Phylogeny and Genetic Diversity of Palolo Worms (*Palola*, Eunicidae) from the Tropical North Pacific and the Caribbean

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Abstract. Palolo worms (*Palola*, Eunicidae) are best known for their annual mass spawnings, or "risings," in the South Pacific. *Palola* currently contains 14 morphologically similar species, mostly from shallow tropical waters. In this study, 60 specimens of *Palola* from nine locations in the tropical North Pacific and the Caribbean were sequenced for the two mitochondrial markers cytochrome *c* oxidase subunit I and 16S ribosomal RNA to infer phylogenetic relationships, genetic diversity, and phylogeography within the taxon. Phylogenetic analysis was performed using Bayesian statistics and parsimony. Vouchers of the same specimens were examined morphologically. Two major clades (A and B) can be distinguished within the monophyletic *Palola*. A number of individuals in clade B bear rows of ventral eyespots in the posterior body region, typical for swarming *P. viridis* and probably a synapomorphy for clade B. No morphological synapomorphy was found for clade A. Haplotypes from divergent clades often co-occur in the same location. Some haplotypes are geographically widespread, in one case covering the entire east-west expansion of the tropical Pacific. These results imply that despite the apparent absence of teleplanic larvae in eunicid polychaetes, long-distance dispersal is possible in at least some lineages of *Palola*.

With the first taste of palolo I understood the Samoans' love for it. Certainly it suggested a salty caviar, but with something added, a strong, rich whiff of the mystery and fecundity of the ocean depths.


Introduction

Early reports of swarming palolo worms and their use as a food source by the native populations of Samoa, Tonga, Fiji, and other South Pacific islands originated from European missionaries dispatched to these remote regions (e.g., Codrington, 1891; MacDonald, 1858; Gray, 1847, cited in Stair, 1897). These authors were primarily interested in the anthropological aspects of the “rising,” but the Reverend J.B. Stair provided the British Museum with specimens of the swarming stages from the Samoan islands, which Gray (1847) used as the basis for the species description of the Pacific palolo worm, *Palola viridis* (he chose a feminine ending for the genus name). Considering that the swarming stages were epitokes and no heads were present, this original description is very short. Friedlaender (1898, 1904) and Woodworth (1903, 1907) both retrieved complete worms and independently assigned them to *Eunice viridis*. The segments of the swarming epitokes of *Eunice viridis* each bear one dark pigment spot on the ventral surface. The pigment spots were examined histologically by Schröder (1905), who concluded that they were light sensors.

The common name, palolo, is based on the Samoan name for the worms. The Fijian name *mbalolo* and the Tongan name *balolo* are very similar. Other names are, or were, used in other swarming locations throughout the South Pacific (Table 1).

Later authors emphasized the regularity of the swarming, which is correlated with the lunar cycle and happens in October, November, or December in the South Pacific (Burrows, 1945, 1955; Caspers, 1961, 1984; Korringa, 1947). Annual risings of palolo worms have also been reported from the island of Ambon in Indonesia. In contrast to the South Pacific risings, in Ambon the event takes place in March or April. The worms are here known as *wawo* (Table

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1), but the swarming stages are actually a mix of 13 polychaete species (Martens et al. 1995). The wawo was identified as *Lysidice oele* by Horst (1904, 1905), but Martens et al. (1995) reported that the mix primarily contained *Palola viridis*. Two other polychaetes have been described as “palolos.” Both also form epitokes and swarm annually. The “Atlantic palolo” is *Eunice fucata*. Its swarming periodicity was described by Mayer (1908). The “Japanese palolo” is the brackish water nereidid *Tylorrhynchus heterochaetus*, which has been used as a model organism in physiology and embryology (e.g., Osanai, 1978; Sato and Osanai, 1990). As neither is a *Palola*, I will not consider them further in this study.

*Palola* is morphologically characterized by the presence of two palps and three antennae, peristomial cirri, and scoop-shaped calcified mandibles, and by the absence of subacicular hooks (Fauchald, 1992). Branchial filaments, if present, are usually simple. Thus, many characters used in other eunicids to distinguish species, such as the shape and coloration of the subacicular hooks and the branching patterns of the branchial filaments, are not useful in *Palola*. Characters used by Fauchald in his review are mostly size ratios: length to maximum width of the specimen, relative length of antennae, palps, peristomial and notopodial cirri, as well as length ratios of appendages and shafts of compound falicigers. These differences are subtle and some of the type material was incomplete or poorly preserved, so Fauchald regarded his taxonomy as only a first step.

Three of the fourteen *Palola* species have wide geographic distributions (Table 2). *P. viridis* occurs all over the South Pacific, while *P. siciliensis* has been reported in all major oceans, roughly between latitudes 43 °N and 32 °S. *P. edentulum* might have a general subantarctic distribution. Apart from its type location on the Juan Fernandez Islands, it has also been reported from the North Island of New Zealand and the Chatham Islands in the Southwest Pacific and from the Magellanic Islands in the Southeast Pacific (Glasby and Alvarez, 1999). All other species are exclusively known from their type locations (Table 2).

Because morphological characters to distinguish species are limited and no information exists about intraspecific variation, I am here using a molecular approach to reconstruct the phylogeny within *Palola* and to assess genetic diversity and historical biogeography. Toward these goals, I sampled *Palola* species from the Caribbean and across the tropical North Pacific, sequenced them for two mitochondrial markers, and analyzed the sequence data in a phylogenetic and phylogeographic context.

### Material and Methods

#### Collections

To retrieve specimens of *Palola* spp., *Eunice antennata*, *Eunice cariboea*, and *Dorvillea similis*, coral rubble was collected from seven Pacific and two Caribbean locations from depths varying from 0 to about 23 m, if necessary by snorkeling or scuba diving (see Appendix for collection information). Specimens were removed by breaking the rubble with hammer and chisel and pulling the worms out with forceps. In this process, most of the worms fragmented. In case of doubt whether two fragments belonged to the same individual, only one of the fragments (usually the one containing the head, or if no head was retrieved, the bigger fragment) was used. Usually, a small portion of each individual was fixed in 95%–100% ethanol for DNA studies. The remainder was treated as a voucher sample, fixed in 4% formalin in seawater, and later transferred to 70% ethanol. The voucher specimens are stored at the National Museum of Natural History in Washington, DC (USNM 1084310–USNM 1084406).

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<th>Location</th>
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Dash (—) signifies that the local name is unknown.
Total genomic DNA was extracted using a CTAB protocol (Thollesson, 2000) or a DNeasy kit (Qiagen). Gene regions of the mitochondrial genes for large subunit ribosomal RNA (16S rRNA) and for cytochrome c oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR). PCR reactions were performed in a volume of 25 or 50 μl. For each 25-μl reaction, 5–10 ng DNA (CTAB protocol) or 1 μl of the extractions (Qiagen protocol) and 0.625 units of taq polymerase (Promega) were used. The concentration of other reagents were 200 μM each of dATP, dGTP, dCTP, and dTTP, 0.5–1 μM of each primer, and 1X sequencing buffer. If amplification was unsuccessful even at lower annealing temperatures, it could often be achieved using the MasterTaq kit (Eppendorf), according to the manufacturer’s instructions. The following primers were employed: 16Sa (5'-CGCCTGTITATCAAAAACAT-3' [Xiong and Kocher, 1991]) and 16Sbr (5'-CCGGTCTGACTCATCAGCT-3' [Palumbi, 1996]) for 16S rRNA; the primer pairs LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGTTGACAAAAATC-3') (Folmer et al., 1994) and COI-7 (5'-ACNAAYCAYAARGAYTYGGNAC-3') and COI-D (5'-TCNGGRTGNCCRAANARYCARAA-3') (Saito et al., 2000) in all possible combinations for COI. PCRs were performed according to standard protocols with annealing temperatures of 42° to 45° C. PCR products were visualized in 1%-1.5% agarose gels stained in ethidium bromide and cleaned using the GENECLEAN II kit (Bio 101) or the QIAquick PCR purification kit (Qiagen).

Sequence reactions were performed in 10-μl volume, using 1 μl (if cleaned with GENECLEAN) or 5 μl (if cleaned with QIAquick) of the sample, 1 μM of primer, 2 μl of ABI BigDye Terminator ver. 3.1 (Applied Biosystems) and 2 μl halfTERM Dye Terminator reagent (Genpak).

Sequence reactions were performed with the same thermal cycler as for PCR reactions, using standard protocols. After cleanup of the sequence reactions using gel filtration cartridges from Edge Biosystems, the sequences were analyzed with an ABI 377 or 3100 automatic sequencer. Electropherograms from the sequencer were visualized in Sequencher 4.0. Forward and reverse fragments were assembled and primer regions cropped and discarded. Outgroup sequences for *Marphysa bellii*, *Marphysa sanguinea*, *Ophryotrocha gracilis*, *Lumbrineris funchalensis*, and *Hyalinoecia tubicola* were from Struck et al. (2005), Dahlgren et al. (2001), and Siddall et al. (2001). All sequences have been deposited in GenBank with accession numbers DQ317807 to DQ317917 (Table 3).
Analysis

The alignment of the COI sequences produced no ambiguities. The ribosomal sequences were submitted to the MAFFT server in Kyoto (http://www.biophys.kyoto-u.ac.jp/webmafft/ [Katoh et al., 2002]) for complete alignment. In addition to the taxa included in this study, the alignment also included the sequence for the chiton Katharina tunicata (GenBank accession code U09810), downloaded with secondary structure annotations from the European ribosomal database (http://www.psb.ugent.be/rRNA/ [Van de Peer et al., 2000]). The hypervariable loop between the stem regions G3 and G3’ (positions 247–288 in the alignment) was excluded from the phylogenetic analysis.

The analysis files with the aligned sequences have been deposited with Tree BASE and are available through the World Wide Web at http://www.treebase.org.

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For the stem regions of the 16S rRNA sequences, a list of nucleotide pairings was assembled manually using the annotated sequence of Katharina tunicata as a reference for secondary structure. The stem regions were analyzed under a doublet model with a single rate parameter and 16 states (Scho¨ninger and von Haeseler, 1994), representing all possible nucleotide pairings. The 16S loop regions and the COI sequences were separately submitted to MrModeltest 2.2 (Nylander, 2004), which tests 24 models of sequence evolution using four hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion. For COI, all four hLRTs as well as the Akaike information criterion favored

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* Sample names for Palola refer to the collecting locations, followed by individual identifiers that refer to the vials of the voucher material; sample names with dashes refer to several specimens from the same vial. The outgroups are specified by their full species names.

† Empty cells indicate that no sequence was obtained.

Analysis

The alignment of the COI sequences produced no ambiguities. The ribosomal sequences were submitted to the MAFFT server in Kyoto (http://www.biophys.kyoto-u.ac.jp/webmafft/ [Katoh et al., 2002]) for complete alignment. In addition to the taxa included in this study, the alignment also included the sequence for the chiton Katharina tunicata 2 (GenBank accession code U09810), downloaded with secondary structure annotations from the European ribosomal database (http://www.psb.ugent.be/rRNA/ [Van de Peer et al., 2000]). The hypervariable loop between the stem regions G3 and G3’ (positions 247–288 in the alignment) was excluded from the phylogenetic analysis. The analysis files with the aligned sequences have been deposited with Tree BASE and are available through the World Wide Web at http://www.treebase.org.

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a general time reversible model (Tavaré, 1986) with a
correction for a gamma distribution of substitution rates and
a proportion of invariable sites (GTR + G+I). For the 16S
loop regions, the Akaike information criterion and two of
the likelihood ratio tests (hLRT2 and hLRT4) favored an
HKY +I +G model (Hasegawa et al., 1985); the default
hLRT1 favored a GTR+G+I model; hLRT3 favored
GTR+G. As it is the more general model, GTR+I+G was
implemented for both gene regions. Dorvillea similis was
set as the outgroup. For the Bayesian analyses, two runs,
with four Monte Carlo Markov chains each, were performed
simultaneously for 5,000,000 generations each, sampling
trees every 500 generations. The temperature parameter was
set to 0.07. The initial 2,500,000 trees from each run were
discarded as "burn-in." The remaining 5000 trees from each
run were combined and summarized in a majority rule
consensus tree.

Parsimony bootstrap analysis was performed with 1000
bootstrap replicates, using the heuristic search option in
PAUP*. For each heuristic search, 10 replicates of random
taxon addition were performed with tree bisection/recon-
nection as the branch-swapping algorithm. Branches with
less than 50% bootstrap support were discarded.

Haplotypes were collapsed using the program Collapse
1.2 (Posada, 2004). Uncorrected genetic distances were
calculated in PAUP* (Swofford, 2003). Molecular diversity
indices were calculated in Arlequin 2.000 (Schneider et al.,
2000). Nucleotide diversity was calculated under the
Kimura 2-parameter model. Geographic surface distances
between sample locations were calculated using the airports
of the respective locations as reference points—with the
exception of Ant Atoll, for which the position was obtained
from the website of the U.S. Geological Survey, and Carrie
Bow Cay in Belize, for which the location was obtained
from the CCRE program, Smithsonian Institution.

Results

Phylogenetic analysis

For 16S rRNA, sequence length varied between 362 bp
and 509 bp. These differences are based on complete
sequences, that is, not on sequences with missing end
regions. For five of the eight outgroups (Hyalonoea tuberculosis, Luminbrineris funchalsensis, Ophryotrocha gracilis,
Marphysa bellii, and Marphysa sanguinea), the 16S se-
quen ces were markedly shorter than all others. The align-
ment with secondary structure annotations indicates that
these five species are missing entire stem/loop regions: the
complementary strands of G3/G3', G8/G8', G9/G9', and
G15/G15' are absent, as are the loop regions between the
respective pairs. The region that aligns with the G7 region
in other species is partially present but does not seem to
have a complement.

In the combined analysis there was no conflict between
the Bayesian and the bootstrap parsimony analysis, but the
Bayesian analysis gave better resolution in some parts of the
tree, especially in the deeper nodes. Palola appeared as monophyletic, with 99% posterior probability and 69%
bootstrap support (Fig. 1). The sister group to Palola re-
mains unresolved. Within Palola, two major clades can be
distinguished: clade A contains eastern and western Pacific
samples, plus one sample from Bocas del Toro in the
Caribbean. Clade B contains a mix of Caribbean and west-
ern Pacific samples. The clades are further subdivided into
nine subclades each, A1–A9 and B1–B9, respectively.

When both genes were analyzed separately (Fig. 2), all
subclades remained supported, with one exception: there
was no support for clade A2 in the Bayesian analysis of 16S
however, the clade had 70% parsimony bootstrap support).
There are some discrepancies between the separate analyses
and the combined analysis, but in all cases of discrepancies
the alternative arrangement to the combined analysis has
low support, both in posterior probabilities and bootstrap
values. The 16S rRNA analysis supports the monophyly of
Palola and resolves clade B. The COI analysis gives low
resolution in the deeper nodes but resolves clade A.

Morphological observations

Due to the incompleteness of much of the material and
the high degree of morphological conservation within the
genus, the clades shown in Figure 1 cannot be clearly
delineated morphologically. However, in some cases char-
acters are restricted to certain clades, although they were not
necessarily observed in every individual. In particular, in
clade B, seven individuals (marked with asterisks in Fig. 1)
showed ventral eyespots in their posterior body regions
(Fig. 3A). In no case have ventral eyespots been observed in
clad e A. Clade A8 contains three individuals that were
unusual with respect to morphology and habitat: they have
unusually long and tapering antennae, palps, and parapodial
cirri (Fig. 3B); and unlike all other samples, they were not
infaunal, but inhabited crevices under rocks. Another mor-
phologically distinct clade is clade A7, containing two spec-
imens from Pohnpei. These are characterized by dark brown
pigment on the dorsal side; in other samples, body pigmen-
tation is usually restricted to the ventral eyes and the
parapodial pigment spots. In addition, the two specimens
have a median sulcus in the prostomium that is markedly
shallow and barely visible dorsally (Fig. 3C).

Haplotype diversity and distribution

The 50 COI sequences grouped into 26 haplotypes; the 55
16S sequences grouped into 32 haplotypes. Number and
percentage of singleton (only found in a single individual),
private (occurring in more than one individual but a single
location), and shared haplotypes (occurring in more than
one location) are listed in Table 4.
Figure 1. Bayesian analysis, 50% majority rule consensus from combined 16S ribosomal RNA and cytochrome oxidase subunit I. All *Palola* are named after their geographic origin, followed by a unique identifier as in Table 3. Asterisks indicate samples with ventral eyespots. Names of outgroup species are given in full. Branch support: posterior probability/parsimony bootstrap (single numbers indicate posterior probabilities at branches for which the bootstrap percentages were < 50%).
No haplotype or nucleotide diversity was found in Ant Atoll or in Las Perlas for either COI or 16S, indicating that all individuals from these locations were identical (however, only three individuals were sequenced from Ant Atoll). The highest haplotype diversity (1 for both genes) was in Pohnpei, where each sampled individual represented a different haplotype. Haplotype diversity was also high in each of the remaining locations (Table 5). Nucleotide diversity was higher in the Caribbean samples than in any of the Pacific samples for both of the genes. Mean nucleotide divergences among and within clades are listed in Table 6. All shared haplotypes spanned distances of over 2000 km.

Figure 2. Cladograms resulting from separate analyses for both genes. Bayesian analysis, 50% majority rule consensus trees. With the exception of clade A2 in the analysis of 16S rRNA, all subclades remained supported in the separate Bayesian analyses. In both trees, clades A1–A9 and B1–B9 correspond to clade designations in Figure 4. Branch support: posterior probability/parsimony bootstrap (single numbers indicate posterior probabilities at branches for which the bootstrap percentages were < 50%). (A) Results of 16S rRNA analysis. In the parsimony analysis, A2 is monophyletic with 70% bootstrap support and appears as the sister group to clade A1 (inset). (B) Results of COI analysis.
The most widespread haplotypes covered the complete east-west expansion of the tropical North Pacific, from Las Perlas in the east to Palau in the west (15,826 km). Private haplotypes occurred in Belize, Bocas, Kosrae, and Yap.

**Discussion**

Despite a high degree of morphological uniformity, the phylogenetic analyses reveal deep genetic divergences within Palola. 16S rRNA is an appropriate marker to resolve the deeper branches within the genus (Fig. 2A), but even more conserved genes, such as the nuclear 18S rRNA, might be necessary to resolve outgroup relationships. Cytochrome c oxidase subunit I (COI) provides better resolution than 16S rRNA within the more derived groups in clade A (Fig. 2B), but faster evolving markers would be necessary to address some phylogeographic questions.

Morphological examinations of the voucher material revealed that few morphological characters distinguish the clades. The only likely morphological distinction between clades A and B are the ventral eyespots, as described in Palola viridis. They are only present in some individuals of clade B but occur in five of the nine subclades. One obvious explanation for why they were not present in every specimen is that often only anterior fragments were collected and examined, the posterior body regions being missing. Another explanation is that eyespots only develop during reproductive time. Schröder (1905) examined the ventral eyespots of *P. viridis* histologically, using the swarming epitokes, but never studied nonreproductive individuals. It is a strong possibility that eyespots are a synapomorphy for clade B. Reproductive specimens in clade A never had ventral eyespots. No morphological synapomorphy for clade A has been detected.

At present, it is impossible to determine which, if any, of

### Table 4

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Individuals</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit I</td>
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</tr>
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<td>Singletons</td>
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</tr>
<tr>
<td>Privates</td>
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<tr>
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<td>16S rRNA</td>
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<tr>
<td>Singletons</td>
<td>24</td>
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<td>Privates</td>
<td>4</td>
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<tr>
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<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
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(Tables 7, 8). The most widespread haplotypes covered the complete east-west expansion of the tropical North Pacific, from Las Perlas in the east to Palau in the west (15,826 km). Private haplotypes occurred in Belize, Bocas, Kosrae, and Yap.
the subclades in clade B is *P. viridis*. All of the subclades are morphologically similar and, whenever relevant characters were observable, more or less conform to the description of *P. viridis* given in Fauchald (1992): palps and antennae are arranged in a horseshoe; the size of the head appendages increases from the palps to the median antenna; the prostomium is about as wide as the peristomium; the prostomium in some specimens is dorsally excavate around the palps and the lateral antennae. Characters of the parapodia and setae widely overlap with the descriptions of other species and have limited value for species identification. Fauchald (1992) describes brown pigmentation in the anterior dorsum of *P. viridis* that was not observed in any samples belonging to clade B. Because of the uncertainties associated with designating any of the subclades of clade B as *P. viridis*, it is desirable to generate DNA sequences for samples originating from the type location of this species in Samoa and throughout the species’ geographic range to determine its true distribution and genetic diversity. Attempts to obtain material from Samoa have so far been unsuccessful.

No clear morphological distinction is apparent among the closely related clades A1, A2, and A3. They may represent a single species with a distribution across the entire east-west expansion of the Pacific and even the Caribbean. If the

<table>
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<tr>
<td>Haplotype (h) and nucleotide diversity (π) for both genes by location</td>
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<td>COI</td>
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<td>Haplotype diversity (h)</td>
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<td>Bocas</td>
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<td>Palau</td>
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<td>Yap</td>
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<td>Pohnpei</td>
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<tr>
<td>Ant</td>
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<td>Kosrae</td>
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<th>Table 6</th>
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<tr>
<td>Mean nucleotide divergence and range (in parentheses) in COI (uncorrected) and 16S rRNA (uncorrected) within and between clades for individuals and haplotypes</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit I</td>
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<tr>
<td>Individuals (%)</td>
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<tr>
<td>Within <em>Palola</em></td>
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<tr>
<td>Within clade A</td>
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<tr>
<td>Within clade B</td>
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<tr>
<td>Between clades A and B</td>
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<tr>
<td>16S rRNA</td>
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<tr>
<td>Within <em>Palola</em></td>
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<tr>
<td>Within clade A</td>
</tr>
<tr>
<td>Within clade B</td>
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<tr>
<td>Between clades A and B</td>
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</table>
| No clear morphological distinction is apparent among the closely related clades A1, A2, and A3. They may represent a single species with a distribution across the entire east-west expansion of the Pacific and even the Caribbean. If the

<table>
<thead>
<tr>
<th>Table 7</th>
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<tr>
<td>List of shared (COI-S1 and COI-S2) and private haplotypes (COI-P1 through COI-P4) for COI with geographic extensions of shared haplotypes</td>
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<td>COI Haplotype name</td>
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<tr>
<td>COI-S1</td>
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<td>COI-P1</td>
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<tr>
<td>COI-P4</td>
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</table>
identification key in Fauchald (1992) is used, the species is identified as *P. siciliensis*; however, the specimens can be distinguished from *P. siciliensis* by their earlier onset of branchiae. In clades A1–A3, branchiae start between setigers 48 and 60 whenever a long enough anterior fragment can be observed. In *P. siciliensis*, the earliest appearance of branchiae is setiger 92 and can be as far posterior as setiger 180 (Fauchald, 1992). As with *P. viridis*, *P. siciliensis* lacks sequence data from the type location in Sicily to test the species designation.

All subclades except A1–A3 may represent distinct species. The species generally seem to be cryptic, although clades A7 and A8 show some characteristic morphological features (Fig. 3) not observed in other specimens or described from any of the known species. However, for clearer morphological delineations, it would be necessary to examine more complete material for all subclades.

The age of *Palola* as a genus is unknown, but the probability is high that it arose in the Paleozoic. Fossil mandibles and maxillae of eunicidan polychaetes date back to the Ordovician (Kielan-Jaworowska, 1966), including the lapi-dognath jaw type found in the Eunicidae. However, the typical scoop-shaped mandibles of *Palola* have not been described from the fossil record. A paleozoic origin would explain the high degree of intrageneric genetic divergence.

Although COI and 16S rRNA are considered two of the more conserved genes in the mitochondrial genome, the relatively high degree of divergence—averaging 14.5% and 12.4% respectively—is not unusual in polychaetes. For 16S rRNA, mean sequence divergence in the syllid genus *Autolytus* is approximately 21% (Nygren and Sundberg, 2003), based on 16 species. For the dorvilleid genus *Ophrytrocha* it is 12%, based on 17 species (Dahlgren et al., 2001). For COI, mean within-family divergence in the Terebellidae is over 20% based on the nine available sequences from Gen-Bank (Colgan et al., 2001; Siddall et al., 2001). For the two terebellid genera of which two species are represented in GenBank, sequence divergence was 20% for the two *Loimia* species and 19% for the two *Amphitrite* species. In view of the fact that taxonomic ranks are arbitrary, these comparisons can be only a rough guide, but they convey that the genetic variation within *Palola* is within a normal range for polychaetes. The genetic variation is only surprising compared with the high degree of morphological conservation among the species.

Most haplotypes (76.9% for COI; 75% for 16S rRNA) detected in this study are singletons (Table 4) and uninformative with respect to phylogeographic questions. Only a small percentage of the haplotypes (7.7% for COI; 12.5% for 16S) are shared among locations, but they represent a large percentage of the sampled individuals (40% for both markers) and cover six of the nine collecting locations (Tables 7 and 8). Several recent studies have shown strong geographic population structure in marine shallow-water invertebrates, even in taxa with high dispersal capabilities (e.g., Kirkendale and Meyer, 2004, and references therein). It is therefore surprising to find widespread, in some cases extremely widespread, haplotypes and clades in *Palola*, especially considering that all known eunicid larvae are short-lived and lecithotrophic (Richards, 1967). In clades A1–A3 with very short branches, the lack of geographic structure may be due to incomplete lineage sorting, suggesting that the islands have been colonized relatively recently in terms of the age of the genus and not enough time has passed for distinct lineages to be established on each island (e.g., Harrison, 1991; Avise, 2000).

Haplotype diversity and nucleotide diversity are both high in all but two collecting locations (Table 5). The phylogenetic reconstructions show that the high nucleotide diversity is not due to local radiations (in which case all haplotypes in one location would form a clade) but to repeated colonization of the islands by members of genetically divergent *Palola* clades. This effect is most pro-

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**Table 8**

List of shared (16S-S1 through 16S-S4) and private haplotypes (16S-P1 through 16S-P4) for 16S rRNA with geographic extensions of shared haplotypes.

<table>
<thead>
<tr>
<th>16S Haplotype name</th>
<th>Samples</th>
<th>Maximum geographic distance (km)</th>
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<tbody>
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<td>16S-S1</td>
<td>Ant158-1</td>
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</tr>
<tr>
<td></td>
<td>Ant158-3</td>
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<td>Ant160</td>
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<td>Pohnpei151-2</td>
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<td>Yap131</td>
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</table>
nounced in the Caribbean locations and is probably related to the complex geological history of the Caribbean region. Multiple models exist for the tectonic history of the Caribbean (Graham, 2003, and references therein), but whichever theory is favored, it is likely that shallow-water marine taxa such as Palola have had numerous opportunities for dispersal and vicariance within the region, allowing divergent clades to co-occur within the same location. Exchange with Pacific waters was possible until approximately 3.5 million years ago, explaining why the two major Palola clades contain samples from both the Caribbean and the Pacific.

No nucleotide and haplotype diversity was detected in Ant Atoll or the Las Perlas Archipelago. Although this might be an artifact of small sample size (only three individuals sampled from Ant Atoll) or a single collecting spot at each island group, it could also indicate that these locations were only recently colonized. If a population had been established for a long time, some local haplotype diversity would be expected. More extensive sampling would be necessary to investigate this question.

More rapidly evolving markers should add resolution to clade A, but the current data indicate that long-distance dispersal has taken place repeatedly in both major clades of Palola. Despite long-held opinions on eunicid development, long-lived planktotrophic larvae might exist in at least some Palola lineages.

Acknowledgments

This research was conducted at the National Museum of Natural History under a Smithsonian Institution postdoctoral fellowship and continued in the Department of Organismic and Evolutionary Biology at Harvard University and at the Smithsonian Marine Station at Fort Pierce, Florida (contribution Nr 630). Additional funding was provided by Caribbean Coral Reef Ecosystems (contribution Nr 738) and the Nando Peretti Foundation. The Smithsonian Tropical Research Institute hosted a short-term visit. I owe special thanks to Kristian Fauchald and Jon Norenburg as special thanks to Kristian Fauchald and Jon Norenburg and many other helpers in Micronesia for their support in the field. Grazia Cantone provided specimens of P. sicilien-sis from the type location for morphological comparison. I also thank two anonymous reviewers whose comments have significantly improved this manuscript.

Literature Cited


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Appendix

Collecting Stations: Geographic co-ordinates refer to airports, with the exception of Ant Atoll and Carrie Bow Cay

Station 1: Carrie Bow Cay area, Belize (16°48'N, 88°05'W)
   1A: Carrie Bow Cay, reef flat, 0.5–1 m, Feb. 2001
   1B: Carrie Bow Cay, reef drop-off, 8–10 m, Feb. 2001
   1C: Blue Ground Range, 0.5–1 m, Feb. 2001
   1D: Twin Cays, 0.5–1 m, Feb. 2001
   1E: Southwater Cay, 1.5 m

Station 2: Saboga Island, Las Perlas Archipelago, Panama (8°35'N, 79°35'W): 2.5–3 m, June 2001

Station 3: Bocas del Toro Archipelago, Panama (9°21'N, 82°15'W)
   3A: Hospital Point, Isla Solarte, 2.5 m, June 2001
   3B: Mangrove Inn, Isla Colón, 1–2 m, June 2001
   3C: Drago Beach, Isla Colón, Panama, 2 m, June 2001

Station 4: Guam, USA (13°29'N, 144°47'E)
   4A: Double Reef, 15–20 m, Oct. 2001
   4B: Shark’s Pit, 15–23 m, Oct. 2001
   4C: Cocos Island, 1.5–3 m, Oct. 2001
   4D: Mangilao, 1 m, Oct. 2001

Station 5: Republic of Palau (7°22'N, 134°32'E)
   5A: Lighthouse Reef, 1.5–2 m, Oct. 2001
   5B: Western Channel, 2 m, Oct. 2001
   5C: Short drop-off, 13 m, Oct. 2001
   5D: Turtle Island, 0.5–2 m, Oct. 2001
   5E: Ngerikuul Channel, 13–17 m, Oct. 2001

Station 6: Yap, Federated States of Micronesia (9°29’N, 138°40’E)
   6A: Colonia, 3–5 m, Nov. 2001
   6B: Mill Channel, 5–15 m, Nov. 2001

Station 7: Pohnpei, Federated States of Micronesia (6°59’N, 158°12’E)
   7A: The Village Hotel, 0.3–1.5 m, Nov. 2001
   7B: Nahpali, 2 m, Nov. 2001
   7C: Black Coral Island, 1.5–3 m, Nov. 2001

Station 8: Tolonmurui Island, Ant Atoll, Pohnpei, Federated States of Micronesia (6°46’N, 157°55’E), 0.1–5 m, Nov. 2001

Station 9: Kosrae, Federated States of Micronesia (5°21’N, 162°57’E)
   9A: Mwot, Kosrae, Federated States of Micronesia, 2–3 m, Nov. 2001
   9B: Buoy 21, Kosrae, Federated States of Micronesia, 13–20 m, Nov. 2001
   9C: Buoy 5, Kosrae, Federated States of Micronesia, 10–15 m, Nov. 2001