PHYLOGEOGRAPHIC PATTERNS OF *TYLOS* (ISOPODA: ONISCIDEA) IN THE PACIFIC REGION BETWEEN SOUTHERN CALIFORNIA AND CENTRAL MEXICO, AND MITOCHONDRIAL PHYLOGENY OF THE GENUS

A Thesis

by

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ABSTRACT

Isopods in the genus *Tylos* are distributed in tropical and subtropical sandy intertidal beaches throughout the world. These isopods have biological characteristics that are expected to severely restrict their long-distance dispersal potential: (1) they are direct developers (i.e., as all peracarids, they lack a planktonic stage); (2) they cannot survive in the sea for long periods of immersion (i.e., only a few hours); (3) they actively avoid entering the water; and (4) they are restricted to the sandy intertidal portion that is wet, but not covered by water. Because of these traits, high levels of genetic differentiation are anticipated among allopatric populations of *Tylos*.

We studied the phylogeographic patterns of *Tylos* in the northern East Pacific region between southern California and central Mexico, including the Gulf of California. We discovered high levels of cryptic biodiversity for this isopod, consistent with expectations from its biology. We interpreted the phylogeographic patterns of *Tylos* in relation to past geological events in the region, and compared them with those of *Ligia*, a co-distributed non-vagile coastal isopod. Furthermore, we assessed the usefulness of the shape of the ventral plates of the fifth pleonite for distinguishing genetically divergent lineages of *Tylos* in the study area. Finally, mitochondrial phylogenenetic analyses to identify the most appropriate outgroup taxa for *Tylos* in the study area, which included 17 of the 21 currently recognized species, provided important insights on the evolutionary history of this genus.

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1. INTRODUCTION

The Gulf of California is one of the most biologically diverse (Brusca et al. 2005) and productive basins of the world (Alvarez-Borrego 2002). More than 4900 nominal species of macroinvertebrates have been reported in this region, of which only 82 (< 2%) correspond to isopods (Brusca and Hendrickx 2010). Nevertheless, a recent phylogeographic study of a common taxon (genus *Ligia*) distributed throughout this region, indicates that isopod diversity of this non-vagile coastal isopod is much higher than previously estimated (Hurtado et al. 2010). Extraordinarily high levels of cryptic diversity were reported in *Ligia*, and its phylogeographic patterns suggest that the complex geological history of this region played a role in the diversification of this non-vagile organism. High levels of cryptic biodiversity are expected in other coastal non-vagile isopods, and their phylogeographic patterns may shed light on past events that contributed to diversification in this region.

The genus *Tylos* Audouin, 1826, an oniscidean isopod within the family Tylidae, is comprised of intertidal species distributed in tropical and subtropical sandy beaches throughout the world (Hayes 1970; Kensley 1974; Brown and Odendaal 1994). The family Tylidae contains only one other genus, *Helleria* Ebner, 1968 (Schmalfuss and Vergara 2000; Schmalfuss 2003), which is terrestrial and endemic to the northern Tyrrhenian area (i.e., Corsica, Sardinia, Tuscan archipelago, and few continental locations in western Italy and southern France). Although it is considered monotypic, the single described species *H. brevicornis* may contain more than one cryptic species

(Gentile et al. 2010). The genus *Tylos* currently has 21 accepted species, with many more originally described, but subsequently synonymized (Schmalfuss and Vergara 2000; Schmalfuss 2003). Only two species have been reported in the northern East Pacific region spanning southern California to central Mexico, including the Gulf of California: *Tylos latreillii* Audouin, 1826 and *Tylos punctatus* Holmes and Gay, 1909 (Mulaik 1960; Hayes 1974; Schultz and Johnson 1984).

Mulaik (1960) reports *T. latreillii* in the northern Pacific coast of the Baja California Peninsula and in the Gulf of California. This species, however, was originally described from the Red Sea Egyptian coast (Audouin 1826). At present, *T. latreillii* is considered a *nomen dubium* (Taiti and Ferrara 1996), and based on its type locality, probably corresponds to one of the two Mediterranean Sea species, *T. europaeus* or *T. ponticus*. The incorrect assignment to *T. latreillii* of specimens from many other localities around the world has contributed to taxonomic confusion within the genus (Audouin 1826; Schmalfuss and Vergara 2000; Schmalfuss 2003; Taiti and Ferrara 2004). Many of the currently valid species of *Tylos* (i.e., *T. ponticus, T. europaeus, T. punctatus*, *T. exiguus*, *T. niveus*, *T. marcuzzii*, and *T. madeirae*) were previously treated as *T. latreillii* (Schmalfuss 2003).

The type locality of *T. punctatus* is San Diego, southern California (Holmes and Gay 1909). This species has been reported at other localities in southern California as far north as Santa Barbara, as well as along the Pacific coast of the Baja California Peninsula, the Gulf of California, and the Galápagos Islands (Hamner et al. 1969; Hayes 1970, 1977; Schmalfuss 2003). It has been suggested, however, that *T. punctatus* from

southern California is taxonomically distinct from *Tylos* reported in the Gulf of California, an issue that needs to be solved (Hamner et al. 1969; Schultz 1970; Hayes 1977; Brown and Odendaal 1994).

Isopods of the genus *Tylos* can be found in the upper intertidal on sand, mud, in cracks and crevices, and under algal detritus or rocks (Hayes 1970; Schultz 1970; Schultz and Johnson 1984; Brown and Odendaal 1994; Schmalfuss and Vergara 2000). Dispersal within water is limited because, as all isopods, *Tylos* does not have planktonic larvae (i.e., they are direct developers). Furthermore, adults actively avoid entering the water, probably because they can survive only for few hours when submerged, due to their extremely limited swimming abilities (Schultz 1970; Kensley 1974; Brown and Odendaal 1994). It has been suggested, however, that juveniles of some species may be able to surf, rolling themselves into a ball, which may facilitate dispersal among nearby beaches (Schultz 1970; Kensley 1974).

Tylos has a 24-hour cycle that is correlated with the tidal cycle, which strongly influences its diurnal and nocturnal behavior (Kensley 1974; McLachlan and Brown 2006). During the day, *Tylos* remains buried and inactive near the previous high tide mark (Hamner et al. 1969; Schultz 1970; Kensley 1974; Hayes 1977). At dusk, they emerge and remain active during all night, foraging on detritus and algae (they are omnivorous scavengers) found in the intertidal portion of the sand that is not covered by water (Hamner et al. 1969; Schultz 1970; Kensley 1974; Hayes 1977). Before sunrise, they bury again at the high tide mark, which appears to be a mechanism to prevent desiccation during the day and avoid being washed away during the next high tide

(Hamner et al. 1969; Schultz 1970; Kensley 1974; Hayes 1977). In the northern Gulf of California, *Tylos* have been found buried in sand with at least 1% moisture, and experiments reveal high mortality rates at sand with 0.1% moisture (Holanov and Hendrickson 1980). In subtropical areas such as the southern California coast, *Tylos* hibernates during the winter, remaining buried and inactive in the sand (Hamner et al. 1969; Schultz 1970; Hayes 1977).

Tylos punctatus from southern California and Baja California have been reported to live at least four years (Hamner et al. 1969). Compared to other isopods, the reproductive rate of this isopod is relatively low, producing 4 to 20 young per brood, and a single brood per year (Hamner et al. 1969). Most females appear to breed only once during their lifespan, usually before three years of age (Hamner et al. 1969). They grow only during a 5-month period in summer and hibernate during winter (Hamner et al. 1969; Hayes 1977).

Given the biological characteristics of *Tylos*, long distance dispersal potential appears to be severely limited. Therefore, high levels of allopatric differentiation are expected. Herein, we studied the phylogeographic patterns of *Tylos* in the northern East Pacific region between southern California and central Mexico, including the Gulf of California; hereafter, the *Study Area*. We discover high levels of cryptic biodiversity for this isopod, as expected from its biological characteristics. We interpret the phylogeographic patterns of *Tylos* in relation to past geological events in the region. We compare the phylogeographic patterns of *Tylos* with those of *Ligia*, a co-distributed non-vagile coastal isopod. We assess the usefulness of the shape of the ventral plates of the

fifth pleonite for distinguishing genetically divergent lineages of *Tylos* in the study area. In addition, to identify the most appropriate outgroup taxa for the *Study Area* phylogenetic analyses, we inferred a mitochondrial phylogeny of the genus *Tylos*, by examination of 17 of the 21 currently recognized species. The results provide insights on the evolutionary history of this genus, a topic that has not been studied before.

2. MATERIALS AND METHODS

2.1 Sampling

Two taxon-sampling schemes were implemented. The first one, *Outgroup Identification*, was aimed at identifying the appropriate outgroup taxa for the second one, *Study Area*. A secondary goal of the *Outgroup Identification* analyses was to infer a mitochondrial phylogeny of the genus *Tylos*, based on 17 out of the 21 currently recognized species (Fig. 1). For the *Study Area* analyses, we examined samples of *Tylos* from 45 rocky and sandy beach localities between central Mexico to Southern California, including the Gulf of California (Fig. 2). Most of the *Study Area* samples were collected during 2008–2011. Collection information for *Study Area* samples is shown in Appendix 1 and for *Outgroup Identification* analyses in Appendix 2.

2.2 Molecular methods

Genomic DNA was isolated from 2–4 legs per specimen with the DNEasy kit (Qiagen, Inc., Valencia, CA). We PCR-amplified six mitochondrial gene fragments and two nuclear gene fragments from one individual per locality in the *Study Area* (primers and annealing temperatures in Appendix 3). The amplified mitochondrial (mt) segments were: 16S ribosomal (r)DNA; 12S rDNA; Cytochrome Oxidase I gene (COI); Cytochrome b gene (Cytb); and a ND6/ND4 segment that includes portions of the 16S rDNA (non-overlapping with the aforementioned segment), NADH4, NADH6 genes, and intervening tRNAs. In addition, we examined the 16S rDNA and COI genes for

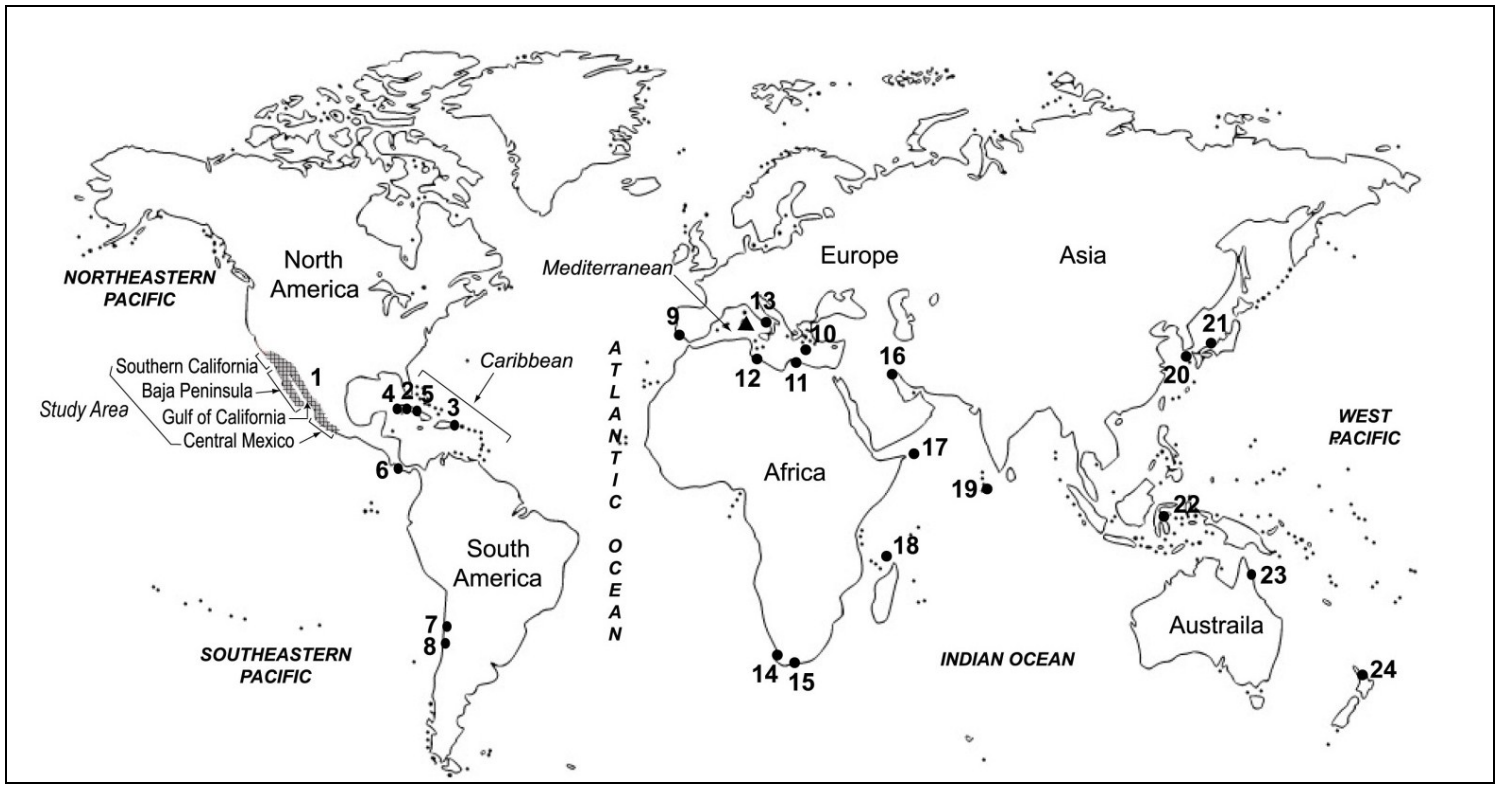

Figure 1. Sampled localities and areas for the *Outgroup Identification* analyses. Each number corresponds to *Tylos* species and sampling locality or region: **1** = ***Study Area*** (Hatched Area); **2** = ***T. sp***; **3** = ***T. niveus;* 4.** ***T. marcuzzii*** (Pinar del Rio); **5** = ***T. marcuzzii*** (Cayo Coco); **6** = ***T. wegeneri***; **7** = ***T. spinulosus***; **8** = ***T. chilensis***; **9** = ***T. ponticus*** (Portugal); **10** = ***T. ponticus*** (Greece); **11** = ***T. ponticus*** (Libya); **12** = ***T. europaeus*** (Libya); **13** = ***T. europaeus*** (Italy); **14** = ***T. granulatus***; **15** = ***T. capensis***; **16** = ***T. maindroni***; **17** = ***T. exiguus***; **18** = ***T. minor***; **19** = ***T. albidus***; **20** = ***T. granuliferus*** (Korea); **21** = ***T. granuliferus*** (Japan); **22** = ***T. opercularis*** (Sulawesi); **23** = ***T. opercularis*** (Australia); **24** = ***T. neozelanicus*** (New Zealand); **Triangle (Outgroup)** = ***Helleria brevicornis*** (Sardinia, Italy).

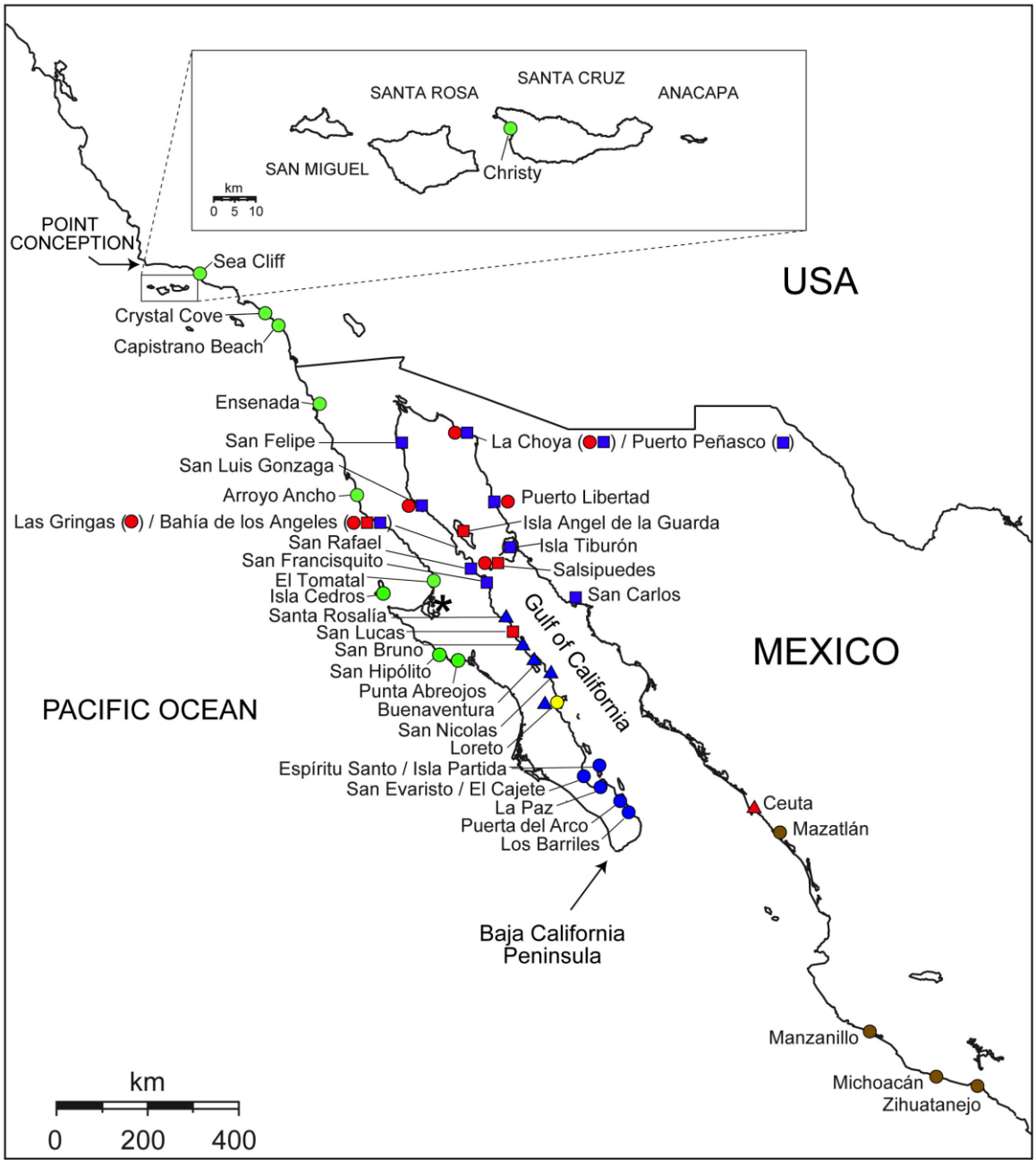

Figure 2. Sampled localities for the *Study Area* analyses. Colors and shapes correspond to clades in Fig 4. * denotes location of Guerrero Negro Lagoon in the central Baja California Peninsula.

additional individuals (up to five) per most localities in the *Study Area* (See Appendix 1). The nuclear genes examined were the highly variable V4 region of the 18S rDNA gene; and Histone 3 (H3A) gene. PCR-amplified products were cleaned with Exonuclease and Shrimp Alkaline Phosphatase, and subsequently cycle sequenced at the University of Arizona Genetics Core. We used Sequencher 4.8 (Gene Codes, Ann Arbor, MI) for sequence editing and primer removal. None of the protein-coding sequences had premature stop codons or frame shifts, suggesting that they are not pseudogenes.

2.3 Sequence alignment and datasets

Alignments for the *Outgroup Identification* and the *Study Area* analyses were conducted separately, due to the high divergence among taxa in the former (see Results). Non-protein-coding sequences were aligned with MAFFT v.6.0 (Katoh and Toh 2008), as implemented in http://mafft.cbrc.jp/alignment/server/, with the Q-INS-I strategy, which considers secondary structure of RNA, and with the L-INS-i strategy with default parameters (e.g. Gap Opening penalty = 1.53). Resulting alignments were edited manually within MacClade v.4.06 (Maddison and Maddison 2003). Regions for which homology could not be confidently established were identified with GBlocks v.0.91b (Castresana 2000), and excluded from the phylogenetic analyses. The following GBlocks parameters were used: "Allowed Gap Positions" = half; "Minimum Length Of A Block" = 5 or 10; and "Maximum Number Of Contiguous Nonconserved Positions" =

4 or 8. Alignments showing included and excluded positions are available in Appendix 4.

For the *Outgroup Identification* analyses, three mitochondrial loci (16S rDNA, 12S rDNA, and Cytb) were concatenated into a single dataset (Table 1). Attempts to amplify and sequence enough taxa for the remaining loci were unsuccessful. These analyses, which used the monotypic genus *Helleria* as the outgroup, suggested that the samples of *Tylos* from the study area represent a monophyletic clade (see Results, Fig. 3). Furthermore, among the taxa examined, two lineages from the Caribbean (*Tylos* sp. from Yaguanabo, Cuba and *T. niveus*, from Puerto Rico) were among the closest relatives of the study area clade, and thus, were used hereafter as outgroup taxa in the *Study Area* analyses. For the *Study Area*, phylogenetic analyses were conducted on the following datasets (see Table 2): (1) concatenated mitochondrial loci (MT); (2) the nuclear 18S rDNA; (3) the nuclear H3A; and (4) concatenated mitochondrial and nuclear loci (MT+NC).

2.4 Phylogenetic analyses

To determine the most appropriate model of DNA substitution among 88 candidate models on a fixed BioNJ-JC tree, we used jModeltest v0.1.1 (Posada 2008) under the Akaike Information Criterion (AIC), corrected AIC(c), and Bayesian Information Criterion (BIC) (Tables 1 and 2). We used the closest more complex model available in the corresponding ML and Bayesian analyses (Table 3 and 4), except that when a proportion of invariable sites (I) and a Gamma distribution of rates among sites

Table 1. Description of characters and substitution models for the *Outgroup Analyses*. Number of characters per gene region that were excluded from and included in the phylogenetic analyses. The number of parsimony informative characters is based on included characters only. Best model selected by jModelTest according to each criterion (AIC, AICc, BIC) and its corresponding weight.

Gene	Samples	Total characters [a]	Excluded characters [ab]	Included characters	Parsimony informative	AICc (weight)	AIC (weight)	BIC (weight)
16S rDNA	72	498	324	174	79	K80+G (1.00)	TPM2uf+I+G (0.28)	HKY+I+G (0.62)
12S rDNA	62	524	351	173	97	HKY+G (0.68)	TPM3uf+G (0.30)	TPM3uf+G (0.61)
Cytb	67	296	0	296	161	TIM2+I+G (0.87)	TIM2+I+G (0.56)	TIM2+I+G (0.92)
MT	72[bc]	1318	675	643	337	TPM2uf+I+G (0.47)	TPM2uf+I+G (0.40)	HKY+I+G (0.56)

[a] Total number of characters in the alignment, including gaps.
[b] Criteria for character exclusion are described in a nexus file in the supporting information.
[c] The number of samples was selected for the combined analyses of mitochondrial genes, including missing genes of *Tylos* species.
MT = combined mitochondrial genes

Table 2. Description of characters and substitution models for the *Study Area* dataset. Number of characters per gene region that were excluded from and included in the phylogenetic analyses. The number of parsimony informative characters is based on included characters only. Best model selected by jModelTest according to each criterion (AIC, AICc, BIC) and its corresponding weight.

Gene region	Samples	Total characters[a]	Excluded characters[b]	Included characters	Parsimony-informative	AICc (weight)	AIC (weight)	BIC (weight)
16S	137	491	145	346	99	HKY+I+G (1.00)	GTR+I+G (1.00)	GTR+I+G (1.00)
12S	44	350	74	276	63	TIM2+G (0.29)	TIM2+I+G (0.24)	TrN+G (0.36)
COI	114	657	57	600	194	TIM1+I+G (0.95)	TIM1+I+G (0.88)	TIM1+I+G (0.93)
Cytb	47	296	0	296	117	TIM2+I+G (0.61)	TIM2+I+G (0.63)	TIM2+I+G (0.57)
ND6/4	43	1639	165	1474	621	TIM2+I+G (0.91)	TIM2+I+G (0.88)	TIM2+I+G (0.99)
MT	50[c]	3433	441	2992	1058	TIM2+I+G (0.66)	TIM2+I+G (0.64)	TrN+I+G (0.49)
H3A	34	285	0	285	20	TPM1+G (0.25)	TPM1uf+G (0.10)	TPM1+G (0.30)
18S	40	1121	601	520	110	TrNef+I+G (0.30)	TrNef+I+G (0.23)	K80+I+G (0.48)
MT+NC	50[d]	4839	1042	3797	1188	TIM2+I+G (0.54)	TIM2+I+G (0.54)	HKY+I+G (0.78)

[a] Total number of characters in the alignment, including gaps.
[b] Criteria for character exclusion are described in a nexus file in the supporting information.
[c] Includes taxa that were missing one or two mitochondrial genes.
[d] Includes taxa that were missing one or more mitochondrial or nuclear genes.
MT = concatenated mitochondrial genes.

Table 3. Models, parameters, and priors used in the Maximum Likelihood and Bayesian phylogenetic analyses of the *Outgroup Analyses* concatenated mitochondrial (MT) dataset.

Method	Model and Priors[1]	Partitioning scheme[2]	iterations generations/ bootstrap replicates	Sample frequency	runs/ chains	burnin	ASDSF[3]	Bayes Factors[4] /ML scores (-lLn)	ESS[4,5] > 200	PSRF[6]
RaxML	GTR G	1	1000	na	na	na	na	-8867.43	na	na
RaxML	(GTR G)[8]	2 (16S+12S+Cytb1 +Cytb2, Cytb3)[8]	1000	na	na	na	na	-8930.53	na	na
RaxML	GTR G	3 (by gene:16S, 12S, Cytb)	1000	na	na	na	na	-8996.85	na	na
RaxML	GTR G	5 (by gene+codon: 16S, 12S, Cytb1, Cytb2, Cytb3)	1000	na	na	na	na	-8867.43	na	na
Garli	HKY G	1	100	na	na	na	na	-8595.55	na	na
Garli	GTR G	1	100	na	na	na	na	-8746.15	na	na
Garli	TPM2uf G	1	100	na	na	na	na	-8901.00	na	na
Garli	Mixed Model best (BIC)[7]	3 (by gene:16S, 12S, Cytb)[7]	100	na	na	na	na	-8596.46	na	na
Garli	Mixed Model best (BIC)[8]	2 (16S+12S+Cytb1 +Cytb2, Cytb3)[8]	100	na	na	na	na	-8896.24	na	na
MrBayes	GTR G	1	2,800,000	1,000	4/4	10%	0.008824	-9176.37	yes	1
MrBayes	HKY G	1	3,012,000	1,000	4/4	10%	0.008782	-9164.62	yes	1
Phycas	GTR G; polytomy prior	1	500,000	100	na	20%	na	-9170.69	na	na
BP	GTR G	1	94,490,000	10,000	8/1	10%	na	-9191.69	yes	na
BP	GTR G	2	39,680,000	10,000	8/1	10%	na	-9106.13	yes	na
BP	GTR G	3	24,960,000	10,000	8/1	10%	na	-9081.95	yes	na

[1] All others default; [2] different partitions separated by comma; [3] Average standard deviation of split frequencies; [4] estimated in Tracer v.1.5; [5] Effective Sample Size; [6] Potential Scale Reduction Factor for all parameters; [7] see Table 1; [8] PartitionFinder 1.0 (HKY+I+G;TIM+I+G; suggested Best Model); BP = BayesPhylogenies

Table 4. Models, parameters, and priors used in the Maximum Likelihood and Bayesian phylogenetic analyses of the *Study Area* concatenated mitochondrial (MT) and nuclear (NC) dataset.

Method	Model and Priors[1]	Partitioning scheme[2]	iterations generations /bootstrap replicates	Sample frequency	runs/ chains	burnin	ASDSF[3]	Bayes Factors[4] /ML scores (-lLn)	ESS[4,5] > 200	PSRF[6]
RaxML	GTR G	1	1000	na	na	na	na	-23426.81	na	na
RaxML	GTR G	**7** (by gene: 16S, 12S, COI, Cytb, ND6/ND4, H3A,18S)	1000	na	na	na	na	-22305.43	na	na
RaxML	GTR G	**9** (16S+12S, COI1+Cytb1, COI2+Cytb2, COI3+Cytb3, ND6/ND4, H3A1, H3A2, H3A3, 18S)	1000	na	na	na	na	-21985.81	na	na
RaxML	(GTR G)[8]	**8** (16S+12S+Cytb1+Cytb2, COI1, COI2, COI3, Ctyb3, ND6/ND4, H3A1+H3A2, H3A3+18S)[8]	1000	na	na	na	na	-22622.06	na	na
Garli	TPM2uf G	1	100	na	na	na	na	-23373.57	na	na
Garli	GTR G	1	100	na	na	na	na	-23359.42	na	na
Garli	HKY G	1	100	na	na	na	na	-23030.02	na	na
Garli	Mixed Models best (BIC)[7]	**7** (by gene: 16S, 12S, COI, Cytb, ND6/ND4, H3A, 18S)[7]	100	na	na	na	na	-23259.96	na	na
Garli	Mixed Models best (BIC)[8]	**8** (16S+12S+Cytb1+Cytb2, COI1, COI2, COI3, Ctyb3, ND6/ND4, H3A1+H3A2, H3A3+18S)[8]	100	na	na	na	na	-23007.07	na	na
MrBayes	GTR G	1	17,000,000	1,000	4/4	10%	0.002379	-23492.78	yes	1
MrBayes	HKY G	1	17,000,000	1,000	4/4	10%	0.002517	-23497.31	yes	1
Phycas	GTR G; polytomy prior	1	500,000	100	na	20%	na	-23503.20	na	na
BP	GTR G	1	40,600,000	10,000	8/1	10%	na	-23502.21	yes	na
BP	GTR G	2	43,000,000	10,000	8/1	10%	na	-23332.23	yes	na
BP	GTR G	3	46,340,000	10,000	7/1	10%	na	-23291.97	yes	na

[1] All others default; [2] different partitions separated by comma; [3] Average standard deviation of split frequencies; [4] estimated in Tracer v.1.5; [5] Effective Sample Size; [6] Potential Scale Reduction Factor for all parameters; [7] see Table 2; [8] PartitionFinder 1.0 (HKY+I+G;TrNef+G;F81;K81uf+G;TrN+G;TrN+I+G;JC+I;K80+G; suggested Best Model); BP BayesPhylogenies

(G) was selected according to jModeltest, we excluded parameter I to avoid problems related to dependency between two parameters [see RaxML manual and (Yang 2006)]. In addition, to assess robustness of the results to substitution model, we also used the complex model GTR+G. Several data partitioning schemes were implemented, including: (a) all positions within a single partition; (b) the best partitioning scheme according to the BIC implemented in PartitionFinder v.1.0 (Lanfear et al. 2012); and (c) 1–3, partitions not specified a priori (i.e., BayesPhylogenies; Tables 3 and 4). The following parameters were used in PartitionFinder: branch lengths = linked; models = all; model selection = BIC; search = greedy; and a priori partitioning by a combination of each gene and codon position.

For the ML analyses, two approaches were employed: (a) a Rapid Bootstrap followed by ML search in RaxMLGUI v. 1.0, which includes the executable files of RAxML 7.3.0 (Stamatakis 2006; Silvestro and Michalak 2011); and (b) GARLI v.2.0 (Zwickl 2006), which uses genetic algorithms for the ML search. Clade support was examined by non-parametric bootstrap analyses (100–1000 replicates) summarized with 50% majority rule consensus trees by SumTrees script implemented in DendroPy 3.10.1 (Sukumaran and Holder 2010).

For the Bayesian analyses, three programs were used. The first one was MrBayes v 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012), but such analyses have been reported to return high clade posterior probabilities in certain cases of known polytomies (a.k.a., the “star-tree paradox”) (Suzuki et al. 2002). Therefore, we also applied one of the proposed strategies to

alleviate this problem; i.e., the polytomy prior (Lewis et al. 2005) as implemented in Phycas v.1.2.0 (Lewis et al. 2010). Finally, we used BayesPhylogenies v.1.1 to fit more than one substitution model to different positions in the dataset without the need for identifying the data partitions a priori (Pagel and Meade 2004). Analyses of 1–3 partitions (i.e., patterns) were conducted.

The following criteria were used to evaluate convergence and adequate sampling of the posterior distribution: (a) Stable posterior probability values; (b) a high correlation between the split frequencies of independent runs as implemented in AWTY (Nylander et al. 2008); (c) small and stable average standard deviation of the split frequencies of independent runs; (d) Potential Scale Reduction Factor close to 1; and (e) an Effective Sample Size (ESS) > 200 for the posterior probabilities, as evaluated in Tracer v. 1.5 (Rambaut and Drummond 2009). Samples prior to reaching a stationary posterior distribution were discarded (i.e., "burnin"; Tables 3 and 4).

Pairwise genetic distances with Kimura-2-parameter (K2P) correction were estimated with MEGA v.5 (Tamura et al. 2011) for COI gene and selectively combined datasets. All ambiguous positions were removed for each sequence pair.

2.5 Examination of pleon ventral shapes

The shape of the ventral plates of the fifth pleonite is commonly used as a diagnostic character for different species of *Tylos* (Schultz and Johnson 1984). We examined this trait in individuals from the *Study Area* belonging to genetically differentiated lineages, as indicated by the phylogenetic results. Specimens were

photographed under a dissecting microscope. We also compared this trait with that of other *Tylos* species.

3. RESULTS

3.1 Outgroup identification and mitochondrial phylogeny of *Tylos*

The concatenated dataset of three mitochondrial genes for the worldwide *Tylos* samples was comprised of 69 taxa and 643 characters, of which 337 were parsimony informative (Table 1). Figure 3 shows the phylogenetic reconstruction for the worldwide *Tylos* samples based on three mitochondrial genes and using *Helleria*, the only other genus of the family Tylidae, as outgroup. Monophyly of the *Tylos* samples from the study area (i.e., between southern California and Pacific Mexico, including the Gulf of California) was supported by 100 Bayesian posterior probabilities (PP), and 61–79 ML bootstrap support (BS). All phylogenetic analyses indicated that the closest relatives of the study area clade were a *Tylos* sample from Yaguanabo (Cuba), and a *T. niveus* sample from Aguada (Puerto Rico). Monophyly of these two samples was suggested by the ML analyses with 67–79 BS and by MrBayes analyses with 72 PP; the other two Bayesian methods, however, did not recover this relationship with > 50 PP. Monophyly of the group formed by these two Caribbean samples and the study area clade was highly supported by all methods (100 PP; 86–95 BS). According to Bayesian analyses, this group was sister to *T. marcuzzii* from the Caribbean (85–92 PP), but support for this relationship was low in the ML analyses (< 50–65 BS).

Tylos from the Mediterranean Sea and South Africa formed a distinct group (79–89 BS; 100 PP). In this clade, the Mediterranean species *T. europaeus* and *T. ponticus* were sister lineages (98–100 BS; 100 PP), the two South African species *T. granulatus*

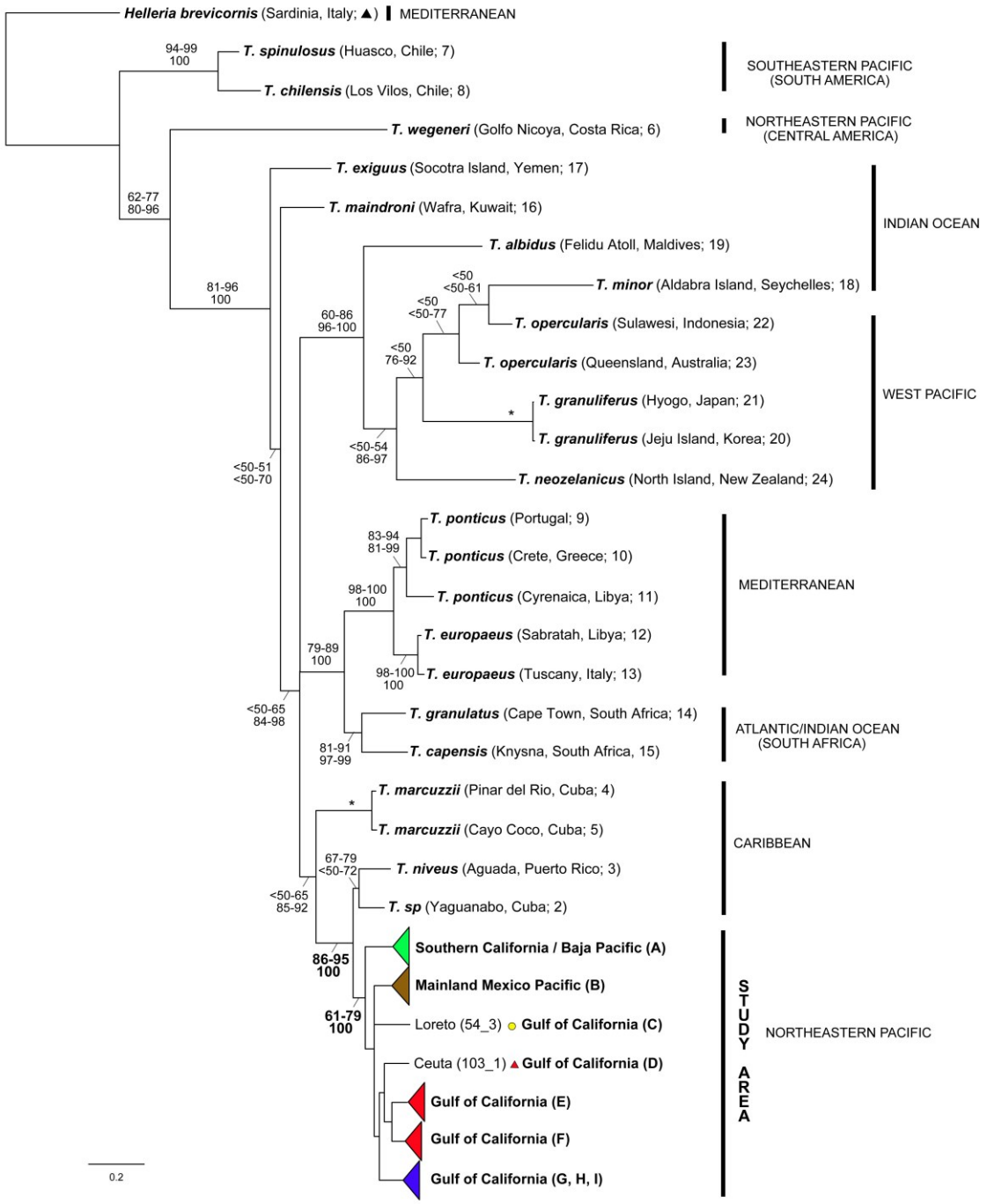

Figure 3. MrBayes majority rule consensus tree of the genus *Tylos* (*Outgroup Identification* analyses). Color and shape correspond to clades in Figs. 2 and 4. Numbers by nodes indicate the corresponding range of Bootstrap Support (BS; top) for Maximum likelihood (RaxML, Garli, PartitionFinder); and Posterior Probabilities (PP; bottom) for Bayesian inference methods (MrBayes, Phycas, BayesPhylogeny), including all partitioning schemes: * denotes nodes that received 100% support for all methods. Nodes receiving less than 50% support for all methods were collapsed and denoted with < 50.

and *T. capensis* were also sister lineages (81–91 BS; 97–99 PP), and the Mediterranean and the South African groups were reciprocally monophyletic.

Tylos from the West Pacific and Indian Ocean, with the exception of *T. maindroni* (Kuwait) and *T. exiguus* (Socotra Island), formed another distinct group (60–86 BS; 96–100 PP). ML methods found very low support for the relationships among members within this clade. Bayesian methods, however, indicated that *T. albidus* (Maldives) diverged first from the other lineages (86–97 PP), followed by *T. neozelanicus* (New Zealand; 76–92 PP). Relationships among the remaining taxa were not resolved.

The monophyly of a clade comprising the three main groups described above (i.e., Study area-Caribbean, Mediterranean-South Africa, and West Pacific-Indian Ocean) was supported by Bayesian analyses (84–98 PP), but received low support from ML analyses (< 50–65 BS). This clade, along with *T. maindroni* (Kuwait) and *T. exiguus* (Socotra Island) formed a well-supported group (81–96 BS; 100 PP), sister to *T. wegeneri* (Pacific Costa Rica; 62–77 BS; 80–96 PP). This group was in turn sister to a well-supported clade (94–99 BS; 100 PP) formed by *T. chilensis* and *T. spinulosus*, both from Chile.

3.2 Phylogenetic relationships of *Tylos* within the study area

The concatenated mitochondrial dataset (MT) of the study area included 50 taxa and 2992 characters, of which 1058 were parsimony informative (Table 2). The nuclear H3A dataset included 34 taxa and 285 characters, of which 20 were parsimony

informative. The nuclear 18S rDNA dataset included 40 taxa and 520 characters, of which 110 were parsimony informative. Finally, the combined mitochondrial and nuclear (MT+NC) dataset included 50 taxa and 3797 characters, of which 1188 were parsimony informative. Figure 4 depicts the inferred phylogenetic relationships based on the MT+NC dataset among the samples of *Tylos* from the study area and two outgroup taxa (i.e., the *Tylos* sp. sample from Yaguanabo, Cuba, and the *T. niveus* sample from Aguada, Puerto Rico). Phylogenetic reconstructions based on the mitochondrial-only and individual nuclear genes are presented in Appendix 5A–C (parameters for analyses in Appendix 6–7). The analyses recognized five main monophyletic lineages (A, B, C, DEF, and GHI; identified by different colors in Fig. 4) that were separated by ~10–19% COI K2P divergence (Table 5). The first main lineage (clade A; green in Figs. 2–4), supported by 100 PP and BS values, includes all Pacific samples between the Baja California Peninsula and southern California. This clade is characterized by very shallow divergences (< 1% COI K2P; Table 5). The second main lineage (clade B; brown in Figs. 2–4), supported by 100 PP and BS values, includes all the samples collected in Mexico between Mazatlán, at the southern mainland limit of the Gulf of California, and Zihuatanejo, in southern Mexico. In this clade, a deep divergence (~10% COI K2P; Table 5) was observed between the sample from Mazatlán and the other localities (which exhibit divergences < 2% COI K2P; Table 5). The third main lineage (lineage C; yellow in Figs. 2–4) was found only in the locality of Loreto, in the central Gulf portion of the Baja California Peninsula. The fourth main lineage (clade

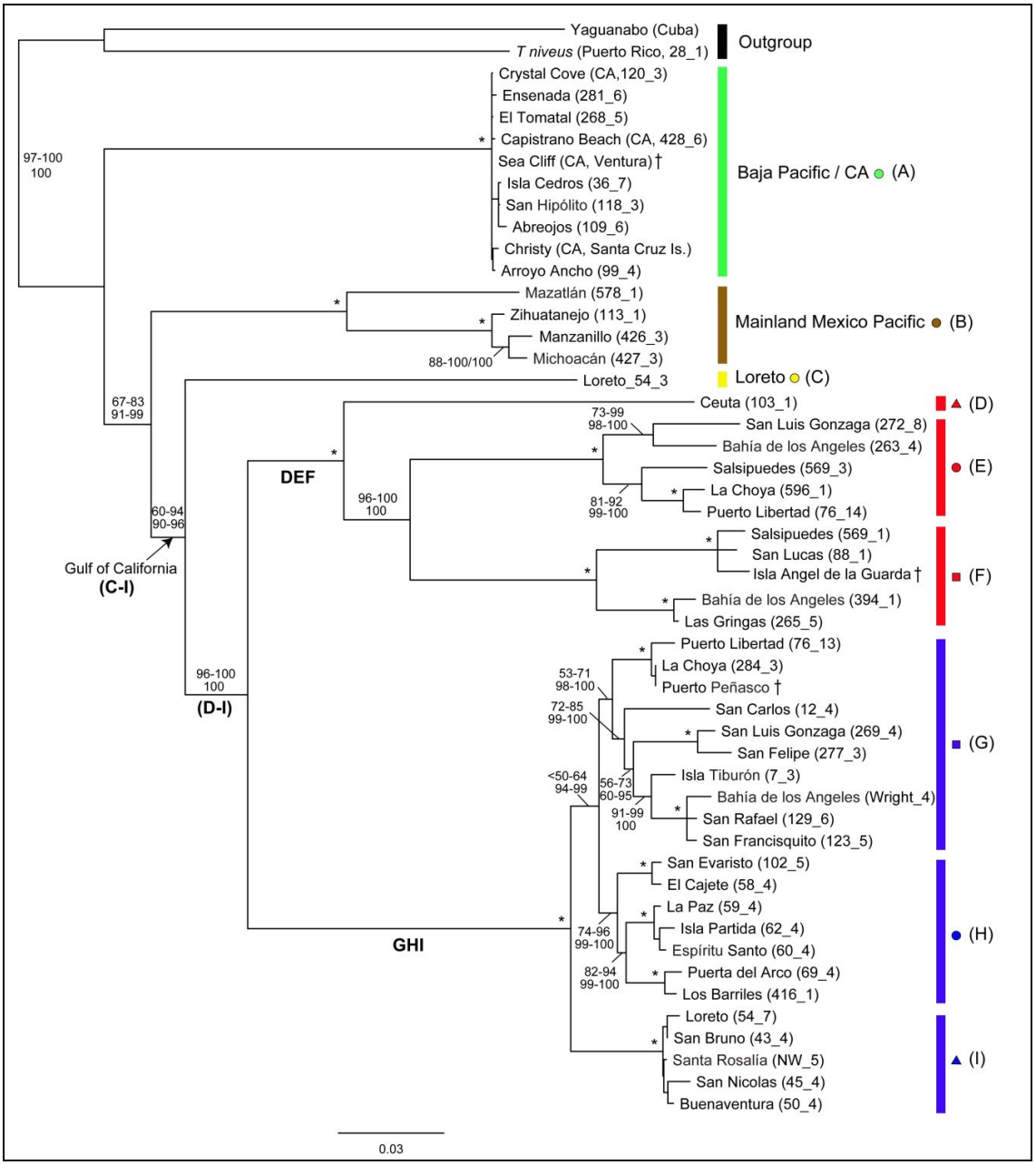

Figure 4. Majority-rule consensus tree (RaxML bootstrap) of the *Study Area* dataset based on concatenated mitochondrial and nuclear datasets (MT+NC). Color and shape correspond to clades in Figs. 2 and 3. Numbers by nodes indicate the corresponding range of Bootstrap Support (BS; top or left) for Maximum likelihood (RaxML, Garli, PartitionFinder); and Posterior Probabilities (PP; bottom or right) for Bayesian inference methods (MrBayes, Phycas, BayesPhylogeny), including all partitioning schemes. * denotes nodes that received 100% support for all methods. Nodes receiving less than 50% support for all methods were collapsed and denoted with < 50. †: relationship based on 16S sequence only: Sea Cliff (CA, Ventura), Isla Angel de la Guarda, and Puerto Peñasco.

Table 5. Ranges of percent Kimura-2-parameter distances among the main *Tylos* clades found in the *Study Area* analyses. Lower matrix; COI gene distances. Upper matrix; the combined mitochondrial gene (16S rDNA+12S rDNA+Cytb+ND4/6) distance. Values on diagonal show maximum within-clade divergence (left: COI gene; right: the combined mitochondrial genes)

	1	2	A	B-I	B-II	C	D	E	F	G	H	I
T. niveus (1)	**na**	18.85	19.15-21.06	19.72	16.29-17.03	21.39	26.65	17.67-22.21	20.37-23.15	16.14-23.20	21.17-21.80	21.66-22.54
Yaguanabo (2)	15.60	**na**	20.65-22.15	22.56	23.12-23.24	23.43	26.55	17.19-24.15	22.16-23.33	16.37-24.98	22.14-23.96	24.15-24.59
A (Pacific-CA)	15.53-15.93	16.62-16.93	**(0.20/0.72)**	18.93-19.52	18.20 19.07-	19.10-19.69	20.98-21.39	15.64-22.07	19.59-22.50	13.86-21.22	18.73-20.45	19.34-20.72
B-I (Mazatlan)	13.64	17.20	12.22-12.47	**na**	9.30-9.89	18.61	22.73	14.88-21.48	20.00-21.57	13.04-19.75	18.82-19.24	19.95-20.44
B-II (Mexico)	15.29-16.63	15.09-15.40	12.22-13.58	8.95-9.07	**(1.30/1.90)**	18.01-18.86	22.70-23.16	14.72-21.58	18.60-20.93	13.51-21.04	18.98-20.65	20.13-20.82
C (Loreto)	16.90	19.43	14.95-15.36	12.50	13.18-14.18	**na**	21.21	15.27-21.70	19.45-20.38	14.17-18.21	17.33-18.68	17.78-17.90
D (Ceuta)	16.06	19.06	11.43-12.15	13.20	12.42-12.93	13.71	**na**	14.46-20.47	18.78-21.64	14.29-22.00	20.01-21.25	20.74-21.11
E (BA-I)	16.95-19.11	15.48-18.69	14.00-16.58	14.86-15.22	13.66-15.22	15.15-17.30	12.07-14.42	**(7.66/5.90)**	8.07-16.99	12.65-19.67	13.92-18.95	14.32-19.22
F (BA-II)	13.45-14.71	16.36-18.18	11.68-15.62	10.22-12.39	13.23-14.57	12.60-14.34	10.69-11.51	11.14-13.34	**(6.86/7.40)**	13.33-20.00	16.84-19.53	18.11-20.14
G (North)	14.72-16.64	16.66-18.94	12.47-14.65	12.52-14.80	11.86-14.31	12.70-14.78	10.93-13.53	12.75-17.02	11.09-14.97	**(6.08/4.86)**	2.80-4.87	2.79-6.67
H (South)	16.74-18.23	16.24-18.23	13.18-15.74	13.79-15.18	12.66-14.48	13.02-15.33	11.94-13.20	13.78-16.93	12.16-16.64	3.56-6.76	**(4.64/2.84)**	5.07-5.60
I (Middle)	16.43-17.05	17.96-18.28	13.45-15.02	12.96-13.56	12.91-13.83	13.77-14.27	13.15-13.47	14.30-16.32	11.98-15.56	4.63-7.05	4.24-5.43	**(0.54/0.90)**

DEF; red in Figs. 2–4), supported by 100 PP and BS values, was divided into three lineages (D, E, and F) that differed from each other by ~11–14.5% COI K2P (Table 5). The fifth main lineage (clade GHI; blue in Fig. 2–4), supported by100 PP and BS values, was also divided into three main clades (G, H, and I) that differed from each other by ~3.5–7% COI K2P (Table 5).

Phylogenetic relationships among the five main lineages suggest that the earliest divergence occurred between clade A (green; Pacific localities between southern California and Baja California) and the remaining lineages B–I (Fig. 4). The monophyly of the clade B–I obtained 91–99 PP and 67–83 BS. Within this clade, the earliest divergence occurred between clade B (brown; Pacific localities between Mazatlán and Zihuatanejo) and the clade containing all of the Gulf lineages (C–I). Support for the monophyly of the Gulf clade (C–I) was 90–96 PP and 60–94 BS. Within the Gulf lineages, the earliest divergence occurred between lineage C (yellow; from Loreto), and the other lineages (D–I). Support for the monophyly of clade D–I was 100 PP and 96–100 BS. Within this group, Clades D–F (red) and G–I (blue) were reciprocally monophyletic with 100% support in all analyses.

Within clade DEF, several distinct and divergent lineages were identified. This clade was divided into three main lineages: two reciprocally monophyletic clades distributed in the northern Gulf (E and F; red circles and squares, respectively) that differed by ~11–13% COI K2P (Table 5); and their sister lineage (D; red triangle), found only at Ceuta in the mainland southern Gulf, and divergent from E and F by ~11–14.5% COI K2P (Table 5). Clade E was divided into two reciprocally monophyletic groups

divergent by ~7.5% COI K2P (not shown): one was found in the northern Gulf Baja localities of San Luis Gonzaga and Bahía de los Angeles; the other was found in the northern Gulf mainland localities of Puerto Libertad and Choya, and the midriff island of Salsipuedes off northern Gulf Baja. Clade F was divided into two reciprocally monophyletic groups divergent by ~7.0% COI K2P (not shown): one was found in Bahía de los Angeles; the other was found in the midriff islands of Angel de la Guarda and Salsipuedes, and in the central Gulf Baja locality of San Lucas.

Many differentiated lineages were observed in clade GHI. This clade was divided into three main lineages distributed allopatrically: clade G (blue squares) distributed in the northern Gulf of California; clade H (blue circles) distributed in the Baja California Cape Region; and clade I (blue triangles) distributed in the central Gulf portion of the Baja California Peninsula. Divergence among clades G, H, and I ranged between ~3.5–7% COI K2P (Table 5). A closer relationship between clades G and H is suggested by Bayesian analysis with 94–99 PP (< 50–64 BS). Divergences within clade G were as high as ~6% COI K2P (Table 5). In this clade, the localities from Puerto Libertad, Puerto Peñasco and Choya formed a differentiated group. Samples from San Luis Gonzaga and San Felipe formed another differentiated group. Bahía de los Angeles, San Francisquito, and San Rafael corresponded to another clade, whose sister lineage was Isla Tiburón. The sample from San Carlos is highly divergent (3.1–6.1 COI K2P; not shown) from the others. Divergences within clade H were as high as ~4.6% COI K2P. In this clade, evidence for a phylogeographic break between El Cajete and La Paz was observed. The localities in Espiritu Santo Island and Isla Partida were closely

related to La Paz. Evidence for another phylogeographic break was observed between La Paz and Puerta del Arco. Within clade I, observed divergences were < 0.6% COI K2P (Table 5).

3.3 Morphology of the ventral plates of the fifth pleonite

Differences in the shapes of the ventral plates of the fifth pleonite were detected among some of the main *Tylos* lineages found in the study area (Appendix 8). Individuals from clade A (Pacific localities between Baja California Peninsula and southern California) can be easily distinguished from those of other clades. Their pleon ventral shape morphology is highly similar to that of *T. punctatus* syntype specimens from San Diego, California, indicating they correspond to this species. The ventral plates of the fifth pleonite of clade A are characterized by a curvilinear upper edge, a narrowly rounded tip, and a distal part that is not much wider than the basal portion. In clade B (Pacific localities between Mazatlán and Zihuatanejo), the ventral plates of the fifth pleonite are wider and square-shaped at the distal part. Clade C (from Loreto) has the largest ventral plates, with a more pronounced square shape. Finally, individuals from clades E to I (we did not have complete body from the only individual collected for lineage D, which was found in Ceuta) can be distinguished from the previous clades, but no obvious differences were found among them. The ventral plates of the fifth pleonite of clades E to I are more raised than in clade B, similar to clade C, but less square and smaller than in clade C.

4. DISCUSSION AND CONCLUSION

4.1 Outgroup identification and mitochondrial phylogeny of the genus *Tylos*

Our outgroup analyses included 17 of the 21 *Tylos* species currently accepted within this genus (Schmalfuss 2003). One limitation of our analyses is that we were able to obtain sequences from only three mitochondrial genes, despite numerous attempts to obtain sequences from additional mitochondrial and nuclear genes. It is possible that DNA was too degraded, as many of the samples were relatively old. Nonetheless, phylogenetic relationships among members of *Tylos* were partially resolved providing important insights about the evolutionary history within this genus, a topic that has not been studied before.

According to our results, *Tylos* samples from the study area have a close relationship with lineages from the Caribbean. This is congruent with paleontological studies in the Gulf of California, which have reported that most fauna-rich sediments found to date in this region show affinities with Caribbean fauna (Escalona-Alcázar et al. 2001). Marine fossils with Caribbean affinities in the Gulf of California date back to Miocene times (Smith 1991). The *Tylos* samples from the study area, Yaguanabo, Cuba, and Aguada, Puerto Rico (the latter identified as *T. niveus*), formed a well-supported monophyletic group. The specimen from Cuba could not be identified based upon morphology because it was severely damaged, but due to its geographic location and genetic affinity with *T. niveus*, it probably represents *T. niveus* or an undescribed related species. The ancestor of the Study area-Yaguanabo-Puerto Rico clade was likely

distributed in the Caribbean, as *T. marcuzzii*, its sister lineage suggested by Bayesian analyses, has a Caribbean distribution. Colonization of the study area thus, appears to have proceeded from the Caribbean. The only other *Tylos* species known from the Caribbean, *T. wegeneri*, was found to be very divergent from the Study area-Yaguanabo-Puerto Rico clade. Our *T. wegeneri* specimen, however, is from the Pacific coast of Costa Rica. Future work should examine Caribbean populations of this species, which may represent a Caribbean/Pacific geminate sister pair.

The other main clades that were found within *Tylos* correspond with geographic regions. One of these included the Mediterranean species (*T. europaeus* and *T. ponticus*; which were sister lineages), and the two species from South Africa (*T. granulatus* and *T. capensis*; which were also sister species), indicating a West Africa vs. Mediterranean Sea split. Considering its geographic location, it seems likely that the species *T. madeirae* from Madeira Island, one of the four species not included in this study, also belongs to this clade. Another main clade included all samples from the West Pacific (*T. opercularis* from Australia and Sulawesi; *T. neozelanicus* from New Zealand; *T. minor* from Seychelles; and *T. granuliferus* from Japan and Korea) and two species from the Indian Ocean (*T. albidus* from the Maldives and *T. minor* from the Seychelles). Given the geographic distribution of this clade, it seems very likely that the three other species that were not included in this study belong to this clade: *T. australis* (from Eastern Australia); *T. nudulus* (from Christmas Island, Java); and *T. tantabiddyi* (from Western Australia). The Indian Ocean species *T. maindroni* (Kuwait) and *T. exiguus* (Socotra Island) appear to represent lineages that diverged a long time ago, and their relationships

with other *Tylos* species were largely unresolved. Lastly, the two species from Chile, *T. chilensis* and *T. spinulosus*, formed a well-supported monophyletic group. Divergence of the Chile lineage represents the most basal split within *Tylos*, suggesting a long history of isolation for *Tylos* in the southern East Pacific. The divergence of *T. wegeneri*, which is also found in the East Pacific region, also represents a very deep separation. Comparatively, the history of *Tylos* in the study area (i.e., East Pacific region from southern California to central Mexico) appears to be much more recent.

Intra-specific genetic divergence values of species for which more than one locality was examined indicate that, as observed in the study area, high levels of cryptic diversity in *Tylos* also occur in other parts of the world. The high genetic divergence observed between the samples from Yaguanabo and Puerto Rico (12.5% K2P at 16SrDNA + 12S rDNA + Cytb; Table 6) suggest that further cryptic diversity may be found at other Caribbean locations in what appears to be a cryptic complex of *T. niveus*. The two samples of *T. opercularis* (from Sulawesi and Australia) were highly divergent (14.4% K2P; Supplement 1), possibly representing different species. Large intra-specific genetic divergences were also observed in *T. ponticus* from Libya vs. Greece and Portugal (11.3–12.9% K2P; Table 6); whereas divergence between *T. europaeus* from Libya and Italy was 2.7% K2P (Table 6). Divergence between the two *T. marcuzzii* localities collected within Cuba is 1.5% K2P (Table 6). Low genetic divergence was observed between *T. granuliferus* from Japan and Korea (0.5% K2P; Supplement 1).

Table 6. Averages and ranges of percent Kimura-2-parameter distances (16S rDNA, 12S rDNA and Cytb concatenated dataset) among and within *Tylos* nominal species and selected clades examined. Lower matrix: average distance. Upper matrix: distance range. Values on diagonal show minimum and maximum within-clade divergence. Letters and numbers correspond to the ones in Figures 1, 3, and 4. Empty cells: no ranges available because selected lineage was represented by a single sample (See Supplement 1 for more details).

	A	B	C	DEF	GHI	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	**(0.0-1.1)**	12.9-13.7	14.2-14.8	11.4-16.3	12.2-15.0	12.1-12.7	13.8-14.2	21.7-22.8	14.8-16.3	40.9-42.1	33.4-34.0	33.0-33.5	23.7-24.9	23.0-24.2	24.6-25.8	23.1-23.4	22.9-23.1	20.5-21.4	25.0-25.7
B	12.9	**(0.9-8.0)**	13.1-14.7	12.1-16.4	12.7-17.4	14.2-14.6	12.6-15.0	19.9-22.6	15.6-16.0	38.2-40.4	31.6-34.1	34.0-36.3	26.1-27.5	24.4-26.0	25.2-25.5	25.1-26.9	25.7-26.8	21.1-24.2	27.3-28.5
C	14.0	13.7	**na**	12.6-15.4	13.3-15.0	16.4	16.2	23.3	17.4	41.8	35.4	35.4	25.1	24.9	27.2	25.8	25.3	23.2	28.1
DEF	13.4	13.8	13.1	**(0.5-16.0)**	11.3-16.0	13.7-17.1	15.1-18.3	20.2-25.2	12.7-18.7	37.5-42.6	30.1-35.2	34.1-36.0	26.0-29.7	23.9-28.6	25.2-27.7	24.6-27.2	25.3-27.5	23.4-26.9	26.5-29.4
GHI	12.3	14.3	13.8	12.3	**(0.2-5.6)**	13.9-16.1	14.0-16.0	22.7-25.5	12.0-18.0	34.2-39.5	30.9-36.5	31.6-36.2-	24.9-26.6	23.0-25.8	26.8-29.0	24.9-26.8	25.2-27.3	21.7-24.2	26.8-29.3
T. sp (2, Yaguanabo)	12.0	14.4	16.4	14.8	14.7	**na**													
T. niveus (3)	13.3	13.3	16.2	16.3	14.8	12.5	**na**												
T. marcuzzii (4, Pinar de Rio)	21.1	21.0	23.3	22.8	23.3	19.5	20.8	**na**											
T. marcuzzii (5, Cayo Coco)	14.8	15.7	17.4	17.0	15.8	13.1	12.7	1.5	**na**										
T. wegeneri (6)	40.5	39.7	41.8	39.2	37.3	39.8	42.7	44.4	45.9	**na**									
T. spinulosus (7)	32.6	32.5	35.4	32.7	33.9	37.0	37.1	38.5	36.8	37.7	**na**								
T. chilensis (8)	32.0	34.9	35.4	34.5	34.5	36.8	37.2	38.3	39.8	42.1	14.0	**na**							
T. ponticus (9, Portugal)	23.1	26.8	25.1	26.5	25.4	27.5	24.8	26.3	19.7	45.8	39.2	38.4	**na**						
T. ponticus (10, Greece)	22.5	25.1	24.9	25.0	24.6	26.3	25.0	26.6	21.8	44.8	38.6	38.1	3.4	**na**					
T. ponticus (11, Libya)	23.8	25.4	27.2	25.1	27.2	28.1	27.1	28.2	19.7	44.5	38.1	38.7	12.9	11.3	**na**				
T. europaeus (12, Libya)	22.8	26.2	25.8	25.1	25.5	24.8	25.5	24.0	17.8	45.2	36.9	36.9	13.6	14.6	15.9	**na**			
T. europaeus (13, Italy)	22.5	26.2	25.3	25.6	26.0	25.2	26.7	24.9	17.4	44.3	36.3	35.8	13.6	14.2	14.1	2.7	**na**		
T. granulatus (14)	20.3	22.0	23.2	24.7	22.8	22.9	22.0	24.4	17.9	41.3	38.6	38.6	23.7	22.7	24.7	20.8	21.0	**na**	
T. capensis (15)	24.4	27.9	28.1	27.0	27.5	25.2	25.1	24.1	18.1	45.9	36.8	38.5	23.3	24.7	24.3	19.7	20.8	17.9	**na**

4.2 Phylogeographic patterns of *Tylos* in the study area

4.2.1 Southern California-Baja Pacific clade

The shallow divergences of Clade A (< 1% COI K2P; Table 5), which included all Pacific coast samples from the Baja California Peninsula to southern California, may be indicative of a drastic past bottleneck followed by a recent range expansion. The distribution of *Tylos* is likely influenced by a limited thermal tolerance to low temperatures, as these isopods are found worldwide only in tropical and subtropical coasts. In the East Pacific, their northern limit is in the region of Santa Barbara and Los Angeles, California (Hamner et al. 1969; Hayes 1970). This region is characterized by a transition between northern-cold and southern-warm water masses around what is known as the Point Conception biogeographic boundary (Newman 1979). The cyclical pattern of rising and falling global temperatures and transgressing and regressing seas that occurred during the Quaternary (Graham et al. 2003), likely provided numerous opportunities for range expansions/contractions of Clade A. Sea Surface Temperature (SST) in the Southern California Bight during the last glacial maximum (~18,500 ya) was 6–10°C lower than at present (Mortyn et al. 1996); thus, the northern limit of Clade A at that time was probably located farther south in the Baja California Peninsula. Evidence for post-glacial range expansions might therefore be expected in other lineages of *Tylos* whose distribution limit occurs at the subtropical-temperate boundary.

With each glacial-interglacial cycle, the Southern California coast also experienced continuous fluctuations in the distribution and size of sandy and rocky shores, which likely affected also the distribution of the sandy-beach organisms such as

Tylos. During most of the late Pleistocene and early Holocene, the coast of the Southern California Bight is suggested to have been composed of extensive cold, rocky, cobbled shores, interrupted by estuarine embayments (Graham et al. 2003). The minimum sea level of this region during the last glacial maximum was ~117 m below present (Graham et al. 2003). The present-day sand-dominated coastlines of the Southern California Bight appear to have developed only over the past 4000–6000 years (Inman 1983), which may have facilitated the recent expansion of *Tylos* in southern California.

One of the Clade A populations, Christy Beach, was sampled on the western portion of Santa Cruz Island, in the northern Channel Islands. The small divergence between this locality and the Clade A mainland localities suggest that colonization was recent. It is possible that colonization of this locality occurred over land, when the four present-day Northern Channel Islands presumably formed a large contiguous land mass, ~17,000 years ago, whose eastern end may have been connected to the mainland (Schoenherr et al. 1999). Rafting and drifting have also been suggested as a potential mechanism for long distance dispersal in *Tylos* (Hayes 1977; Brown and Odendaal 1994). Nevertheless, this may be a rather ineffective mechanism for dispersal as *Tylos* isopods drown if submerged even for short periods (Brown and Odendaal 1994). SST in the western portion of Santa Cruz Island is low compared to the California mainland portion south of Los Angeles, suggesting that the population of this island may have adapted to colder conditions. Remarkably, no other populations of *Tylos* were found on the Channel Islands despite substantial searching effort.

4.2.2 Gulf of California-Central Pacific Mexico clades

In contrast to the shallow pattern observed in the Pacific region spanning southern California to the Baja California Peninsula, the phylogeographic patterns of *Tylos* in the region encompassing the Gulf of California and central Pacific Mexico are deeper and more complex. Many highly divergent lineages are observed, indicating long-standing isolation of numerous populations. Deep phylogeographic breaks are probably related to vicariant events that occurred during the formation of the Gulf of California and the Baja California Peninsula. Unfortunately, key aspects of the geological history of this region remain controversial, limiting our ability to interpret phylogeographic patterns. Two main stages during the evolution of the Gulf of California are recognized (Carreño and Helenes 2002; Ledesma-Vásquez and Carreño 2010). The first stage involves the presence of a northern proto-Gulf basin, which occupied the northern portion of today's Gulf and an extensive area to the north (Karig and Jensky 1972), and is estimated to have existed at least 11.61 Ma (Helenes et al. 2009). A Late Miocene seaway that connected the Pacific with the proto-Gulf basin through the central part of Baja has been proposed (Helenes and Carreño 1999; Holt et al. 2000; Ledesma-Vásquez and Carreño 2010). In the second stage, the Gulf of California-Baja Peninsula region attained its present form (Carreño and Helenes 2002). A formation of a southern basin 5.5–3.5 Ma is suggested (Ledesma-Vásquez 2002), which then joined the northern proto-Gulf to form the present-day Gulf (Johnson and Ledesma-Vásquez 2009). The Cape region is suggested to have been the last portion of the peninsula to separate from mainland, when the modern mouth of the Gulf formed

(Larson et al. 1968; Ledesma-Vásquez and Johnson 2009). Accordingly, some extant Gulf taxa may have colonized and remained in the Gulf since northern proto-Gulf times (Hurtado et al. 2010). Some geologists, however, consider that marine incursions in the southern portion of the Gulf of California occurred earlier (~8 Ma) than in the northern portion (~6.5 Ma) (Oskin and Stock 2003).

The deep divergences observed within clade C–I (up to 17.3% K2P COI; Table 5) suggest that colonization of the Gulf by *Tylos* may have occurred as early as proto-Gulf times. It is possible that the ancestor of Clade B also colonized during these early stages, and that the sister group to Mazatlán (e.g. Manzanillo to Zihuatanejo) colonized the area south of the Gulf more recently; e.g. once the Gulf acquired its present-day configuration. Unfortunately, obtaining reliable divergence date estimates is not feasible because well-established calibration points (e.g. fossils or vicariant events) are not available, and the substitution rates of *Tylos* in the study area are unknown.

Lineage C is highly divergent and was found only at a small beach ~14 Km south of Loreto. The limited distribution of this lineage suggests that either it has not dispersed from this restricted area or it has gone extinct in other areas. The age of the Loreto Basin appears too young to explain the divergence of Clade C by isolation in this basin. The oldest marine incursions in the Loreto basin are estimated at ~2.4–2.0 Ma (Dorsey et al. 1997) or less than 3.3 Ma (Ledesma-Vásquez and Carreño 2010). Divergence in older basins and subsequent movement to the Loreto basin appears a more likely explanation. Marine deposits 6.0–5.3 Ma old are found in Carmen and Monserrat

islands in the proximity of Loreto Bay, whereas the Santa Rosalía basin, north of Loreto in the central Peninsula, has an age of ~10–8 Ma (Ochoa-Landín et al. 2000).

Lineage D is another highly divergent lineage that appears to have a very restricted distribution, as it was only found in Ceuta, north of Mazatlán. The region between San Carlos-Guaymas and Ceuta has been poorly explored, thus, further examination of this region is needed to assess whether lineage D has a broader distribution.

The deep divergence between clades E and F suggests a long presence of *Tylos* in the northern Gulf of California, which probably occurred during proto-Gulf times. Interestingly, both clades exhibit splits involving a lineage containing Bahía de los Angeles and a lineage containing the midriff island of Salsipuedes (and Angel de la Guarda in the case of Clade F). These independent splits share similar divergences (5.8–7.7% K2P COI for Clade E and 6.2–6.9% for Clade F), which is consistent with a common vicariant or dispersal event. Separation of midriff islands in the proximity of Bahía de los Angeles (i.e., Angel de la Guarda, Salsipuedes, and San Lorenzo) could have provided opportunities for such an event. The island of Angel de la Guarda is suggested to have separated from the Puertecitos area (~190 km NW of Bahía de los Angeles) ~3–2 Ma and migrated southeast to its current position (Stock 2000). The San Lorenzo Archipelago was located across from Bahía Las Animas and Sierra Las Animas, just south of Bahía de los Angeles, during Pliocene time, before its southeastward migration (Escalona-Alcázar et al. 2001). The basin located between the eastern Sierra Las Animas and the San Lorenzo Archipelago is suggested to have formed during the

late Miocene–early Pliocene, ~8–4 Ma (Escalona-Alcázar et al. 2001). Dispersal however, may have occurred between populations of Angel de la Guarda and Salsipuedes, and between these and populations at non-insular localities with which they are closely related. For example, the close relationship between Angel de la Guarda and Salsipuedes with San Lucas appears to be the result of dispersal.

The shallower divergences of clade GHI compared to clade DEF suggest that clade GHI has a more recent history, possibly after proto-Gulf times (i.e., once the present-day peninsula was completely formed). Clade GHI shows, however, a higher number of lineages and broader distribution. The three main clusters in Clade GHI are distributed according to geography, and groupings within each of these clusters also correspond with geography. Among the three clusters, clade G, which is distributed in the upper half of the Gulf, has the highest diversity of lineages and broadest distribution. Clade I has a more limited distribution in the central part of the peninsula, in the region between Loreto and Santa Rosalía. This region includes a series of basins that have separated at different times and may have contributed to the isolation and differentiation of *Tylos* lineages. These include the basins of Santa Rosalía, Bahía Concepción, San Nicolás, and Loreto (Dorsey et al. 1997; Meldahl et al. 1997; Ochoa-Landín et al. 2000; Ledesma-Vásquez and Johnson 2009). Divergences within Clade I are < 1%, however, suggesting a recent expansion. Clade H is found in the southern Peninsula. Within this clade a basal phylogeographic break is observed between a lineage containing the localities of El Cajete-San Evaristo and a lineage containing the Cape region localities. A phylogeographic break in this specific area has also been observed in another upper

intertidal isopod and in a lizard (Lindell et al. 2005; Hurtado et al. 2010). La Paz and the lineages in Isla Espiritu Santo and Isla Partida share some COI haplotypes, which is congruent with their common recent history (Carreño and Helenes 2002).

4.2.3 Comparison of *Tylos* and *Ligia* phylogeographic patterns

Phylogeographic patterns of the supralittoral rocky intertidal isopod *Ligia* in the study area have been reported (Hurtado et al. 2010). Comparison of these patterns with the ones observed in *Tylos* is interesting because these two non-vagile isopods share similar biological characteristics and are restricted to the upper intertidal. Furthermore, the majority of the beaches where *Tylos* was collected for the present study were also included in the *Ligia* study. *Ligia* is restricted to the dry and splash portion of the rocky intertidal, and movements of this isopod outside rocky beaches are highly restricted. Therefore, *Ligia* isopods appear to be highly constrained throughout their life cycle to the same rocky beach (Hurtado et al. 2010). Consistent with this, long-standing isolation and high levels of cryptic allopatric differentiation were detected for *Ligia* populations in the study area (Hurtado et al. 2010).

Remarkably, despite their biological similarities and high levels of allopatric genetic differentiation, *Ligia* and *Tylos* in the study area show very distinct phylogeographic patterns. Many differences are observed in the Pacific region spanning southern California to the Baja California Peninsula. In this region, *Ligia* shows many highly divergent lineages, whereas *Tylos* shows very shallow divergences (< 1%) that suggest a recent expansion of the latter into this region. A drastic decrease in genetic

divergences is observed in *Ligia* populations, however, north of Point Conception, suggesting a recent post-glacial expansion for this isopod north of this biogeographic boundary (Eberl et al., unpublished). Differences between *Ligia* and *Tylos* in their tolerance to low SST may explain the distinct latitudes at which each of these isopods exhibits signatures of post-glacial expansions. The tolerance of *Ligia* to lower SST may also explain its higher abundance in the Northern Channel Islands, where it occurs in many localities on all islands, whereas *Tylos* was found at a single locality. In the Baja Peninsula, a deep phylogeographic break coincident with the Guerrero Negro Lagoon was observed in *Ligia*, indicating long-standing separation between populations at both sides of the lagoon, which probably resulted from a lack of rocky habitat. The presence of this lagoon, however, does not appear to have impeded the recent expansion of *Tylos* in this region, where the sandy beaches of the lagoon may have facilitated its dispersal.

Within the Gulf of California, *Ligia* shows two reciprocally monophyletic clades that are highly divergent (15–26% COI). One is distributed in the northern Gulf (Gulf North clade) and the other in the southern Gulf and Central Pacific Mexico (Gulf South clade). Lineages in the Gulf's southern Peninsula are, thus, most closely related to mainland lineages between the central Gulf and central Pacific Mexico. In addition, lineages in the southernmost portion of the peninsula (Cape Region) are most closely related to the southernmost portion of mainland. These phylogeographic patterns were interpreted to be congruent with expectations from several geological hypotheses on the formation of the Gulf of California. Hurtado et al. (2010) suggest that the divergence between the two main Gulf clades probably occurred during the Miocene and that the

Gulf North clade represents a lineage that colonized the northern proto-Gulf. They also indicate that a closer relationship between Cape region and southern mainland lineages is congruent with the hypothesis that the Cape region was the last portion of the peninsula to separate from mainland. Such patterns were not observed in *Tylos*. The *Tylos* lineages found in the southern portion of the peninsula (Clades H and I) are not more closely related to any lineage in the southern mainland. Nonetheless, Clade EF in *Tylos* might represent a proto-Gulf colonization, as suggested for the Gulf North clade in *Ligia*. Another interesting contrast between *Tylos* and *Ligia* is that whereas sympatry of distinct lineages of *Tylos* occurs at several localities and regions in the northern half of the Gulf (see Fig. 2), essentially all distinct lineages of *Ligia* appear to be allopatric.

4.3 Cryptic biodiversity and taxonomic implications for *Tylos* in the study area

The generally high levels of allopatric genetic differentiation observed among *Tylos* from the study area are consistent with expectations stemming from the limited vagility of this isopod and the fragmented nature of its habitat. The high divergences observed among many of the lineages suggest that *Tylos* in the study area corresponds to a complex of cryptic species. This result contrasts with the previous recognition of only two species, *T. latreillii* and *T. punctatus*, in the study area (Mulaik 1960; Hamner et al. 1969; Hayes 1970, 1977; Schmalfuss 2003).

Tylos latreillii is the type species of the genus and is presently a *nomen dubium* (Taiti and Ferrara 1996). This species was originally described in the Red Sea Egyptian coast (Audouin 1826), and based on its type locality, it may correspond either to *T.*

europaeus or *T. ponticus*. The identification of many specimens around the world as *T. latreillii* has contributed to taxonomic confusion. Species previously assigned to *T. latreillii* are currently treated as different valid species [i.e., *T. ponticus, T. europaeus, T. punctatus*, *T. exiguus*, *T. niveus*, *T. marcuzzii*, and *T. madeirae*; (Schmalfuss 2003)].

We consider that the species *T. punctatus* corresponds to the lineage identified as Clade A. The type locality of this species is San Diego, southern California (Holmes and Gay 1909), in the distribution range of Clade A. Furthermore, the morphology of the ventral plates of the fifth pleonite between the *T. punctatus* syntype (USNM 89583) and specimens of the Clade A is highly similar, and differentiated from the other lineages found in the study area. Given the extremely reduced genetic divergences within Clade A (i.e., < 1%), it is reasonable to consider all members of this clade as *T. punctatus*. This species has also been reported in the Gulf of California and the Galápagos Islands (Hamner et al. 1969; Hayes 1970, 1977; Schmalfuss 2003). The lineages within the Gulf of California, however, are very divergent and appear to correspond to different species. The morphology of the ventral plates of the fifth pleonite of the Galápagos samples (Van Name 1924) is very different to that of Clade A members, the *T. punctatus* syntype, and the other lineages from the study area. The Galápagos samples have been suggested to represent a different species based on morphology [i.e., *T. insularis*; (Van Name 1936)]; we expect genetic characterization to confirm this.

Morphology of the ventral plates of the fifth pleonite is diagnostic for several, but not all of the divergent lineages identified in the study area. Clades A, B, and C are

distinct from each other and from clades E–I (clade D was not examined). Nevertheless, clades E–I share similar morphology, despite high divergences. Thus, this character fails to consistently distinguish what appear to be cryptic species. Metazoan barcode studies using the same COI fragment examined in our study have found that intra-specific divergences are typically < 3% (Hebert et al. 2003). Accordingly, clades A, B, C, D, E, F, and GHI, are highly differentiated (~11–17%), and probably correspond to different species. Additionally, within some of these clades other cryptic species may be present. In Clade B, the sample from Mazatlán may correspond to one species, whereas the samples from Zihuatanejo, Manzanillo, and Michoacán to another (divergence of Mazatlán vs. the others is 9.3–9.9%; whereas divergence among the others is < 2%). Deep divergences within clades E and F are also observed, which may also correspond to distinct species. Divergences among clades G, H, and I, are 3.5–7%, potentially representing also different species. Within clades E, F, G, and H, lineages with divergences > 3% occur, which may also correspond to distinct species. It is possible that further cryptic *Tylos* biodiversity remains to be discovered, especially within the Gulf of California. The coastline between San Carlos-Guaymas and Ceuta, which is dominated by sandy beaches, and was largely unexplored, may have additional *Tylos* populations.

4.4 Conclusion

The phylogeographic patterns of *Tylos* revealed high levels of cryptic biodiversity for this isopod in the northern East Pacific region between southern

California and central Mexico, including the Gulf of California. This is consistent with expectations from the biological characteristics of this isopod. The deep divergences observed among several lineages suggest a long-standing isolation. Within the Gulf of California, divergence among certain *Tylos* lineages is so high that they appear to have colonized this basin during proto-Gulf times. Remarkably, the phylogeographic patterns of *Tylos* in the study area are very distinct from those of the coastal isopod *Ligia*, despite their biological similarities and high levels of allopatric genetic differentiation. Morphology of the ventral plates of the fifth pleonite, a character that is used to distinguish *Tylos* species, is diagnostic for several, but not all of the divergent lineages identified in the study area. Thus, this character fails to consistently distinguish what appear to be cryptic species. Finally, the inferred mitochondrial phylogeny of 17 of the 21 currently recognized *Tylos* species, suggested very early divergences for some members of the genus and identified clades that group, in general, according to geography.

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APPENDIX 1

Information on *Tylos* localities used in the *Study Area* analyses (i.e., between Central Mexico to Southern California, including the Gulf of California). Unless otherwise noted, all samples collected by Dr. Luis A. Hurtado (Texas A&M University). The COI and 16S columns correspond to the number of individuals sequenced for each of these genes.

NO	COI	16S	Localities			Latitude	Longitude	Date
1	4	4	Abreojos (109)	Baja California Sur	Mexico	26°44'1.3N	113°32'58.1W	07/18/2009
2	3	2	Arroyo Ancho (99)	Baja California	Mexico	29°54'44.88N	115°42'40.50W	08/19/2009
3	1	1	Bahía de los Angeles (394)	Baja California	Mexico	28°56'19.7N	113° 33'7.3W	08/16/2009
4	2	4	Bahía de los Angeles South	Baja California	Mexico	28°50'37.25N	113°28'23.37W	06/19/2009
5	4	3	Bahía de los Angeles (263)	Baja California	Mexico	28°54'19.62N	113°32'2.40W	07/19/2009
6	2	4	Buenaventura (50)	Baja California Sur	Mexico	26°38'38.88N	111°50'46.68W	11/19/2008
7	4	5	Capistrano Beach	California	USA	33°29'19.4N	117°40'13.6W	08/28/2009
8	1	1	Ceuta (103)	Sinaloa	Mexico	23°53'39.5N	106°56'52.2W	08/25/2009
9	3	3	Choya (284)	Baja California	Mexico	31°21'42.8N	113°38'23.5W	07/22/2009
10	1	1	Choya (596)	Baja California	Mexico	31°21'42.8N	113°38'23.5W	08/27/2010
11	2	2	Christy: Santa Cruz	California	USA	34°2'36.8N	119°51'34.1W	03/20/2008
12	3	3	Ensenada (281)	Baja California	Mexico	31°47'23.40N	116°36'57.36W	07/22/2009
13	3	3	Crystal Cove Beach	California	USA	33°34'15.4N	117°50'14.9W	06/07/2010
14	2	2	El Cajete (58)	Baja California Sur	Mexico	24°15'21.06N	110°36'41.82W	11/21/2008
15	2	2	El Tomatal (268)	Baja California Sur	Mexico	28°29'12.5N	114°4'5W	11/10/2009
16	2	2	Isla Cedros (36)	Baja California	Mexico	28°7'8.22N	115°20'34.80W	11/14/2008
17	2	4	Isla Espíritu Santo (60)	Baja California Sur	Mexico	24°24'13.08N	110°20'53.28W	11/23/2008
18	4	5	Isla Partida (62)	Baja California Sur	Mexico	24°31'56.58N	110°23'0.00W	11/23/2008
19	3	3	Isla Tiburón (7)	Sonora	Mexico	28°52'57.42N	112°34'3.90W	10/29/2008
20	4	4	La Paz (59)	Baja California Sur	Mexico	24°13'45.60N	110°18'33.54W	11/22/2008
21	1	4	Las Gringas (265)	Baja California	Mexico	29°1'20.82N	113°33'38.94W	70/20/2009
22	4	3	Loreto (54)	Baja California Sur	Mexico	25°52'43.50N	111°20'31.56W	11/20/2008

NO	COI	16S	Localities			Latitude	Longitude	Date
23	2	2	Los Barriles (416)	Baja California Sur	Mexico	23°44'4.14N	109°42'46.92W	11/26/2008
24	2	4	Mazatlán (578/579)	Sinaloa	Mexico	23°13'54.8N	106°24'26.6W	08/28/2009
25	1	3	Michoacán (427)	Michoacán	Mexico	18°34'31.9N	103°39'53.1W	08/28/2009
26	2	3	Manzanillo (426)	Colima	Mexico	19°6'19.68N	104°21'6.24W	08/28/2009
27	4	4	Puerta del Arco (69)	Baja California Sur	Mexico	23°53'43.7N	109°48'9.9W	11/29/2008
28	3	3	Puerto Libertad (76)	Sonora	Mexico	29°54'9.61N	112°43'36.31W	12/02/2008
29	2	3	Salsipuedes (569)	Baja California	Mexico	28°43'29.7N	112°57'8.9W	08/19/2010
30	3	6	San Bruno (43/104/405)	Baja California Sur	Mexico	27°9'46.26N	112°9'34.14W	11/16/2008
31	4	4	San Carlos (12)	Sonora	Mexico	27°56'21.42N	111°5'19.26W	11/02/2008
32	2	2	San Evaristo (102)	Baja California Sur	Mexico	24°21'59.8N	110°40'48.7W	08/22/2009
33	3	3	San Felipe (277)	Baja California	Mexico	31°1'31.97N	114°49'52.01W	07/21/2009
34	3	3	San Hipólito (118)	Baja California Sur	Mexico	26°59'26.76N	113°58'38.94W	07/18/2009
35	4	4	San Lucas (88/44)	Baja California Sur	Mexico	27°13'31.6N	112°12'32.9W	07/17/2009
36	2	3	San Luis Gonzaga (269)	Baja California	Mexico	29°47'43.32N	114°23'47.16W	07/20/2009
37	3	2	San Luis Gonzaga (272)	Baja California Sur	Mexico	29°47'43.32N	114°23'47.16W	07/20/2009
38	4	4	San Nicolás (45)	Baja California Sur	Mexico	26°32'37.02N	111°32'24.08W	11/17/2008
39	3	4	San Rafael (129)	Baja California	Mexico	28°34'18.78N	113°7'12.66W	07/19/2009
40	1	3	San Francisquito (123)	Baja California	Mexico	28°24'25.3N	112°51'31.8W	07/19/2009
41	3	3	Santa Rosalía (NW)	Baja California Sur	Mexico	27°21'40.62N	112°16'47.82W	11/16/2008
42	2	2	Zihuatanejo (113)	Guerrero	Mexico	17°37'29.22N	101°32'44.40W	08/29/2009
43[1]	-	1	Sea Cliff	California	USA			06/00/1935
44[2]	-	1	Puerto Peñasco	Sonora	Mexico			04/17/1965
45[3]	-	1	Isla Angel de la Guarda	Baja California	Mexico			

[1] Museum specimen: Smithsonian Institution National Museum of Natural History (specimen # USNM 236471)
[2] Museum specimen: Smithsonian Institution National Museum of Natural History (specimen # USNM 112670)
[3] Museum specimen: Natural History Museum of Los Angeles County (specimen # LACM 71-540.2)

APPENDIX 2

Information on *Tylos* specimens from outside the *Study Area* used for the outgroup analyses.

No	Source	Locality	Country	ID	Species	Year
1	LACM[1]	Golfo Nicoya	Costa Rica	#08	*T. wegeneri*	1985
2	Dr. Hurtado	Aguada	Puerto Rico	28	*T. niveus*	2011
3	Liz Carrera	Yaguanabo, Cienfuegos	Cuba	CU	*T. sp*	2010
4	Dr.Taiti	Ciego de Avila, Cayo Coco	Cuba	Tmar1	*T. marcuzzii*	2002
5	Dr.Taiti	Maria La Gorda, Pinar del Rio	Cuba	Tmar2	*T. marcuzzii*	2002
6	Dr.Taiti	Atacama	Chile	Ts1	*T. spinulosus*	1980
7	Dr.Taiti	Los Vilos, Punta Tablas	Chile	Tch1	*T. chilensis*	1980
8	Dr.Taiti	Cape Town, Rondeberg	South Africa	Tg1	*T. granulatus*	1980
9	Dr.Taiti	Knysna	South Africa	Tc1	*T. capensis*	1980
10	Dr.Taiti	Sabratah	Libya	Te1	*T. europaeus*	2005
11	Dr.Taiti	Wadi El Hamasa, Cyrenaica	Libya	Te2	*T. europaeus*	2005
12	Dr.Taiti	Susah, Cyrenaica	Libya	Tp1	*T. ponticus*	2008
13	Dr.Taiti	Qalansiyah, Socotra Island	Yemen	Tex1	*T. exiguus*	2000
14	Dr.Taiti	Wafra	Kuwait	Tma1	*T. maindroni*	1988
15	Dr.Taiti	Aldabra Island, Grande Terre	Seychelles	Tm1	*T. minor*	1975
16	Dr.Taiti	Piha Beach, North Island	New Zealand	Tnz1	*T. neozelanicus*	2004
17	Dr.Taiti	Palu, Sulawesi	Indonesia	To1	*T. opercularis*	1980
18	Dr.Taiti	Cape Tribulation, Queensland	Australia	To2	*T. opercularis*	2004
19	Dr.Taiti	Felidu Atoll	Maldives Island	Ta1	*T. albidus*	1994

No	Source	Localities	Country	ID	Species	Year
20	Dr.Taiti	Burano, Tuscany	Italy	24	*T. europaeus*	
21	Dr.Taiti	Burano, Tuscany	Italy	25	*T. europaeus*	
22	Dr.Taiti	Preveli Beach, Crete	Greece	26	*T. ponticus*	
23	Dr.Taiti	Preveli Beach, Crete	Greece	27	*T. ponticus*	
24	Dr. Wright		Portugal	428-1	*T. ponticus*	2009
25	Dr.Kwon	AnDeok, Jeju island	South Korea	CY	*T. granuliferus*	1993
26	Dr.Kwon	YoungIl, Gyeongsang-do	South Korea	KY	*T. granuliferus*	1992
27	Miyuki Niikura	Matsue, Shimane	Japan	SHI	*T. granuliferus*	2011
28	Miyuki Niikura	Toyooka, Hyogo	Japan	HYO	*T. granuliferus*	2011
29	Dr. Griffiths	Cape Town	South Africa	Gr	*T. granulatus*	1980
30	Dr. Taiti	Burcei, Sardinia	Italy	Hb	*H. brevicornis*	2011

[1] Natural History Museum of Los Angeles County

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Dr. Charles Griffiths (University of Cape Town, South Africa)

APPENDIX 3

PCR primers information and annealing temperature (Tm).

Gene	Name	Primer sequences	Tm	References
16S	16s SAR	5’-CGCCTGTTTATCAAAAACAT-3’	48-49	Palumbi (1996)
	16s SBR	5’-CCGGTCTGAACTCAGATCACGT-3’		
	16S Tyhe-F	5'-ATATTGACTGTGCTAAGGTAGC-3'		Gentile et al. (2010)
	16S Tyhe-R	5'-CTTAATCCAACATCGAGGTC-3'		
	16s TA	5’-CTG TGC TAA GGT AGC RTA AT-3’		newly designed
	16s TB	5’-TTA ARR GTC GAA CAG AC-3’		
12S	12SCRF	5’-GAGAGTGACGGGCGATATGT-3’	48-49	Wetzer (2001)
	12SCRR	5’-AAACCAGGATTAGATACCCTATTAT-3’		
	Crust-12f	5’-CAGCAKYCGCGGTTAKAC-3’		Podsiadlowski and Bartolomaeus (2005)
	Crust-12r	5’-ACACCTACTWTGTTACGACTTATCTC-3’		
Cytb	Cytb151F	5’-TGTGGRGCNACYGTWATYACTAA-3’	48-49	Merrit et al. (1998)
	Cytb144F	5’-TGAGSNCARATGTCNTWYTG-3’		
	Cytb270R	5’-AANAGGAARTAYCAYTCNGGYTG-3’		
	Cytb272R	5’-GCRAANAGRAARTACCAYTC-3’		
16S2-ND4	N4	5’-GGAGCTTCAACATGAGCTTT-3’	50	Roehrdanz et al. (2002)
	16S2	5’-GCGACCTCGATGTTGGATTAA-3’		
	N4-specific2	5’-CYCCRWCYTGGAGKCWYTCSGGY-3’		newly designed
COI	HCO-2198	5’-TAAACTTCAGGGTGACCAAAAAATCA-3’	50-55	Folmer et al. (1994)
	LCO-1490	5’-GGTCAACAAATCATAAAGATATTGGGG-3’		
	M13FLCO-1490	5’-TGTAAAACGACGGCCAGTGGTCAACAAAT CATAAAGATATTGG-3’		
	M13RHCO-2198	5’-CAGGAAACAGCTATGACTAAACTTCAGGG TGACCAAAAAATCA-3’		
18S	18s-3F	5’-GTTCGATTCCGGAGAGGGA-3’	49	Giribet et al. (1996)
	18s-5R	5’-CTTGGCAAATGCTTTCGC-3’		
H3	H3-AF	5’-ATGGCTCGTACCAAGCAGACVGC-3’	49	Colgan et al. (1998)
	H3-AR	5 -ATATCCTTRGGCATRATRGTGAC-3’		

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APPENDIX 4

Sequences alignments in Nexus format (a separate file, Supplement 2).

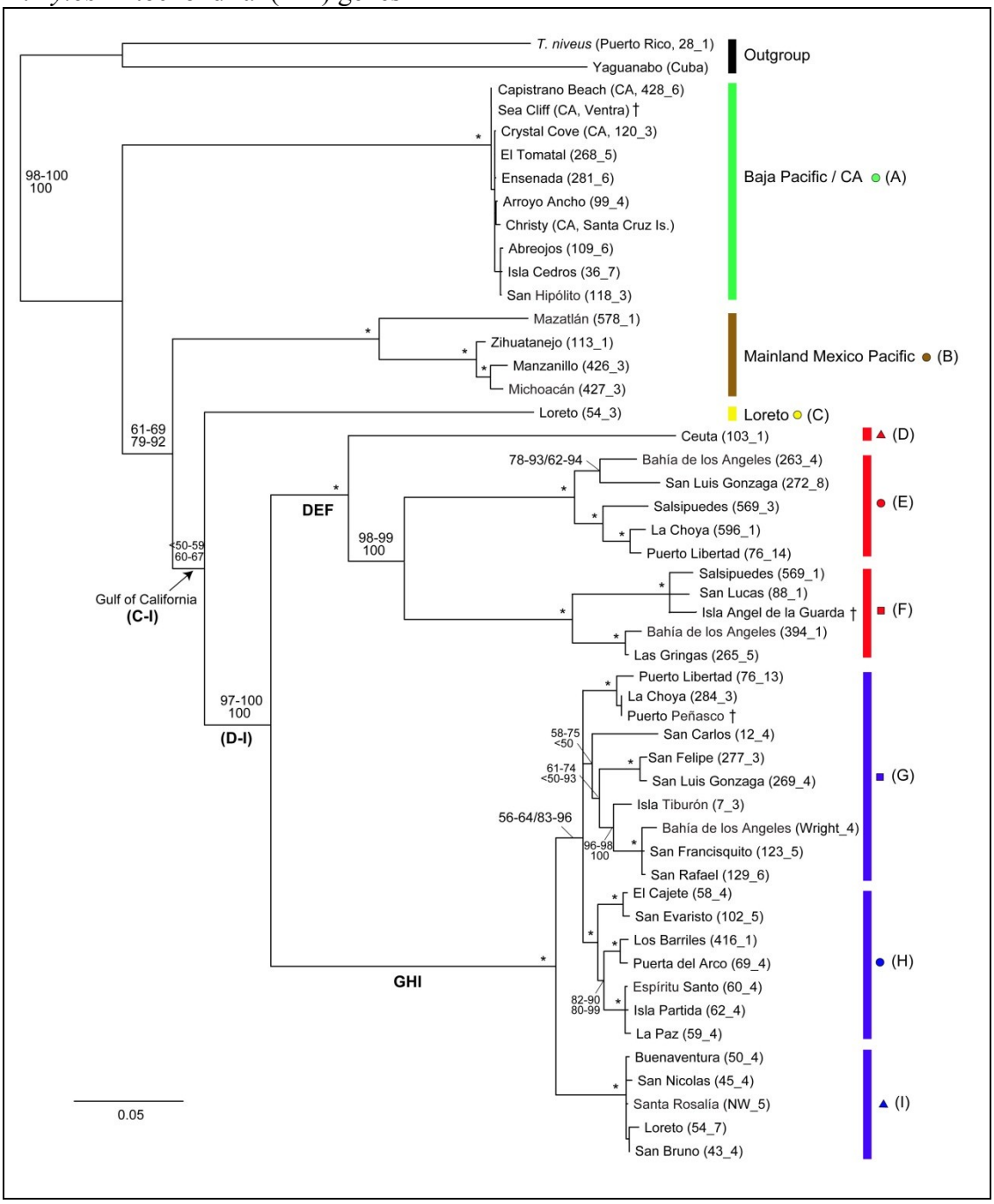
APPENDIX 5

Phylogenetic trees of concatenated mitochondrial loci (MT), 18S, and H3 genes.

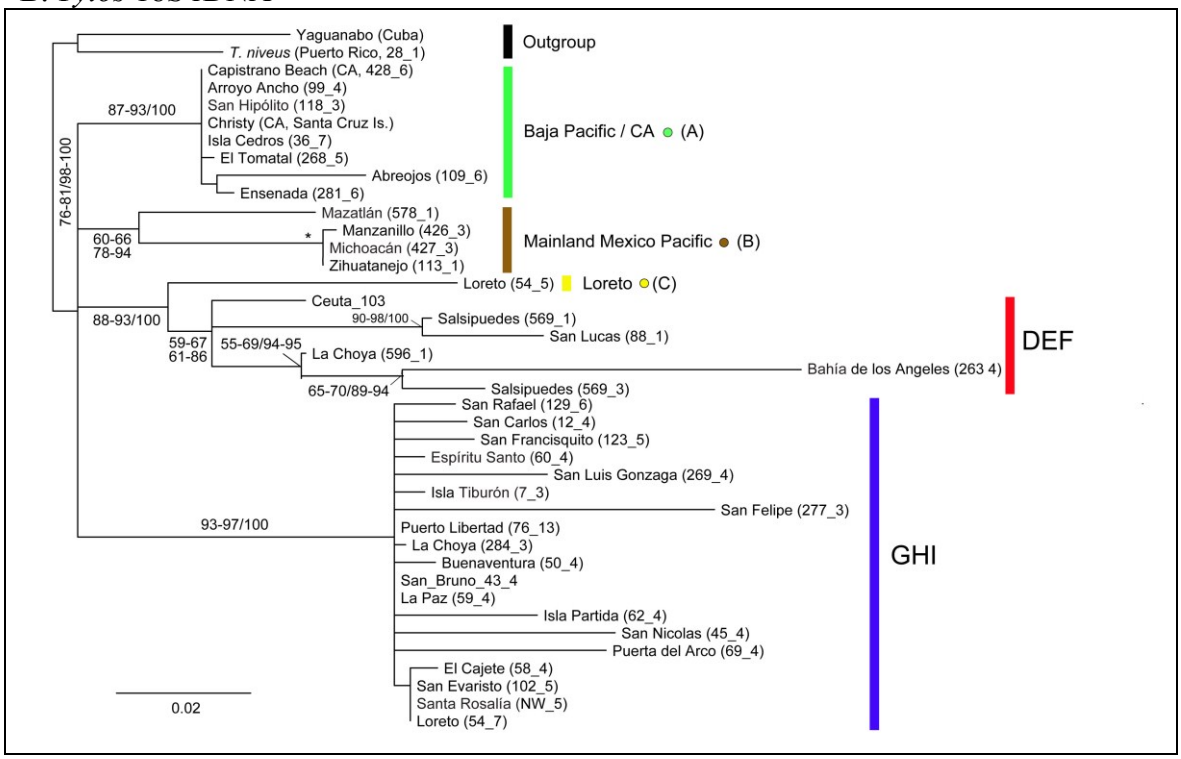
A. Majority-rule consensus tree (RaxML bootstrap) of the *Study Area* dataset based on concatenated mitochondrial loci (MT). Color and shape correspond to clades in Figs. 2 and 3. Numbers by nodes indicate the corresponding range of Bootstrap Support (BS; top or left) for Maximum likelihood (RaxML, Garli, PartitionFinder); and Posterior Probabilities (PP; bottom or right) for Bayesian inference methods (MrBayes, Phycas, BayesPhylogeny), including all partitioning schemes. * denotes nodes that received 100% support for all methods. Nodes receiving less than 50% support for all methods were collapsed and denoted with < 50. † = relationship based on 16S sequence only: Sea Cliff (CA, Ventura), Isla Angel de la Guarda, and Puerto Peñasco. **B and C.** Majority-rule consensus trees (RaxML bootstrap) of the *Study Area* dataset based on (B) 18S rDNA gene, and (C) Histone gene (H3A). Color and shape correspond to clades in Figs. 2 and 3. Maximum likelihood (RaxML, Garli, PartitionFinder); and Posterior Probabilities (PP; bottom or right) for Bayesian inference methods (MrBayes, Phycas, BayesPhylogeny). * denotes nodes that received 100% support for all methods. Nodes receiving less than 50% support for all methods were collapsed and denoted with < 50.

Appendix 5 Continued.

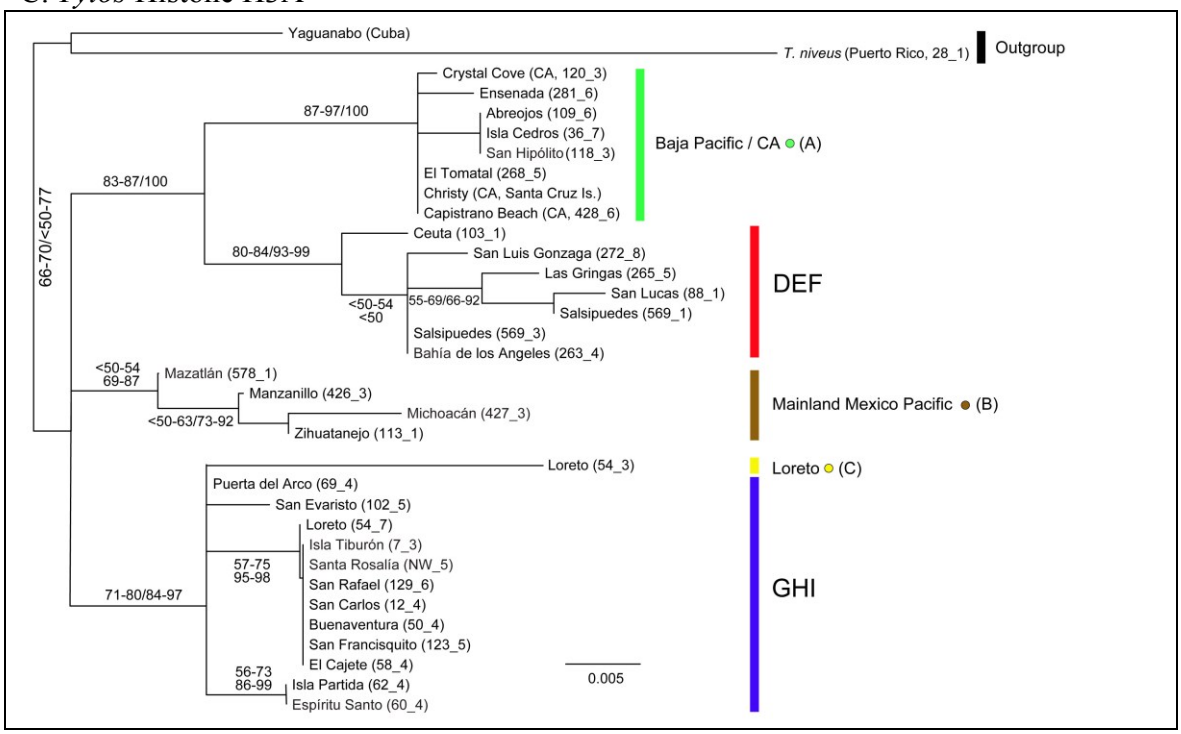
A. *Tylos* mitochondrial (MT) genes



B. *Tylos* 18S rDNA



C. *Tylos* Histone H3A



APPENDIX 6

Models, parameters, and priors used in the Maximum Likelihood and Bayesian phylogenetic analyses of the *Study Area* concatenated mitochondrial (MT) dataset.

Method	Model and Priors[1]	Partitioning scheme[2]	iterations generations/bootstrap replicates	Sample frequency	runs/ chains	burnin	ASDSF[3]	Bayes Factors[4] /ML scores (-lLn)	ESS[4,5] > 200	PSRF[6]
RaxML	GTR G	1	1000	na	na	na	na	-19413.81	na	na
Garli	TIM2 I G	1	100	na	na	na	na	-18935.40	na	na
Garli	HKY G	1	100	na	na	na	na	-19262.02	na	na
Garli	GTR G	1	100	na	na	na	na	-19833.38	na	na
MrBayes	GTR G	1	100,000,000	1,000	4/4	10%	0.000781	-19470.73	yes	1
MrBayes	HKY G	1	100,000,000	1,000	4/4	10%	0.000936	-19492.27	yes	1
Phycas	GTR G; polytomy prior	1	500,000	100	na	20%	na	-19485.60	na	na

[1] All others default; [2] different partitions separated by comma; [3] Average standard deviation of split frequencies; [4] estimated in Tracer v.1.5; [5] Effective Sample Size; [6] Potential Scale Reduction Factor for all parameters.

APPENDIX 7

Models, parameters, and priors used in the Maximum Likelihood and Bayesian phylogenetic analyses of the *Study Area* nuclear (NC) datasets.

Gene	Method	Model and Priors[1]	Partitioning scheme[2]	iterations generations/bootstrap replicates	Sample frequency	runs/ chains	burnin	ASDSF[3]	Bayes Factors[4] /ML scores (-lLn)	ESS[4,5] > 200	PSRF[6]
18S rDNA	RaxML	GTR G	1	1000	na	na	na	na	-2565.73	na	na
	Garli	K80 G	1	100	na	na	na	na	-2593.56	na	na
	Garli	HKY G	1	100	na	na	na	na	-2581.50	na	na
	Garli	GTR G	1	100	na	na	na	na	-2409.38	na	na
	MrBayes	GTR G	1	30,000,000	1,000	4/4	10%	0.003538	-2657.08	yes	1
	MrBayes	HKY G	1	30,000,000	1,000	4/4	10%	0.003554	-2656.50	yes	1
	Phycas	GTR G; polytomy prior	1	500,000	1,000	na	20%	na	-2644.56	na	na
H3A	RaxML	GTR G	1	1000	na	na	na	na	-711.72	na	na
	RaxML	(GTR G)[7]	2 (by codon; H3A1+2, H3A3)[8]	1000	na	na	na	na	-670.41	na	na
	Garli	TPM1 G	1	100	na	na	na	na	-648.94	na	na
	Garli	HKY G	1	100	na	na	na	na	-697.45	na	na
	Garli	GTR G	1	100	na	na	na	na	-691.27	na	na
	Garli	Mixed Model best (BIC)[7]	2 (by codon; H3A1+2, H3A3)[8]	100	na	na	na	na	-664.60	na	na
	MrBayes	GTR G	1	30,000,000	1,000	4/4	10%	0.002061	843.55	yes	1
	MrBayes	HKY G	1	30,000,000	1,000	4/4	10%	0.002243	-845.62	yes	1
	Phycas	GTR G; polytomy prior		500,000	1,000	na	20%	na	-782.60	na	na

[1] All others default; [2] different partitions separated by comma; [3] Average standard deviation of split frequencies; [4] estimated in Tracer v.1.5; [5] Effective Sample Size; [6] Potential Scale Reduction Factor for all parameters; [7] PartitionFinder 1.0 (JC+I;K80; suggested Best Model); BP = BayesPhylogen

APPENDIX 8

Ventral shape of the fifth pleonite for selected *Tylos* specimens from the *Study Area* and worldwide samples (a separate file, Supplement 3).