



Six genetically distinct clades of *Palola* (Eunicidae, Annelida) from Lizard Island, Great Barrier Reef, Australia

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Abstract

A total of 36 lots of *Palola* spp. (Eunicidae, Annelida) were collected during the Lizard Island Polychaete Workshop on Lizard Island, Great Barrier Reef, Queensland, Australia. Of these, 21 specimens were sequenced for a portion of the mitochondrial cytochrome *c* oxidase I gene. These sequences were analysed in conjunction with existing sequences of *Palola* spp. from other geographic regions. The samples from Lizard Island form six distinct clades, although none of them can clearly be assigned to any of the nominal species. Four of the six Lizard Island clades fall into species group A and the remaining two into species group B (which also includes the type species, *Palola viridis*). All sequenced specimens were characterized morphologically as far as possible and a dichotomous key was assembled. Based on this key, the remaining samples were identified as belonging to one of the clades.

Key words: Lizard Island, Eunicidae, *Palola*

Introduction

This study investigates the molecular and morphological diversity of the genus *Palola* (Eunicidae, Annelida), from Lizard Island, Great Barrier Reef, Queensland, Australia. To date, *Palola* spp. have rarely been reported from Australian waters although some records exist from the Great Barrier Reef as prey items of cone snails (Marsh 1970, 1971; Taylor & Lewis 1995) and from Sydney Harbor (Hutchings *et al.* 2013). These have been identified as *Palola siciliensis* but, as discussed below, these species identifications are doubtful.

The term “palolo worm” is often loosely applied to any type of polychaete exhibiting mass spawning, also called “swarming” or “rising”. Originally, however, the term refers to the eunicid *Palola viridis* Gray in Stair, 1847 from Samoa. Members of this species are generally cryptic inhabitants of coral rubble, except for once a year, when they cast off their posterior ends, or epitokes, into the water column. The epitokes can be up to a meter long and are packed with eggs and sperm. Within minutes to hours of entering the water column they disintegrate to release the gametes into the environment. These swarming events have great cultural significance in many locations throughout the South Pacific and in Indonesia, where the epitokes are harvested as a delicacy and sometimes seasons are named after the worms (e.g., Craig *et al.* 2008; Hauenschild *et al.* 1968; Levine & Sauafea-Le'au 2013; Mondragón 2004; Pamungkas 2011, 2015).

Characteristic for the epitokes of *P. viridis* are the ventral eyespots. These round to oval eyes are located on the ventral side of each segment and are equipped with cuticular lenses (Schröder 1905; Schulze & Timm 2012; Woodworth 1903). Sometimes ventral eyes are detected in the benthic stages (see Schulze 2006), but they are probably transient features that start forming when swarming is imminent. They are also probably often lost when the worms fragment while being extracted from their habitat.

Palola is easily diagnosable by a combination of features, even from incomplete specimens (Fauchald 1992; Schulze 2006; Schulze & Timm 2012). The most characteristic features in the anterior region are the scoop-shaped mandibles. These also occur in *Lysidice*, although usually less heavily calcified than in *Palola*. The two genera can also be distinguished by the number of prostomial appendages: *Palola* has three antennae and two lateral palps,

whereas in *Lysidice* the palps are missing. Parapodial features are simplified in *Palola*, compared to other eunicids. Most notably, *Palola* lacks subacicular hooks and pectinate chaetae along the entire body length. Branchiae, if present, are simple, unbranched filaments which start in the mid-body region. Due to this simplified morphology, not many features are available for species-level identification, since many of the characters commonly used in other eunicids, such as the shape, coloration and onset of the subacicular hooks or the branching pattern of the branchiae along the body, are inapplicable. According to the recent molecular phylogenetic analyses by Zanol *et al.* (2010, 2014), *Palola* is monophyletic within Eunicidae.

Fourteen species of *Palola* are formally recognized (Fauchald 1992; Morgado & Amaral 1981). Most of them are only known from their type localities but two species, *Palola viridis* Gray, 1847 and *Palola siciliensis* (Grube, 1840), are more widely reported. Matching the nominal species with clades based on molecular data is not straightforward. Using mitochondrial sequence data, Schulze (2006) and Schulze & Timm (2012) found roughly twenty, largely divergent lineages of *Palola* throughout the Pacific, Caribbean and Mediterranean which can be divided into two species groups. Species group A occurs throughout the tropical eastern and western Pacific whereas group B is found throughout the Western Pacific, Caribbean and Mediterranean Seas. While some of the lineages making up these groups are localized, others are extremely widespread. Morphologically, the only character that appears to separate the two groups is the presence of ventral eye spots in clade B which are presumably indicative of epitokous reproduction (Schulze 2006). The eyespots are never present in members of species group A, suggesting that epitoky does not occur in these lineages. Sequence data from specimens collected at the type locality clearly indicate that *P. viridis* falls into species group B (Schulze & Timm 2012).

The situation for *Palola siciliensis* is less obvious. The type locality for this species is Sicily but no tissues useable for DNA sequencing are currently available from there. The only *Palola* specimen from the Mediterranean Sea that has been sequenced to date is from Catalonia (Zanol *et al.* 2010) and falls into species group B. No ventral eyespots have ever been mentioned for *P. siciliensis* although the species does produce epitokes (Hofmann 1972, 1974, 1975). While reports of *P. viridis* are limited to the South Pacific, *P. siciliensis* has been reported worldwide from temperate to tropical waters. Many of these reports are probably erroneous, given the large degree of genetic diversity within the genus and the difficulties with species identifications. It appears that *P. siciliensis* has become the default identification for any *Palola* which is not clearly recognizable as *P. viridis*.

This study examines how many different clades of *Palola* sp. are present on Lizard Island and whether they can be identified as any of the nominal species. Due to the incompleteness of most specimens collected from coral rubble, no formal descriptions or re-descriptions are provided, but the morphology is documented as far as possible and a dichotomous key to the *Palola* clades at Lizard Island is provided.

Material and methods

Palola spp. were collected during 20 different collecting events on Lizard Island, Great Barrier Reef, Queensland, Australia between August 13 and 24, 2013. Locality data for material examined during this Lizard Island Polychaete Workshop are provided in the Appendix of Ribas & Hutchings (2015, *Zootaxa* 4019) together with maps showing sites. Number of specimens under each registration number is one unless otherwise specified.

Palola spp. were retrieved from pieces of coral rubble by carefully breaking the rubble and removing the worms or fragments thereof with forceps. Specimens were preserved either in 4% buffered formalin and transferred to 70% ethanol or in 95% ethanol. Whenever a lot contained several specimens, they were later separated into individual vials.

DNA was extracted from ethanol-preserved small (< 1mm³ or 1–3 segments) pieces of tissue using the Qiagen DNEasy Blood and Tissue kit. A ~600 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR). PCRs were either performed using a “primer cocktail” of four different primers, following the protocol of the authors for this method (Carr *et al.* 2011; Ivanova *et al.* 2007) or a standard protocol with an annealing temperature of 45 °C using the following primers originally developed for Megascopidae by Nancy Schult, Colgate University (pers. comm.): Mega-F: 5'-TAY TCW ACW AAY CAY AAA GAY ATT GG-3' and Mega-R: 5'-TAK ACT TCT GGR TGM CCA AAR AAT C-3'. PCR products were cleaned with ExoSapIt (Affymetrix) following the manufacturer's protocol. PCR products were sequenced in both directions. Cycle sequencing reactions were performed either with M13 primers (after PCR with the “primer

cocktail”) or with the “Mega” primers. Cycle sequencing reactions used 1 µl cleaned PCR product, 0.5–1 µl BigDye Terminator™ reaction mix, 2 µl 5X cycle sequencing buffer, 1 µl primer (10 µM) and ddH₂O to a total volume of 10 µl. Cycle sequencing products were cleaned using the Zymo Research DNA Sequencing Clean-up Kit™. Sequences were run through capillary gel electrophoresis on an ABI3130 Genetic Analyzer (Life Technologies).

Forward and reverse sequences for each individual were assembled in Sequencher 4.8 (GeneCodes). Primer sequences were removed on both ends and individual nucleotide positions were edited as necessary. Sequences were aligned with MEGA 6.06 (Tamura *et al.* 2013) using the Muscle algorithm. MEGA 6.06 was also used to calculate average genetic distances among clades using the Kimura-2-parameter model. All sequences were deposited in GenBank under accession numbers KT124718 through KT124738.

The sequences from Lizard Island *Palola* spp. were combined with existing sequences from previous studies (Schulze 2006; Schulze & Timm 2012) available on GenBank. A phylogenetic analysis was conducted using Bayesian Inference in MrBayes 3.2.1 (Ronquist *et al.* 2012) through the CIPRES Science Gateway v 3.3 (Miller *et al.* 2010), using two runs with four Metropolis Coupled Markov Chains Monte Carlo (MCMCMC) each for 5,000,000 generations under a General Time Reversible Model plus Gamma, with the first 1,000,000 generations discarded as burn-in. The correct model for sequence evolution had previously been determined (Schulze & Timm 2012). Trees were sampled every 100 generations from the posterior distribution after the burn-in period and a 50% majority rule consensus tree was generated. *Leodice antennata* was chosen as the outgroup based on the analyses by Zanol *et al.* (2010, 2014). Additional eunicid taxa representing *Lysidice*, *Nicidion* and *Marphysa* were included to test the monophyly of *Palola*. Clade numbers were assigned to groups of Lizard Island sequences whenever they formed monophyletic lineages separated from each other in the phylogenetic tree by sequences from other locations.

Morphological examinations were conducted using a Leica S8APO stereomicroscope and a Leica DM2500 equipped with a DFC420 digital camera. Helicon Focus v 6.3.3 (Kozub *et al.* 2000–2015) was used to merge image stacks with different focus depths. The descriptions in the taxonomic account are based on the sequenced specimens, because only their membership to a clade could be confirmed without doubt. A dichotomous key to the clades of Lizard Island *Palola* was assembled based on the morphological descriptions and used to identify the remaining samples which are also included under “Material Examined”.

Results

The molecular analysis revealed that six largely divergent genetic lineages of *Palola* spp. are present on Lizard Island, four representing species group A and two representing species group B (Fig. 1). Only species group A was recovered as monophyletic.

Taxonomic account

Genus *Palola* Gray in Stair, 1847

Palola Gray in Stair, 1847: 1718.—Fauchald 1992: 1179–1181.

Type-species. *Palola viridis* Gray in Stair, 1847, by monotypy.

Diagnosis. Eunicids with calcified, scoop-shaped mandibles. Three antennae (1 medial, 2 lateral) and one pair of palps arranged in a horseshoe shape on prostomium. Peristomium consisting of two rings, with peristomial cirri on second peristomial ring. Chaetal arrangement lacking subacicular hooks and pectinate chaetae along the entire body; branchiae simple, if present, usually from the mid-body region.

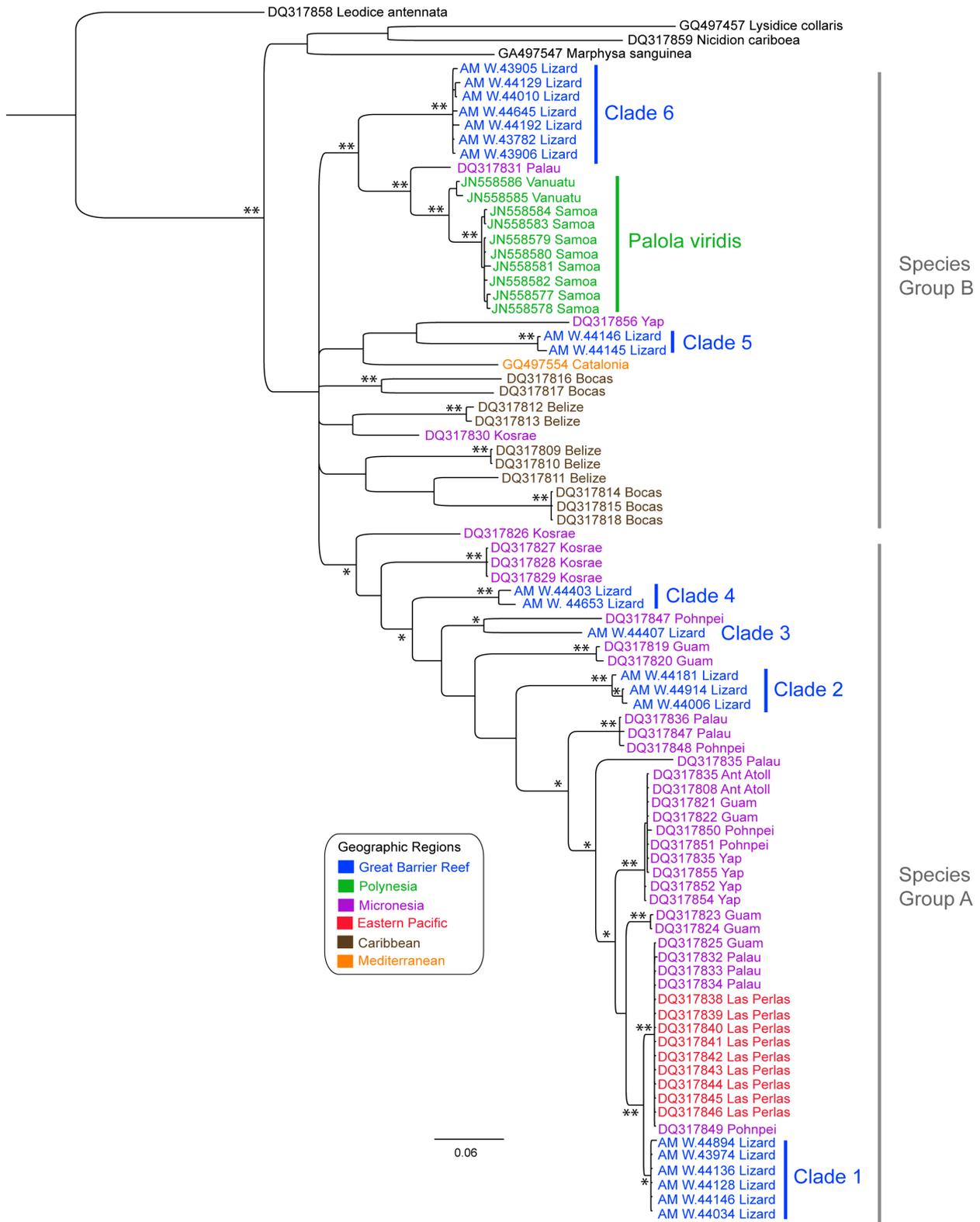


FIGURE 1. 50% majority rule consensus tree resulting from Bayesian analysis of 601 bp of COI. Branch support is given as Bayesian posterior probability. Double asterisks (**) indicate 100% posterior probability; single asterisks (*) indicate 95–99% posterior probability; branch support < 95% posterior probability not shown. Samples sequenced for previous studies are listed with their genbank accession numbers and collecting locality. Samples sequenced for the present study are listed as “Lizard” with their AM voucher numbers.

***Palola* Lizard Island Clade 1**

(Fig. 2A–C)

Material examined. AM W.43974, MI QLD 2344, sequenced; AM W.44034, MI QLD 2352 (3, 1 sequenced and photographed); AM W.44146, MI QLD 2356 (3, 1 sequenced and photographed); AM W.44128, MI QLD 2358, sequenced; AM W.44136, MI QLD 2358, sequenced; AM W.44894, MI QLD 2401, sequenced; AM W.44655, MI QLD 2398; AM W.44656, MI QLD 2398; AM W.44925, MI QLD 2390; AM W.44202, MI QLD 2359; AM W.43912, MI QLD 2335.

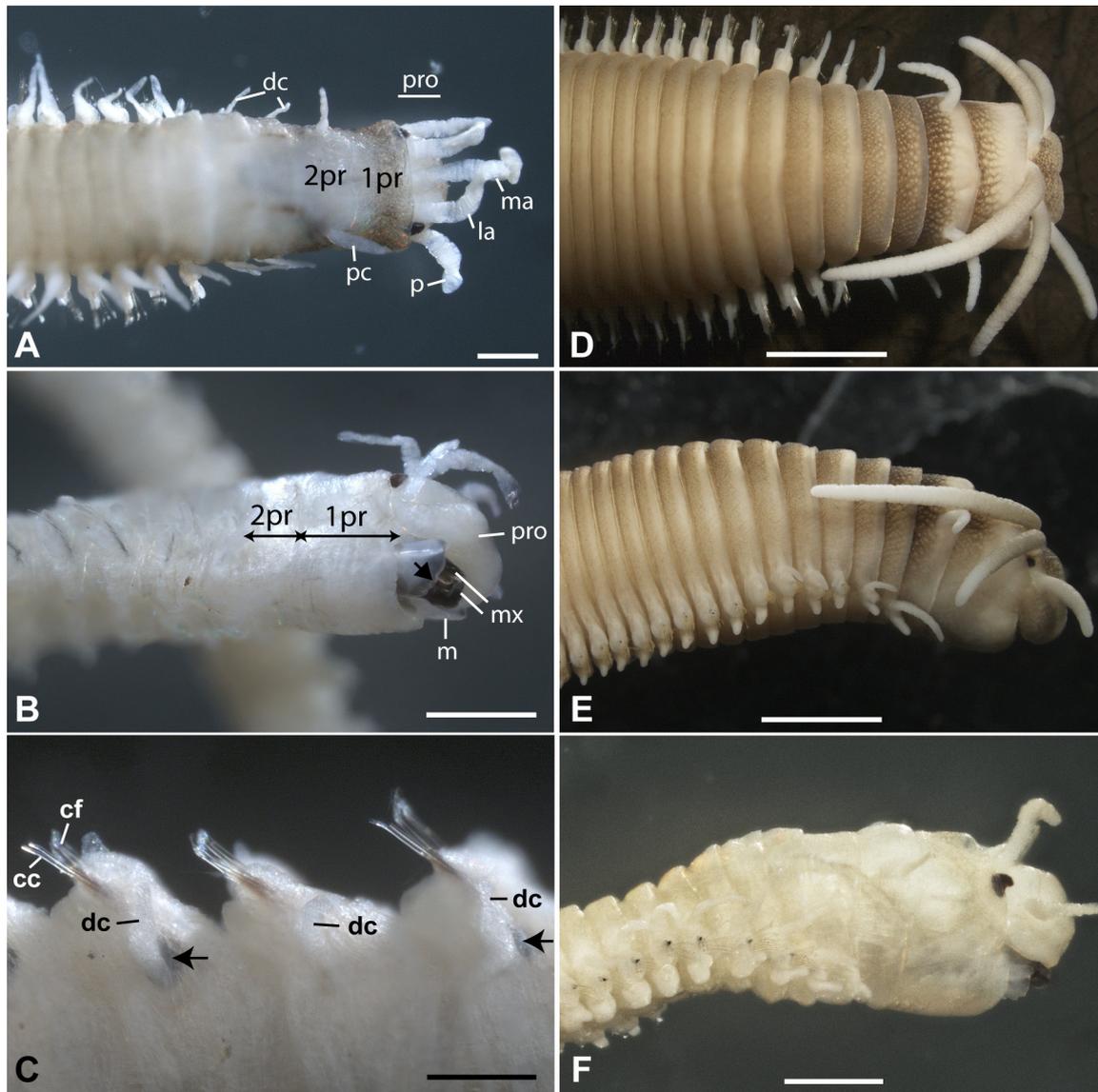


FIGURE 2. *Palola* Lizard Island clades 1 (A–C) and 2 (D–F). Specimens shown in A–C and F ethanol preserved; specimen shown in D and F fixed in formalin and later transferred to to 70% ethanol. Labels in A–C serve as a reference for all following figures. A. *Palola* Lizard Island clade 1, AM W.44034, dorsal view; B. *Palola* Lizard Island clade 1, AM W.44146, ventrolateral view, note serrated anterior margin of mandible and length ratio between first and second peristomial ring; C. *Palola* Lizard Island clade 1, AM W.44034, dorsal view of parapodia in posterior region of the fragment, arrows indicate the black pigment spots on the dorsal side of the parapodia; D. *Palola* Lizard Island clade 2, AM W.44914, dorsal view; E. *Palola* Lizard Island clade 2, W.44914, lateral view; F. *Palola* Lizard Island clade 2, AM W.44006, lateral view. Abbreviations: cc = capillary chaetae, cf = compound falcigers, dc = dorsal cirrus of parapodium, la = lateral antenna, m = mandible, ma = median antenna, mx = maxillae, p = palps, pc = peristomial cirrus, 1pr = first peristomial ring, 2pr = second peristomial ring, pro = prostomium. Scale bars: A, B, F = 0.1 mm; C = 0.05 mm; D, E = 0.5 mm.

Description. Specimens small and threadlike. Anterior fragments examined for all sequenced specimens, ranging in length from 7 to 18 mm and from 0.6 to 1.7 mm in width; with 38–78 chaetigers. Branchiae only observed in one specimen (AM W.44034) from chaetiger 62 to the end of the fragment. No ventral eyespots. Head and body generally without pigmentation but with iridescent shine, or, if pigmentation is present light brown and restricted to prostomium and peristomium (Fig. 2A). Mandibles usually protruding from mouth, thin and nearly transparent with serrated anterior margin (Fig. 2B). Maxillae not examined. Antennae, palps and peristomial and parapodial cirri without pigment. Antennae and palps wrinkled, in preserved material, tapering and pointy tip. Median antenna reaches to chaetiger 2 or 3, lateral antennae reach to chaetiger 1 or 2 and palps reach to first or second peristomial ring. Tapering peristomial cirri reach forward to about 3/4 of the length of first peristomial ring. Eyes dark, oval or with ventral notch and nestled between lateral antennae and palps. Acicula brown. Other chaetae not examined. Dark pigment spots on dorsal side of posterior parapodia observed in two specimens (AM W.44034; AM W.44.136) (Fig. 2C).

Remarks. Although only distantly related in the phylogenetic tree and representing different species groups, *Palola* Lizard Island clade 1 is morphologically most similar to clade 5. Members of both clades are thin and threadlike, almost entirely lack pigmentation and have relatively thin and transparent mandibles. They can be distinguished by the relative length of the first and second peristomial rings: in clade 1, the first peristomial ring is 1.5 to 2 times as long as the second when viewed in the midlateral line where as in clade 5 they are more similar in length. Despite the morphological similarities, COI sequences are 26.9% divergent (Kimura-2-Parameter model) between clades 1 and 5. *Palola* Lizard Island Clade 1 falls into species group A and is the sister group to a clade with an extremely wide geographic distribution throughout the tropical Eastern and Western Pacific, referred to as clade A1 in Schulze (2006) and Schulze & Timm (2012). The average genetic distance to this clade is only 1.9% and the two might represent the same species, although they are reciprocally monophyletic.

***Palola* Lizard Island Clade 2**

(Fig. 2D–F)

Material examined. AM W.44914, MI QLD 2401, sequenced and photographed; AM W.44006, MI QLD 2341, sequenced; AM W.44181, MI QLD 2359, sequenced; AM W.44337, MI QLD 2359, juvenile.

Description. One of the specimens (AM W.44914) much larger (Fig. 2D, E) than the others which are thin and threadlike (Fig. 2F). Anterior fragments closely examined for the three sequenced specimens. The larger specimen (AM W.44914) is 52 mm long and up to 5 mm wide with 165 chaetigers. The smaller specimens are 5 mm long and 0.7 mm wide (AM W.44181) and 9 mm long and 1 mm wide (AM W.44006) with 34 and 63 chaetigers, respectively. Branchiae present in AM W.44914 from chaetiger 123 to end of fragment. No ventral eyespots. Mandibles protruding from mouth in smaller specimens; thin and almost transparent. Head and body in larger specimens with brown pigment dorsally on prostomium and posterior portions of peristomial rings and anterior chaetigers (Fig. 2D–E). Antennae, palps and peristomial and parapodial cirri without pigment. Smaller specimens without pigmentation. Antennae and palps slightly medially inflated in larger specimen and with a blunt tip. In AM W.44914 median antenna reaches to chaetiger 4, lateral antennae reach to chaetiger 2 and palps to first peristomial ring. Tapering peristomial cirri reach forward to first peristomial ring. Eyes dark, round with a ventral notch and nestled between lateral antennae and palps. Acicula brown. Other chaetae not examined.

Remarks. The smaller specimens belonging to this clade are difficult to distinguish from members of clades 1 and 5 which are also largely without pigmentation. However, in clades 1 and 5 the anterior margins of the mandibles are serrated, whereas they are smooth in clades 3 and 4. The larger specimens show a distinctive pigmentation pattern with brown pigment in the posterior, dorsal portion of the peristomial rings and anterior chaetigers and distinctive shape of antennae and palps. The latter are slightly medially inflated and have blunt tips, as opposed to tapering with sharp tips as in the other clades. *Palola* Lizard Island Clade 2 falls into species group A and the mean genetic distance to any of the other clades in species group A is 20% and over.

***Palola* Lizard Island Clade 3**

(Fig. 3A–B)

Material examined. AM W.44407, MI QLD 2390, sequenced and photographed; AM W.44829, MI QLD 2424; AM W.44641, MI QLD 2413.

Description. Specimens thin and threadlike. Anterior fragment of AM W.44407 was closely examined (Fig. 3A, B): 15 mm long, 1.5 mm wide, with 67 chaetigers. No branchiae or ventral eyespots present. Mandibles protruding from mouth and relatively thin with a smooth, unserrated anterior margin (Fig. 3B). Maxillae not examined. Head and body with faint, relatively uniform brown pigment dorsally on prostomium, peristomium and anterior chaetigers. Antennae, palps and peristomial and parapodial cirri without pigment. Antennae and palps wrinkled, tapering and with pointy tip. Median antenna reaching chaetiger 2, lateral antennae chaetiger 1 and palps second peristomial ring. Tapering peristomial cirri reaching forward to about $\frac{3}{4}$ of the anterior peristomial ring. Eyes dark, round to oval shaped and nestled between lateral antennae and palps. Acicula brown. Other chaetae not examined.

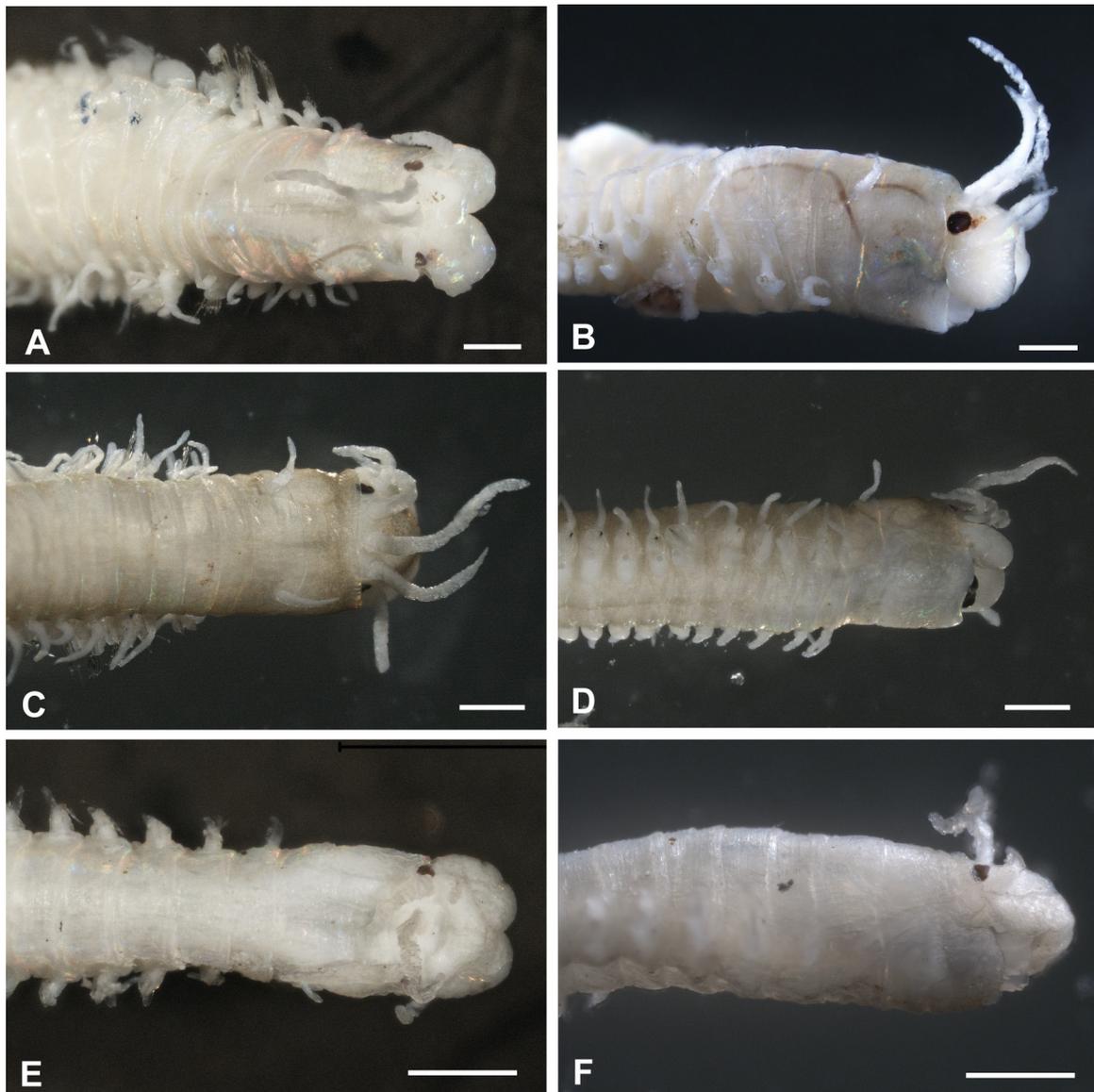


FIGURE 3. *Palola* Lizard Island clades 3 (A, B), 4 (C, D) and 5 (E, F). All material ethanol-preserved. A. *Palola* Lizard Island clade 3, AM W.44407, dorsal view; B. *Palola* Lizard Island clade 3, same specimen as in A, lateral view; C. *Palola* Lizard Island clade 4, AM W.44653, dorsal view; D. *Palola* Lizard Island clade 4, same specimen as in C, ventro-lateral view; E. *Palola* Lizard Island clade 5, AM W.44146, dorsal view; F. *Palola* Lizard Island clade 5, same specimen as in E, lateral view. Scale bars: 0.1 mm.

Remarks. Clade 3 is morphologically very similar to clade 4. Both belong to species group A, are thin and threadlike and have faint brown dorsal pigmentation in the anterior body regions, and have mandibles with smooth anterior margins. The two can be distinguished by the relative length of their antennae. Whereas in clade 3 the

median and lateral antennae are similar in length, in clade 4 the lateral antennae are only ~2/3 the length of the median antenna. The two clades differ by over 20% in COI sequence divergence.

***Palola* Lizard Island Clade 4**

(Fig. 3 C, D)

Material examined. AM W.44403, MI QLD 2371, sequenced; AM W.44653, MI QLD 2413, sequenced and photographed; AM W.44324, MI QLD 2375; AM W.44642, MI QLD 2413.

Description. Specimens thin and threadlike. Anterior fragments of the two sequenced specimens closely examined (Fig. 3C, D). Fragments 9–13 mm long and 1 mm wide, with 35 and 75 chaetigers, respectively. No branchiae or ventral eyespots present. Mandibles protruding from mouth and relatively thin with a smooth, unserrated anterior margin (Fig. 3D). Maxillae not examined. Head and body with faint, relatively uniform brown pigment dorsally on prostomium, peristomium and anterior chaetigers. Antennae, palps, peristomial and parapodial cirri without pigment. Antennae and palps wrinkled, tapering and with pointy tip. Median antenna reaching chaetiger 2 or 3, lateral antennae to chaetiger 1 or 2 and palps to first or second peristomial ring. Tapering peristomial cirri reaching forward to about ¾ of the anterior peristomial ring. Eyes dark, round with a ventral notch and nestled between lateral antennae and palps. Acicula brown. Other chaetae not examined.

Remarks. *Palola* Lizard Island Clade 4 falls into species group A. As mentioned above, clades 3 and 4 are morphologically very similar to each other but can be distinguished by the relative lengths of their antennae.

***Palola* Lizard Island Clade 5**

(Fig. 3E, F)

Material examined. AM W.44146, MI QLD 2356 (2, 1 sequenced and photographed); AM W.44155, MI QLD 2353, sequenced; AM W.44202, MI QLD 2359; AM W.44352, MI QLD 2371; AM W.45119, MI QLD 2435.

Description. Specimens thin and threadlike. Only the sequenced fragment of specimen AM W.44146 is described in detail here (Fig. 3E). The only other sequenced specimen (AM W.44155) is minuscule, broken into several fragments and probably a juvenile. Specimen AM W.44146 is 11 mm long and 0.7 mm wide with 50 chaetigers. No branchiae or ventral eyespots present. Mandibles protruding from mouth and very thin with a serrated anterior margin (Fig. 3F). Maxillae not examined. Head and body without pigmentation. Antennae and palps wrinkled, tapering and with pointy tips. Median antenna reaching chaetiger 2, lateral antennae chaetiger 1 and palps first peristomial ring. Peristomial cirri absent or lost. Eyes dark, round with a ventral notch and nestled between lateral antennae and palps. Acicula brown. Other chaetae not examined.

Remarks. Clade 5 belongs to species group B. As mentioned above, clades 1 and 5 are morphologically very similar but can be distinguished by the relative length of the peristomial rings in the mid-lateral line. In clade 1 the anterior peristomial ring is substantially longer while they are nearly the same length in clade 5.

***Palola* Lizard Island Clade 6**

(Fig. 4)

Material examined. AM W.43782, MI QLD 2335, sequenced; AM W.43905, MI QLD 2331, sequenced and photographed; AM W.44010, MI QLD 2341, sequenced; AM W.44129, MI QLD 2356, sequenced; AM W.44192, MI QLD 2359, (2, 1 sequenced); AM W.44645, MI QLD 2410, sequenced and photographed; AM W.44330, MI QLD 2359 (2); AM W.44334, MI QLD 2371; AM W.44652, MI QLD 2413; AM W.43914, MI QLD 2337.

Description. Most specimens relatively large with strongly calcified mandibles protruding from mouth. Anterior fragments closely examined for all sequenced specimens, ranging in size from 11 mm to 50 mm, 1.5–3 mm wide; with 41 to 153 chaetigers. Dorsoventrally flattened with a deep ventral groove. Some larger fragments with branchiae, starting from chaetiger 77 (AM W.44645) to chaetiger 98 (AM W.43906). No ventral eyespots. Head and body pigmented, particularly dorsally, with fairly uniform brown pigment (Fig. 2A, B). Antennae, palps and peristomial cirri without pigment. Antennae and palps wrinkled, in preserved material, tapering and pointy tip. Median antenna generally reaches to chaetiger 4, lateral antennae to chaetiger 2 or 3 and palps to posterior end of first or to second peristomial ring. Tapering peristomial cirri reach forward at least to anterior margin of first

peristomial ring or beyond. Eyes dark, round with a ventral notch and nestled between lateral antennae and palps. Bases of ventral cirri strongly inflated after chaetiger 10. Chaetiger 10 with single dark acicula, single capillary chaeta and multiple compound falcigers (Fig. 4C, D).

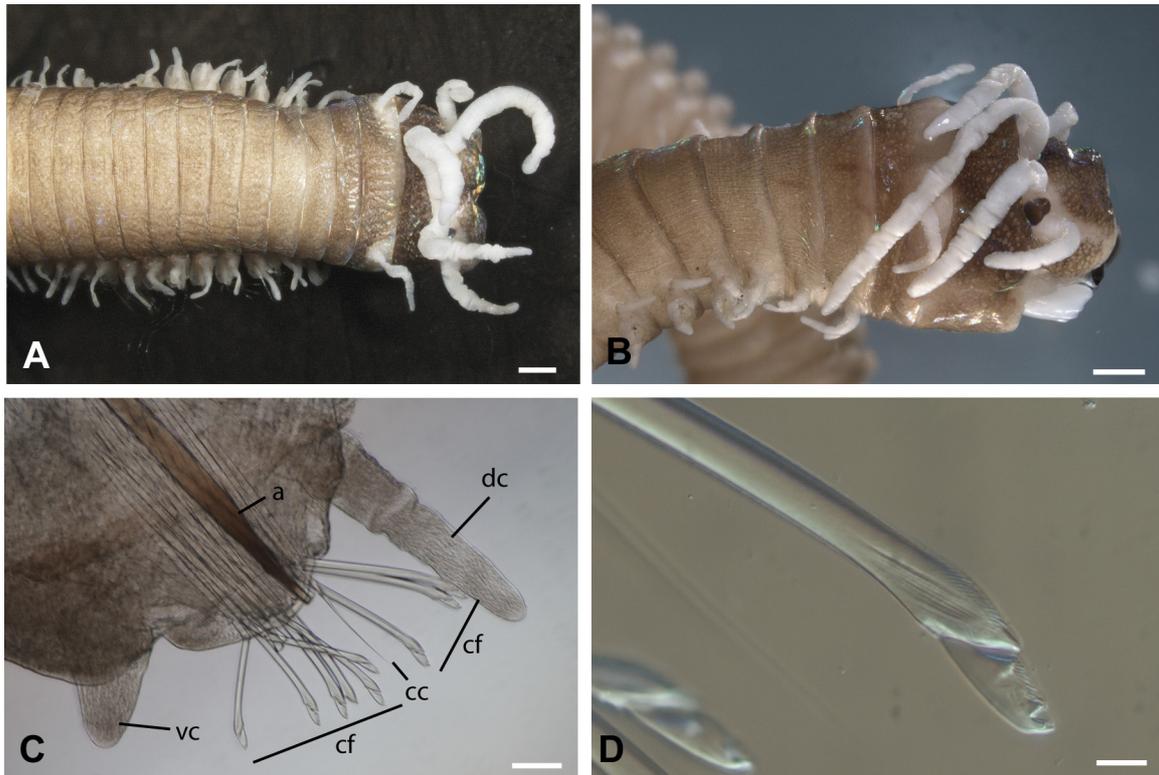


FIGURE 4. *Palola* Lizard Island clade 6. All material ethanol preserved. A. AM W.43905, dorsal view; B AM W.44645, dorso-lateral view. C. AM W.44645, parapodium 10; D. AM W.44645, parapodium 10, detail of compound falciger. Abbreviations: a = aciculum, cc = capillary chaeta, cf = compound falciger, dc = dorsal cirrus of parapodium, vc = ventral cirrus of parapodium. Scale bars: A, B, C = 0.1 mm, D = 20 μ m.

Remarks. This is the most morphologically distinctive of the six clades found on Lizard Island. Members of *Palola* clade 6 can be distinguished from the other clades by the relatively uniform dark brown pigmentation and the long peristomial cirri which often reach forward to the eyes or beyond. This clade is the most closely related to the type species of the genus, *Palola viridis*, but can be distinguished from it by the shorter antennae: in *P. viridis*, the median antenna reaches to chaetiger 10 (Fauchald 1992) and only to chaetiger 4 in *Palola* Lizard Island clade 6. The average genetic divergence between the two lineages is 20% (Kimura-2-parameter model) for COI. Posterior ends are missing in all of the specimens, but even the longer fragments do not have ventral eyespots as reported in *P. viridis*.

Key to *Palola* clades from Lizard Island

- 1 Peristomial cirri reach at least to or beyond anterior margin of peristomium; fairly uniform brown pigmentation dorsally. *Palola* clade 6
- Peristomial cirri not reaching to anterior margin of anterior peristomial ring; pigmentation, if present, faint or limited to posterior margins of segments 2
- 2. (1) Antennae and palps smooth and blunt-tipped *Palola* clade 2
- Antennae wrinkled and with a pointy tip 3
- 3. (2) Anterior margins of mandibles serrated 4
- Anterior margins of mandibles with a smooth edge 5
- 4. (3) First peristomial ring 1.5 to 2 times as long as second peristomial ring in lateral mid-line *Palola* clade 1
- First and second peristomial rings nearly equal in length in lateral midline. *Palola* clade 5
- 5. (3) Lateral antennae about 2/3 the length of the median antenna. *Palola* clade 4
- Lateral and median antennae more similar in length to each other. *Palola* clade 3

Discussion

Despite relative morphological uniformity, *Palola* spp. from Lizard Island show high genetic diversity in COI. With six genetically divergent clades, Lizard Island actually has the highest diversity of *Palola* lineages of any location sampled to date, reflecting the intense collecting effort during the Lizard Island Polychaete Workshop.

COI shows a high degree of divergence within *Palola* (and other eunicids) which allows delineation of individual clades. On the other hand, because of its rapid substitution rate, the marker is quite limited in resolving deeper-level relationships. This study did not aim to fully reconstruct the phylogeny of the genus and no attempt was made to utilize other, more conserved markers to achieve that goal. Doing this would probably add resolution to the base of the phylogenetic tree. One of the markers used by Zanol *et al.* (2010, 2014) for eunicid phylogeny is 18S ribosomal RNA (18S rRNA). However, examination of the 18S rRNA sequences from these studies deposited in Genbank revealed that they were all identical and thus would be too conserved to resolve phylogenetic relationships within *Palola*. A marker with a substitution rate intermediate between COI and 18S rRNA would be desirable. Another mitochondrial marker, 16S ribosomal RNA, has a slightly lower substitution rate than COI in *Palola* (Schulze 2006; Schulze & Timm 2012), but presents alignment problems as many insertions and deletions occur.

Trying to match the clades found at Lizard Island with any of the nominal species is nearly impossible given that most of the material, including the existing type material (reviewed by Fauchald 1992) is incomplete. This problem is not unique to *Palola*, or to eunicids in general, but it is particularly severe here because of the great length of these worms and because the distribution of parapodial characteristics along the body may provide the most phylogenetically informative characters. The ventral eyes, if present, are a very distinctive feature. They are sometimes observed in benthic specimens (see Schulze 2006), but are probably transitory and often lost with posterior fragments.

It is further questionable whether some of the characters used in this and previous studies, such as the relative length and shape of the antennae, may be affected by the preservation method, as they may contract to various degrees during the fixation process. Likewise, the pigmentation will fade in preserved material, although distinctive patterns can often still be detected for extended time periods after formalin or ethanol fixation. For consistency, the morphological descriptions in this study are based on material preserved in 95% ethanol for approximately the same amount of time (~ 2 years). However, no significant differences were observed with regard to the pigmentation between the ethanol and the formalin preserved samples. Compared to other eunicids, pigmentation is relatively uniform in *Palola*, but certain trends do seem to exist (e.g., consistent brown pigmentation in clade 5 and “striped” pattern in the larger specimen in clade 2). Photographs of live specimens can therefore greatly aid in taxonomic work in this genus as well as other eunicids.

Size-related variation also deserves further study. For example, clade 2 included a large individual with distinctive pigmentation and antennal features and several smaller individuals. The smaller individuals would probably not have been recognizable as belonging to the same clade without the molecular evidence.

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References

- Carr, C.M., Hardy, S.M., Brown, T.M., Macdonald, T.A. & Hebert, P.D.N. (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE*, 6, e22232.
<http://dx.doi.org/10.1371/journal.pone.0022232>
- Craig, P., Green, A. & Tuilagi, F. (2008) Subsistence harvest of coral reef resources in the outer islands of American Samoa: Modern, historic and prehistoric catches. *Fisheries Research*, 89, 230–240.
<http://dx.doi.org/10.1016/j.fishres.2007.08.018>

- Fauchald, K. (1992) Review of the types of *Palola* (Eunicidae: Polychaeta) *Journal of Natural History*, 26, 1177–1225.
<http://dx.doi.org/10.1080/00222939200770681>
- Hauenschild, C., Fischer, A. & Hoffmann, D.K. (1968) Untersuchungen am pazifischen Palolawurm *Eunice viridis* (Polychaeta) in Samoa. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 18, 254–295.
<http://dx.doi.org/10.1007/BF01613354>
- Hofmann, D.K. (1972) Nachweis der Epitokie als Fortpflanzungsmodus des Polychaeten *Eunice siciliensis* (Polychaeta: Annelida). *Marine Biology*, 14, 341–344.
<http://dx.doi.org/10.1007/BF00348182>
- Hofmann, D.K. (1974) Maturation, epitoky and regeneration in the polychaete *Eunice siciliensis* under field and laboratory conditions. *Marine Biology*, 25, 149–161.
<http://dx.doi.org/10.1007/BF00389263>
- Hofmann, D.K. (1975) Reproductive forms in eunicid polychaetes inhabiting the "fonds coralligènes in the region of Banyuls-s-m. (Mediterranean Sea) with particular reference to *Eunice siciliensis*. *Pubblicazioni della stazione zoologica di Napoli*, 39, 242–253.
- Hutchings, P., Ah Yong, S.T., Ashcroft, M.B., McGrouther, M.A. & Reid, A.L. (2013) Sydney Harbor: Its diverse biodiversity. *Australian Zoologist*, 36, 255–320.
<http://dx.doi.org/10.7882/AZ.2012.031>
- Ivanova, N.V., Zemlak, T.S., Hanner, R.H. & Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7, 544–548.
<http://dx.doi.org/10.1111/j.1471-8286.2007.01748.x>
- Kozub, D., Khmelik, V., Shapoval, J., Chentsov, V., Yatsenko, S., Litovchenko, B. & Starikh, V. (2010–2015) Helicon Focus v. 6.3.3. Computer Program. Available from: <http://www.heliconsoft.com> (accessed 21 July 2015)
- Levine, A. & Sauafea-Le'au, F. (2013) Traditional knowledge, use, and management of living marine resources in American Samoa: Documenting changes over time through interviews with elder fishers. *Pacific Science*, 67, 395–407.
<http://dx.doi.org/10.2984/67.3.7>
- Marsh, H. (1970) Preliminary studies of the venoms of some vermivorous Conidae. *Toxicon*, 8, 271–277.
[http://dx.doi.org/10.1016/0041-0101\(70\)90002-4](http://dx.doi.org/10.1016/0041-0101(70)90002-4)
- Marsh, H. (1971) Observations on the food and feeding of some vermivorous conus on the Great Barrier Reef. *Veliger*, 14, 45–53.
- Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) *Creating the CIPRES Science Gateway for the inference of large phylogenetic trees*. Gateway Computing Environments Workshop, New Orleans, LA, 8 pp.
- Mondragón, C. (2004) Of winds, worms and mana: The traditional calendar of the Torres Islands, Vanuatu. *Oceania*, 74, 289–308.
<http://dx.doi.org/10.1002/j.1834-4461.2004.tb02856.x>
- Morgado, E.H. & Amaral, A.C.Z. (1981) Anelídeos poliquetos associaods a um briozoário i. Eunicidae, Lumbrineridae, Lysaretidae e Dorvilleidae. *Iheringia Série Zoologia*, 60, 33–54.
- Pamungkas, J. (2011) Delicious! Marine worms from Ambon Island, Indonesia. *Habitat Marine*, 35–37.
- Pamungkas, J. (2015) Species richness and macronutrient content of wawo worms (Polychaeta, Annelida) from Ambonese waters, Maluku, Indonesia. *Biodiversity Data Journal*, 3, e4251.
<http://dx.doi.org/10.3897/bdj.3.e4251>
- Ribas, J. & Hutchings, P. (2015) Lizard Island Polychaete Workshop: sampling sites and a checklist of polychaetes. *Zootaxa*, 4019 (1), 7–34.
<http://dx.doi.org/10.11646/zootaxa.4019.1.4>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
<http://dx.doi.org/10.1093/sysbio/sys029>
- Schröder, O. (1905) Beiträge zur Kenntnis der Bauchsinnesorgane (Bauchaugen) von *Eunice viridis* Gray sp. (palolo). *Zeitschrift für wissenschaftliche Zoologie*, 79, 132–149.
- Schulze, A. (2006) Phylogeny and genetic diversity of palolo worms (Palola, Eunicidae, Polychaeta) from the tropical North Pacific and the Caribbean. *Biological Bulletin*, 210, 25–37.
<http://dx.doi.org/10.2307/4134534>
- Schulze, A. & Timm, L.E. (2012) *Palolo* and *un*: Distinct clades in the genus *Palola* (Eunicidae, Polychaeta). *Marine Biodiversity*, 42, 161–171.
<http://dx.doi.org/10.1007/s12526-011-0100-5>
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A. & Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
<http://dx.doi.org/10.1093/molbev/mst197>
- Taylor, J.D. & Lewis, A. (1995) Diet and radular morphology of *Peristernia* and *Latirolagena* (Gastropoda: Fascioliariidae) from Indo-Pacific coral reefs. *Journal of Natural History*, 29, 1143–1154.
<http://dx.doi.org/10.1080/00222939500770481>

- Woodworth, W.M. (1903) Preliminary report on the "palolo" worm of Samoa, *Eunice viridis* (Gray). *American Naturalist*, 37, 875–818.
<http://dx.doi.org/10.1086/278371>
- Zanol, J., Halanych, K.M. & Fauchald, K. (2014) Reconciling taxonomy and phylogeny in the bristleworm family Eunicidae (Polychaeta, Annelida). *Zoologica Scripta*, 43, 79–100.
<http://dx.doi.org/10.1111/zsc.12034>
- Zanol, J., Halanych, K.M., Struck, T.H. & Fauchald, K. (2010) Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. *Molecular Phylogenetics and Evolution*, 55, 660–676.
<http://dx.doi.org/10.1016/j.ympev.2009.12.024>