspicuous spines inside); homotrich (holotrich for other authors). Sporadic special spirulae (special b-mastigophores for other authors) (not shown). See table 2 and fig. 3.

**Biological interactions:** This species colonises a paragorgiid coral, most likely *Paragorgia coralloides*. Due to the coexistence of the octocoral and the zoanthid, further studies are necessary to determine the type of relationship between the two organisms.

**Distribution:** The single colony observed was sub-sampled for this description. However, similar zoanthids are frequently observed on samples of *Paragorgia coralloides* throughout the Pacific, further investigation on such specimens are required to confirm the identity between this specimen and these other zoanthids.

**Remark on the diagnoses:** Diagnoses and descriptions were based on single species for each genera, moreover originating from a restricted locality. Therefore, it is likely that these diagnoses will be completed and modified with the discovery of related species around the world, as happened for the genera *Mesozoanthus* and *Alysoanthus* [8,9,62].

**Phylogenetic results**

**Maximum Likelihood and Bayesian trees topologies are similar** (fig. 4), clearly separating the different genera described here. In comparison to the Bayesian tree shown in fig. 4, ML trees (not shown) place H. parrishi as sister group of the *Z. ammophilus*, *Ku. haumeae*, C. tsukaharae group (ML bootstrap support: 56) and isolate “Parazoanthidae clade 1” at the base of all other Parazoanthidae and Hydrozoanthidae (those groups forming a monophyly supported by a ML bootstrap support of 62). The information provided by three markers used distinguishes most specimens at the species level. At family level, the results confirmed the hypotheses published earlier [7,21] with the family Hydrozoanthidae and the Brachycnemina (not shown) diverging from the Parazoanthidae. However, the phylogenetic relationships among Parazoanthidae are mostly unresolved and no reliable information could be obtained in regard to the presumed close relation between the genus *Antipathozoanthus* and the octocoral-associated zoanthids [22]. Within parazoanthids, the monophyly of all octocoral-associated zoanthids is supported. Both analyses place *Mesozoanthus fossis*, another parazoanthoid not associated to specific organisms, as sister group of this clade and *Savalia* species branch at the base of the octocoral-associated clade. *Ku. kerbyi*, whose ecological relation to octocorals or octocoral-associated zoanthids are unclear, appears distinct from the unresolved clade formed by the remaining octocoral-associated zoanthids. However, it must be noted that the basal position of *Savalia* in relation with the position of *Ka. kerbyi* have weak support in the statistical analyses. Within the clade grouping the other octocoral-associated zoanthids, while ML analyses do not show supported evidence of evolutionary relationships, Bayesian analyses separate *Ku. haumeae* and C. tsukaharae as a sister clade to *Z. ammophilus* and distinct from *H. parrishi* and *B. emilyacadiaarum*.

**Discussion**

**How to classify these new species?**

Based on the phylogenetic trees, several options could be considered to define the taxonomy of Hawaiian deep octocoral-related zoanthids.
1) The Hawaiian gold coral, described here as *Ku. haumeaae*, has historically been referred as ‘*Gerardia* sp.’, a younger synonym of *Savalia* Nardo 1844. Based on the close molecular distances between all octocoral-associated zoanthids, all species examined could have been moved to the genus *Savalia* which would then also include the genus *Corallizoanthus*. However, *Savalia* is characterised by its ability to secrete a skeleton and this ability is not shared by *Corallizoanthus*, *H. parrishi* and *Ka. kerbyi* (unclear in *H. ammophilus*). Morphology of the colonies is completely different between those species, with *Corallizoanthus* colonies consisting of individual polyps connected together only temporarily after polyp division, *B. emilyacadiaarum* also presents a poorly developed coenenchyme and groups of polyps scattered along the host, and other species usually present dense colonies with well developed coenenchyme covering most of the host.

2) Strictly based on phylogenetic tree information, Hawaiian species might be placed in the genus *Corallizoanthus*, however, the remarks mentioned above apply also in this situation and while *Savalia* would remain independent, the secretion of a skeleton by *Ku. haumeaae* appears as a character difficult to ignore when grouping this species with non-skeleton secreting zoanthids.

3) Ignoring phylogenetic information at genus level and based on the history of referring to the Hawaiian gold coral as *Gerardia* sp., all the skeleton secreting zoanthids could be grouped into *Savalia*. However, the taxonomic significance of the skeleton is unknown as well as information on biochemical similarities between secretions of *Savalia* and *Ku. haumeaae* is still missing. The absence of sand incrustation in *Ku. haumeaae* is at least as important character to separate *Kulamanamana* from *Savalia* as is the skeleton to group those species together.

4) Each new species, sharing only a few characteristics and possessing several unique features can be placed in their own genus. The main issue of this solution is the creation of numerous monospecific genera. Nevertheless, in such a restricted and isolated geographic location as was investigated in this study, it is not unlikely to find genera represented only by a single species. The situation is also similar to *Mesozoanthus* or *Abyssozanthus*, monospecific when first described, but since then new species have been discovered around the world [7–9,62].

Figure 4. Phylogenetic tree of the Hawaiian gold coral (*Ku. haumeaae*) and related zoanthids. Bayesian tree based on concatenated 18S, COI and 16S genes. Values at the nodes represent posterior probabilities and bootstrap values respectively. “$S$” indicate the ability to secrete a skeleton. Values below posterior probabilities of 0.5 or 50% bootstrap were considered as unresolved. Hawaiian zoanthids described here are indicated in bold. Epizoanthidae are used as outgroup. Vertical bars indicate the species belonging to Epizoanthidae and Parazoanthidae respectively. doi:10.1371/journal.pone.0052607.g004
Based on our knowledge on zoanthid taxonomy and evolution, the hypothesis of the Hawaiian zoanthids examined in this study belonging to 5 genera was retained and is presented here.

Molecular morphology
As mentioned above, morphological characters are sometimes confusing and can be difficult to compare between different authors. Two of the molecular markers used in this study are ribosomal gene fragments. This implies that the DNA sequence will not be translated into a protein, but the sequence of the RNA transcribed from the DNA will directly define the shape of this RNA strand to form the ribosome. The mitochondrial 16S rDNA is particularly interesting for DNA-based taxonomy as most of the variation appears under the form of insertion and deletion of relatively short strings of DNA. Reconstruction of the natural shape of the ribosome based on the sequence is extremely difficult as it is dependent on several external factors, such as temperature and interactions with other molecules among others. Insertion and deletion events are clear signs of ribosome morphological variation as they are directly related to loops or hairpin folding of the RNA. Our results show that the V5 region (sensu Sinniger et al. [21]) can be used to distinguish zoanthids at the generic level (fig. 2). The insertion/deletion pattern of the V5 region could then be considered as the meeting of molecular and morphological characteristics in zoanthids.

Isozoanthus primnoidus and H. parrishi two primnoid-associated zoanthids
Recently Carreiro-Silva et al. [28] described Isozoanthus primnoidus from Azores, a zoanthid ecologically similar to H. parrishi. While unfortunately no DNA information was published or could be obtained from the type specimens of this new species, the morphological similarities and potential placement of H. parrishi within the genus Isozoanthus must be addressed here. I. primnoidus was assigned to the genus Isozoanthus based on sphincter characteristics and absence of several histological structures. The same characters have been used in the description of Hydrozoanthus antumbrosus (originally described as Isozoanthus), however, Isozoanthus as interpreted recently is likely a “catch all” genus for unrelated species, this statement was based on the comparison with I. sulcatus, a widely accepted Isozoanthus species, showing clear divergences from H. antumbrosus [7]. This statement was further supported by the by the examination of the type species of the genus Isozoanthus, I. giganteus [29]. This species also appears completely divergent to any of the zoanthids described here (Fig. S1). The ecology of the original species of Isozoanthus also supports the absence of a relationship between the species here, I. primnoidus (associated with primnoid octocorals) and formerly described Isozoanthus species, encrusting rocks or in some cases hexactinellid stalks. Marked morphological differences between I. primnoidus and other Isozoanthus species are also mentioned in the original description [28]. Preliminary molecular results (not shown) obtained from potential I. primnoidus from Mediterranean Sea support a clear distinction between I. primnoidus and H. parrishi.

Skeleton-secreting zoanthids
The molecular divergence observed between Ku. haumeaeae and Savalia spp. is surprising considering the absence of variation observed in other samples of Savalia collected worldwide and the apparent morphological similarities between those zoanthids. Moreover the presence of zoanthids not secreting a skeleton within the monophyletic group comprising Savalia and Ku. haumeaeae would suggest either a loss or multiple acquisitions of this very particular ability during the evolution of this group. The potential secretion of a skeleton in Z. ammophilus remains to be examined in detail, so no conclusion can be made on this species at the moment. All these zoanthids belong to the same monophyletic clade associated with octocorals, however the potential secretion of a skeleton in the genus Antipathozoanthus, a genus often associated with antipatharians and placed outside of this large octocoral associated monophyletic group requires further investigation [22,63] but see [7,21]. The confirmation of skeleton secretion in Antipathozoanthus would strongly support the hypothesis of multiple evolution of the capacity to build a scleroprotein axis among zoanthids.

“Allen” nematocysts?
The presence of nematocysts types characteristic of medusozoa, such as rounded “special” spirulae [64,65] in Z. ammophilus and mainly in B. emlyancoanaeae could originate from the hypothesized feeding habits of these species. Carlsgen [23] discussed this issue in Epizoanthus incrustatus E. edwardsi concluding, based on the heterogeneity between samples of the same species, a probable external origin of medusozoan nematocysts types. However, until further clarification of the trophic behaviour of these zoanthids we decided to include those nematocysts as part of the cnidome equipment of the zoanthids. This decision is supported by the finding of similar nematocysts categories in other zoanthids [63].

Habitat and ecology
All those zoanthids were found in areas of seamount pinnacles or on the slope of Hawaiian Islands exposed to currents. This habitat was frequently shared with other precious corals such a Corallium lauuense, other octocorals and antipatharians, as described in detail for Ku. haumeaeae in previous studies [3–5]. However, the distribution of those epizoic species is expected to be highly dependent on the distribution of the host organisms.

Molecular phylogeny supports some relationships between the evolution of the order and the group of organisms used as host. However, for species such as Ku. haumeaeae, the invasive and destructive nature of investigations necessary to identify the original host organism limits the number of samples examined. Further studies are necessary to determine host specificity in these zoanthids as well as on the nature of the interaction between the zoanthids and their host. However, due to the slow growth of the colonies and the invasive nature of current methods to investigate the substrate host in adult colonies, caution must be taken not to impact the stocks of these vulnerable species.

Conclusion
Deep-sea diversity
The zoanthid diversity found in the deeper waters surrounding Hawaii is exceptional in comparison to the diversity found on coral reef in the same area. 7 species of zoanthids belonging to the 3 genera usually found in coral reefs (Palythoa, Zoanthus and Isaurus) were identified in Hawaii [66]. Tropical coral reefs are commonly assumed to be hot spots of biological diversity, however, the discovery of such species and genera diversity in octocoral-associated zoanthids at depth ranging from 300–500 meters would suggest an even higher biodiversity in subphotic coral communities along the steep slopes of oceanic islands and seamounts. A similar pattern is observed for octocorals in the Hawaiian Archipelago, with the vast majority of known Hawaiian octocoral species occurring only in deep water [67]. In addition to the epizoic zoanthids examined in this study, at least three other non epizoic, undescribed species, two belonging to the genus Epizoanthus and
one probable Parazoanthidae were found in the same habitat. This discovery illustrates the amount of zoanthid diversity that remains to be discovered on deep-sea hard substrate environments. Understanding the speciation processes and distribution of these zoanthids will certainly contribute to a better understanding of the past oceanographic patterns in the worldwide oceans as well as contributing to developing efficient impact assessment methods in relation to deep-sea resource exploitation.

Supporting Information

Table S1  Sequences information, n/a = not available. FS refers to the sample number in the first author collection. Due to the short length of the sequences or availability, sequences of Microzoanthus and Isozoanthus were used only in the 16S analyses presented in the fig. S1.

(DOC)

Figure S1  Phylogenetic tree based on 16S including Microzoanthus and Isozoanthus. ML phylogenetic tree reconstructed using the partial 16S sequences. The position of Isozoanthus gigantus near Epizoanthus is well supported. ML bootstrap values are indicated at the nodes. Only the groups supported by 50% or more bootstrap are represented. Microzoanthidae are displayed in black. The tree is rooted with Epizoanthus from the north-east Atlantic. ML phylogenetic tree for the deep-sea coral. (PDF)

References

1. Miller K, Neil H, Tracey D (2009) Recent advances in deep-sea coral science and emerging links to conservation and management of deep-sea ecosystems. Mar Ecol Prog Ser 397: 1–5.
2. Krieger KJ, Wing BL (2002) Megafauna associated with deepwater (Pinnax sp.) in the Gulf of Alaska. Hydrobiologia 471: 83–90.
3. Parrish FA, Baco A (2007) State of Deep Coral Ecosystems in the United States Western Pacific Region: Hawaii and the United States Pacific Islands. Int. Lamsden SE, Hourigan TF, et al., editors. The State of Deep Coral Ecosystems of the United States. NOAA Technical Memorandum CERC-P. Silver Spring, MD: National Oceanic and Atmospheric Administration. pp. 155–194.
4. Baco AR (2007) Exploration for deep-sea corals on North Pacific seamounts and islands. Oceanography 20(4): pp. 108–117.
5. Parrish FA (2006) Precious corals and subphotic fish assemblages. Atoll Res Bull 543: 425–430.
6. Murhead A, Tyler PA, Thurston MH (1986) Reproductive biology and growth of the genus Epizoanthus (Zoanthidae) from the north-east Atlantic. J Mar Biol Assoc UK 66: 131–143.
7. Sinninger F, Reimer JD, Pawlowski J (2010) The Parazoanthidae (Hexacorallia: Zoantharia): DNA taxonomy: Description of two new genera. Mar Biodiv 40: 57–79.
8. Reimer JD, Sinniger F (2010) Discovery and description of a new species of Alysiaznathus (Zoantharia: Hexacorallia) at the Japan Trench: the world’s deepest known zoanthid. Cah Biol Mar 51(4): 451–457.
9. Sinniger F, Zehou K, Taviani M, Reimer JD (2010) Presence of Alysiaznathus sp. (Zoantharia: Zoantharia) in the Mediterranean Sea: indication of a new cold seep or of ecological tolerance of Alysiaznathus to non-chemotrophic environments? Cah Biol Mar 51 (4): 475–478.
10. Grigg RW (1974) Distribution and abundance of precious corals in Hawaii. Proc 2nd Int Coral Reef Symp, Brisbane: pp. 235–240.
11. Grigg RW (2002) Precious corals in Hawaii: discovery of a new bed and revised management measures for existing beds. Mar Fish Rev 66: 13–20.
12. Marshel C, Garabao J, Harmelin-Vivien M, Pichon M (2004) A new method for measuring growth and age in the precious red coral Corallium rubrum (L.). Coral Reefs 23: 423–432.
13. Roark EB, Guilderson TP, Dunbar RB, Ingram BL, (2006) Radiocarbon-based ages and growth rates of Hawaiian deep-sea corals. Mar Ecol Prog Ser 327: 1–14.
14. Roark EB, Guilderson TP, Dunbar RB, Fallon SJ, Shuster GS, et al. (2009) Extreme longevity in proteinaceous deep-sea corals. Proc Natl Acad Sci USA 106: 5208–5213.
15. Parrish FA, Roark EB (2009) Growth validation of cold coral Gerardia sp. in the Hawaiian Archipelago. Mar Ecol Prog Ser 397: 163–172.
16. Goodfellow GA (1997) Aspartic acid racemization and amino acid composition of the organic endoskeleton of the deepwater colonial anemone Geonassan from kinetic experiments. Geochim Cosmochim Acta 61: 1931–1939.
17. Druffel ERM, Griffin S, Witter A, Nelson E, Southon J, et al. (1995) Gerardia: Brief review of the genus. Geochim Cosmochim Acta 59: 5031–5036.
18. Grigg RW (1976) Fishery management of precious and stony corals in Hawaii. Report No. UNIHI-SEAGRANT-TR-77-03. Honolulu, HI: University of Hawaii Sea Grant Program.
19. Poche F (1914) Das System der Coelenterata. Arch Naturgesch 80 (3): 47–128.
20. Haussermann V (2003) Zoantharia. In: Hoferichter, R. (ed.), Das Mittelmeer, bestimmungsfu¨hrer Band II/1. Heidelberg: Bekleim Acad. Verl. pp. 501–505.
21. Sinniger F, Montoya-Burgos J, Chevaldonné P, Pawlowski J (2005) Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. Mar Biol 147: 1121–1128.
22. Ocata O, Brío A, Gonzalez G, Herrera R (2007) Additions in relation to Gerardia: The Mediterranean waters and the Mediterranean Sea (Anthozoa: Zoantharia). Vieraes: 35: 163–166.
23. Carlgren O (1913) Zoantharia. The Danish Ingrid Expedition. 5 (4): pp. 1–65.
24. Lovkovsky F (1913) Revision der Gattung Siphon Gray (Euphyllia ant.): Ein Beitrag zur Kenntnis der Zoantharia. Bull Sci Nat: 50: 113–120.
25. Sanamyan NP, Sanamyan KE (2007) Deep-water Actinaria from East Pacific hydrothermal vents and cold seeps. Invert Zoo 4: 83–102.
26. Swain TD (2009) Inozanthus antumbrosus, a new species of zoanthid (Cnidaria: Anthozoa: Zoantharia) symbiotic with Hydrozoa from the Caribbean, with a key to allied and sympatric zoanthid species. Zoosyst 2003: 41–48.
27. Ritt B, Sarrazin J, Caprias J-C, Noël P, Gauthier O, et al. (2010) First insights into the structure and environmental setting of cold-seep communities in the Marmara Sea. Deep-Sea Res Pt I, 57: 1120–1136.
28. Carreiro-Silva M, Braga-Henrique A, Sampaio I, de Mato V, Porteiro FM, et al. (2010) Inozanthus primnoidus, a new species of zoanthid (Cnidaria: Zoantharia) associated with the gorgonian Calliagorgia verticillata (Cnidaria: Aleyonacea). ICES J Mar Sci: doi:10.1093/icesjmsf/kip73.
29. Swain TD (2010) Evolutionary transitions in symbioses: dramatic reductions in bathymetric and geographic ranges of Zoanthidae coincide with loss of symbioses with invertebrates. Mol Ecol 19: 2587–2598.
30. Sinniger F, Haussermann V (2009) Zoantharia (Cnidaria: Hexacorallia) from shallow waters of the southern Chilean fjord region, with descriptions of a new genus and two new species. Org. Div. Evol. 9: 23–36.
31. Fuji T, Reimer JD (2011) Phylogeny of the highly divergent zoanthid family Microzoanthidae (Anthozoa, Hexacorallia) from the Pacific. Zool. Sci: 40: 418–431.
32. Byrdland JS, Beaseer MM, Lancaster JE (2004) Use of cnidae in taxonomy: implications from a study of Actinostola australis (Hexacorallia, Zoantharia). J Nat. Hist. 38: 1193–1223.
33. Reimer JD, Ono S, Iwama A, Takishita K, Tsukahara J, et al. (2006) Morphological and molecular revision of Zoanthus (Anthozoa: Hexacorallia) from southwestern Japan, with descriptions of two new species. Jpn. J. Zool. 61: 625–637.
34. Kozloff KD, Zhang L, Kozloff LA, Henis Y, Reimer JD, et al. (1992) DNA sequence variation in the mitochondrial cytochrome oxidase III gene of a marine sponge symbiont. Mol Mar Biol Biotechnol: 1: 256–262.
35. Sinniger F, Reimer JD, Pawlowski J (2008) Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). Zool Sci 25: 1253–1260.

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Author Contributions

Conceived and designed the experiments: AB FS. Performed the experiments: FS OO. Analyzed the data: FS OO. Contributed reagents/materials/analysis tools: FS AB OO. Wrote the paper: FS. Histological and morphological examinations: OO. Molecular analyses: FS. External morphology: OO AB FS. Sampling: AB.
37. Reimer JD, Hirose M, Nishisaka T, Sinniger F, Itani G (2010) Epizoanthus spp. Associations Revealed Using DNA Markers: A Case Study from Kochi, Japan. Zool. Sci. 27: 729–734.
38. Bertoloni A (1819) Amoenitates Italicae, sistentes Opuscula ad Rem herbariam et zoologiam Italiae spectamina. Bononiae, [1] 472 pp.
39. Cutress CE, Pequegnat WJ (1968) Three new species of Zoantharia from California. Pac Sci 14: 89–100.
40. Schmidt H (1972) Prodromus zu einer Monographie der mediterranen Aktinien. Zoologica, Stuttgart, 121: 1–146.
41. Hartog JC den (1980) Caribbean shallow water Corallimorpharia. Zool Verh Leiden 176: 1–83.
42. Hartog JC den, Ocan˜a O, Brito A (1993) Corallimorpharia collected during the CANCAP expedition (1976–1986) in the south-eastern part of the North Atlantic. Zool Verh, 292: 1–76.
43. Ryland JS, Lancaster JE (2004) A review of zoanthid nematocyst types and their population structure. Hydrobiologia 530/531: 179–187.
44. Medlin L, Elwood HJ, Stickel S, Sogin ML (1980) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71: 491–499.
45. Apakupakul K, Siddall ME, Burrellson E (1999) Higher-level relationships of leeches based on morphology and gene sequences. Mol Phyol Evol 12: 530–539.
46. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98.
47. Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: A powerful graphical analysis environment for molecular phylogenetics. BMC Evol Biol 4: 18.
48. Ronquist F, Huelsenbeck JP (2003) Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
49. Grigg RW (1993) Precious coral fisheries of Hawaii and the US. Pacific Islands. Ecosystems. Berlin: Springer-Verlag. pp. 663–678.
50. Parrish FA (2007) Density and habitat of three deep-sea corals in the lower Hawaiian chain. In: George RY, Cairns SD, editors. Conservation and adaptive management of seamount and deep-sea coral ecosystems. Miami, FL: Rosenstiel School of Marine and Atmospheric Science, University of Miami. pp. 55–65.
51. Baco AR, Shank TM (2005) Population genetic structure of the Hawaiian precious coral, Corallium lauuense. Mar Biodiv Report (86): 317 pp.
52. Etnoyer P, Morgan LE (2005) Habitat-forming deep-sea corals in the Northeast Pacific Ocean. In: Freiwald A, Roberts JM, editors. Cold-Water Corals: The Biology and Geology of Deep-Sea Coral Habitats. Cambridge: Cambridge University Press. 334 p.
53. Clark MR, Kelley C, Baco A, Rowden A (2011) Hauna's cobalt-rich ferromanganese crust seamounts. International Seabed Authority Tech Study No. 8. 92 p.
54. Reimer JD (2010) Unexpected diversity in Canadian Pacific water precious corals from the Hawaiian Archipelago: Gerardiidae, Corallium sp., Corallium unicornium, and Corallium lauuense. Bull Mar Sci 81: 311–322.
55. Pelletier R, Baco AR (2007) Reproductive morphology of three species of deep-water precious corals from the Hawaiian Archipelago: Gerardiidae, Corallium sp., and Corallium unicornium. Bull Mar Sci 81: 333–342.
56. Murray Roberts J, Wheeler AJ, Freiwald A, Cairns SD (2009) Cold-Water Corals: The Biology and Geology of Deep-Sea Coral Habitats. Cambridge: Cambridge University Press. 334 p.
57. Grigg RW, Bayer FM (1976) A review of Hawaiian zoanthids with descriptions of three new species. Zool J Linn Soc-Lon 50: 161–180.
58. Walsh GE, Etnoyer P (1971) A review of Hawaiian zoanthids with descriptions of three new species. Zool J Linn Soc-Lon 50: 161–180.
59. Reimer JD, Sinniger F (2010) Hawaiian Seamount Zoanthid Diversity