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Restriction to large-scale gene flow vs. regional panmixia among cold seep *Escarpia* spp. (Polychaeta, Siboglinidae)

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Abstract :

The history of colonization and dispersal in fauna distributed among deep-sea chemosynthetic ecosystems remains enigmatic and poorly understood because of an inability to mark and track individuals. A combination of molecular, morphological and environmental data improves understanding of spatial and temporal scales at which panmixia, disruption of gene flow or even speciation may occur. Vestimentiferan tubeworms of the genus *Escarpia* are important components of deep-sea cold seep ecosystems, as they provide long-term habitat for many other taxa. Three species of *Escarpia*, *Escarpia spicata* [Gulf of California (GoC)], *Escarpia laminata* [Gulf of Mexico (GoM)] and *Escarpia southwardae* (West African Cold Seeps), have been described based on morphology, but are not discriminated through the use of mitochondrial markers (cytochrome oxidase subunit 1; large ribosomal subunit rDNA, 16S; cytochrome *b*). Here, we also sequenced the exon-primed intron-crossing Haemoglobin subunit B2 intron and genotyped 28 microsatellites to (i) determine the level of genetic differentiation, if any, among the three geographically separated entities and (ii) identify possible population structure at the regional scale within the GoM and West Africa. Results at the global scale support the occurrence of three genetically distinct groups. At the regional scale among eight sampling sites of *E. laminata* ($n = 129$) and among three sampling sites of *E. southwardae* ($n = 80$), no population structure was detected. These findings suggest that despite the patchiness and isolation of seep habitats, connectivity is high on regional scales.

Keywords : deep sea ; hydrocarbon seep ; microsatellite ; population structure ; siboglinid ; vestimentiferan tubeworm

Introduction

A multitude of factors influence speciation and distributional ranges of marine populations, including larval biology, life history, adult physiology, geographic and oceanic barriers, as well as temperature (Jackson 1974). In this context, life history can influence connectivity in marine habitats by either preventing or promoting isolation of populations (Palumbi 1992). Ocean current dynamics add another dimension to the dispersal of marine groups, influencing population ranges by linking fragmented habitat patches (Johnson and Black, 1984; Wares et al. 2001; Hedgecock et al. 2007). True biological ranges of marine invertebrates in the deep sea can be especially difficult to assess given the lack of morphological diversity and the inability to cross and rear offspring (Palumbi 1994). Furthermore, physical limitations of sampling in the deep sea have inhibited extensive study of deep dwelling populations. Molecular genetic tools, combined with environmental and morphological data, are now universally accepted approaches for elucidating evolutionary lineages in deep-sea animals (Vrijenhoek et al. 1994; Etter et al. 1999; Vrijenhoek 2010). Additionally, studies of biogeography, genetic structure and ecological adaptation can provide information on the scales at which panmixia is replaced by gene flow restriction or disruption; if these processes are active over appropriate evolutionary time scales, they may lead to speciation. This question is particularly germane to the deep sea where distinct biogeographic provinces support an expectation of isolated populations in different regions, while the large scale dispersal potential of many taxa, and some population genetic data, suggest the occurrence of regular colonization events over large geographic distances,

77 *Escarpia vestimentiferan tubeworms*

78 Hydrocarbon seeps, which are located on passive ocean margins worldwide, fuel
79 specialized deep-sea ecosystems. In the Gulf of Mexico, the continental slope has
80 developed distinctive topography over time as a result of sediment compaction of a thick
81 salt layer, and subsequent creation of diapirs and faults; it is through these faults that
82 hydrocarbon fluids and gasses migrate to the seafloor (Andersen et al. 1983; Sassen et al.
83 1994a; Cordes et al. 2009; Haas et al, 2010). The constant deformation of the salt layer
84 controls the level of seepage (Roberts and Aharon 1994) which supports specially
85 adapted chemosynthetic fauna, including the vestimentiferan tubeworms (Family:
86 Siboglinidae), a group of polychaete worms that lack digestive tracts and depend on
87 endosymbiotic bacteria for their nutrition (Childress and Fisher 1992). Seep
88 vestimentiferan larvae settle on exposed carbonate rock that is generated as a by-product
89 of bacterial hydrocarbon degradation (Sassen et al. 1993). Tubeworms extend the
90 posterior portion of their bodies (“roots”) deep into the sediment to acquire the supply of
91 sulfide necessary to maintain their symbioses (Julian et al. 1999; Freytag et al. 2001).
92 These special adaptations allow individuals to have long life spans; at least two
93 tubeworm species in the Gulf of Mexico can live for centuries (Bergquist et al. 2000;
94 Cordes et al., 2007a; Fisher et al. 2007). Vestimentiferans are important components of
95 seep habitats, as they cluster in aggregations that form “bush-like” three-dimensional
96 structures (Figure 1A), which provide living space and protection for a variety of other
97 fauna (Bergquist et al. 2003). Furthermore, due to their dependence on hydrocarbons,
98 habitats suitable for tubeworm communities can be separated by hundreds of kilometers
99 (Brooks et al., 1987; Roberts et al., 2007).

Vestimentiferans of the genus *Escarpia* are distributed at seeps below 950m along the temperate and tropical continental margins of the Atlantic and Pacific Oceans (Olu et al. 2010). Three species have been described within the genus. *Escarpia laminata* (Jones 1985) is distributed across the Lower Louisiana Slope in the Gulf of Mexico (GoM), and is found at depths from ~950 – 3300m (McMullin et al. 2003; Miglietta et al. 2010). *Escarpia spicata* (Jones 1985) is known from the western coast of North and Central America and the Gulf of California, and can inhabit seeps, hydrothermal vents and whale falls below 1000m (Feldman et al. 1998; Vrijenhoek et al. 2007). *Escarpia southwardae* (Andersen et al. 2004) occurs at pockmark depressions at West African Cold Seeps (WACS) distributed along the Congo River Canyon at depths below 3000m (Ondréas et al. 2005; Olu-Le Roy et al. 2007).

All three *Escarpia* have similar gross morphology (Figure 1B). Members of each group have an axial rod on the top of the obturaculum, and the lack of large flare rings on their tubes contrasts with a related species, *Seepiophila jonesi* (Gardiner et al. 2001). Each described species differs in the shape of the axial rod, and *E. spicata* has a ventral ridge that is not present in *E. laminata* (Jones 1985). Additionally, *E. southwardae* is the only vestimentiferan known that does not have pinnules on the plume. Pinnules are thought to be the primary site of O₂ and CO₂ uptake in the other species (Andersen et al. 2004). Although the three described *Escarpia* species are geographically distant and morphologically distinguishable, they do not form monophyletic clades with commonly used mitochondrial markers Cytochrome Oxidase subunit 1 (COI) and the large ribosomal subunit rDNA (16S) (Black et al. 1997; McMullin et al. 2003; Andersen et al. 2004; Miglietta et al. 2010). COI has been suggested as a global standard to provide

species level resolution in animals, hence its nickname, the “DNA barcoding” marker (Herbert et al. 2003; Hajibabaei et al. 2007). However, there are limitations to using barcoding molecules (see Moritz and Cicero, 2004). The inability of this marker to differentiate the described *Escarpia* species has led to suggestions that they are either a single morphologically plastic species or that there is low COI divergence between the *Escarpia*, which may be the result of the long life spans and the slow evolutionary rate seen in seep vestimentiferan mitochondrial genes (McMullin et al. 2003). The large distance between populations of a single “species” would require that *Escarpia* offspring travel thousands of kilometers along continental margins or across the Atlantic abyssal plain to maintain integrity of a single panmictic population, possibly using an undiscovered continuum of chemosynthetic communities as “stepping stones” (Feldman et al. 1998; Cordes et al. 2007b).

Vestimentiferan larval biology

The investigation of life history traits in tubeworms is important for understanding how speciation occurs and how seep communities are established and maintained (Tyler and Young 1999). Adult vestimentiferans are sessile and have separate sexes. The females are known to store sperm bundles; eggs of the seep tubeworms from the upper Louisiana Slope of the Gulf of Mexico, *Lamellibrachia luymesii* and *Seepiophila jonesi*, are fertilized internally, and zygotes are released into the water column allowing the lecithotrophic larvae to disperse on ocean currents (Vrijenhoek 2010; Hilario et al. 2005). *S. jonesi* larvae reared in the laboratory survived for 21 days before metamorphosis, suggesting a possible dispersal range of 40 – 60km for tubeworms

at < 1000m in the Gulf of Mexico (Young et al. 1996; Tyler and Young 1999). In comparison, *Riftia pachyptila*, the giant hydrothermal vent tubeworm found on the East Pacific Rise, is estimated to have dispersal distances of about 100km along the ridge axis with a larval lifespan of five weeks (Marsh et al. 2001).

Use of molecular tools to define vestimentiferan population structure

Gene flow and connectivity processes of vestimentiferans living at hydrothermal vents have been the subject of several studies because the linear arrangement of vents along a ridge crest provides a very tractable model system for these studies (Tunnicliffe 1988, Mullineaux and France, 1995; Kim and Mullineaux, 1998; Marsh et al. 2001). Several authors used allozymes to uncover varying levels of genetic variation in vent vestimentiferans *R. pachyptila*, *Tevnia jerichonana*, and *Oasisia alvinae* across 4000km at the East Pacific Rise (Black et al. 1994; Black et al. 1998). Furthermore, Hurtado et al. (2004) used mtCOI to identify subdivision in *R. pachyptila* across the Easter Microplate Region, indicating restricted gene flow across this region.

Shank and Halanych (2007) proposed that genetic markers such as COI have under sampled genetic diversity in *R. pachyptila*, and investigated genetic structure using amplified fragment length polymorphisms (AFLP). With this approach, 630 polymorphic loci were uncovered to identify clustering of *R. pachyptila* individuals according to locality (9°N EPR, Guaymas Basin, and Southern Eastern Pacific Rise) illustrating greater population subdivision than previously seen. Additionally, Vrijenhoek (2010) estimated within species gene flow as high to moderate in *R. pachyptila* and *Ridgeia piscesae*, which was reported as number of migrants transferred along contiguous ridge

segments.

In contrast to vents, studying seep vestimentiferan connectivity is more complex as regions of active hydrocarbon seepage are patchily and haphazardly distributed, often in areas with very complex current regimes. McMullin et al. (2010) investigated genetic structure in seep populations of *L. luymesii* and *S. jonesi*, two species that often co-occur at seep sites in the northern GoM at depths < 1000m. Based on twelve polymorphic microsatellite markers, a pattern of isolation by distance in *L. luymesii* and a genetic clustering fitting age of aggregation in *S. jonesi*. It is, however, unclear if vestimentiferan populations exhibit similar population subdivisions at seeps > 1000m where the currents are even less understood.

To better understand the scale of population connectivity and the factors affecting gene flow in *Escarpia*, we ask 1) On a global scale, are the geographically separated *Escarpia* taxa as genetically similar as suggested by mitochondrial sequences, or do they form genetically distinct populations and 2) On a regional scale, is there evidence of genetic differentiation between populations at distinct isolated sites within the GoM and WACS?

To address the first question we used the classic mitochondrial DNA molecular markers mtCOI and mt16S rRNA, supplemented with another mitochondrial (mt) gene Cytochrome B (CYTB), an Exon Priming Intron Crossing (EPIC) nuclear marker Hemoglobin subunit B2 intron (HbB2i), as well as nine polymorphic microsatellite loci. Cytochrome B has been found to be phylogenetically informative in many groups (Johns and Avise 1998). The utility of CYTB as an informative marker in deep-sea invertebrates has not been investigated in detail.

At the regional scale, the existence and pattern of effective larval dispersal *versus* genetic divergence was addressed by analyzing 11 microsatellites on samples collected from eight sampling sites in the GoM and 16 microsatellites on samples collected from three sampling sites at WACS. Because all *E. spicata* individuals were collected from a single site, a regional scale analysis was not conducted for *E. spicata*.

Methods

Sample Collection and Preparation

Tubeworms were collected via a manned submersible and remotely operated vehicles (ROV) from seep sites during several research cruises occurring from 2003 to 2011. *Escarpia laminata* individuals were collected from eight locations on the Lower Louisiana Slope in the Gulf of Mexico using ROV *Jason II* on the National Oceanic and Atmospheric Administration vessel Ronald Brown or the Deep Sea Vehicle *Alvin* on the R/V *Atlantis*. *Escarpia southwardae* individuals were retrieved from three pockmark locations near the Congo River Canyon off of the west coast of Africa using ROV *Victor 6000* on the N/O *Pourquoi Pas?*, operated by IFREMER, France (Table 1 and Figures 2 and 3). *Escarpia spicata* individuals were collected from the Transform Fault 4 (TF4) site in the Gulf of California and provided by Robert Vrijenhoek of the Monterey Bay Aquarium Research Institute, USA (see Vrijenhoek et al. 2007 for details on the collection localities). Sample preparation and DNA extraction protocols were performed as described in Cowart et al. 2012. Tissue samples are stored at -80°C and in 96% ethanol in Fisher Deep Sea lab at Penn State.

Sequencing and EPIC development

A 658bp fragment of mtCOI was amplified in 42 *Escarpia* individuals using primers HCO and LCO (Folmer et al. 1994); a 435bp fragment of mt16S was amplified in 45 individuals using primers 16Sar and 16Sbr (Palumbi 1996), and a 401bp fragment of mtCYTB was amplified in 46 *Escarpia* and *Seepiophila* individuals using primers Cytbf and Cytbr (Boore and Brown 2000). Cytb primers contained many degenerate bases, and *Escarpia* specific CYTB primers were designed for this study.

EPIC sequencing involves designing primers in the conserved exon regions of nuclear genes to sequence across the introns, and has been previously applied to clarify population relations in both marine, freshwater, and terrestrial animals (Palumbi 1996, Palumbi and Baker, 1994; Bierne et al. 2000; Schaeffer et al. 2003; Li et al. 2010). To amplify the intron region within the hemoglobin subunit B2, the HbB2i fragment was first identified in *R. piscesae*, by designing PCR primers based on the cDNA sequence of *R. piscesae* globin B2 (accession AY250083; Bailly et al. 2003). Cloning and sequencing of the resulting product revealed the 550bp intron sequence. These same primers were tested in *R. pachyptila*, and a similar analysis was conducted to obtain a 531bp sequence. The sequences obtained from *R. piscesae* and *R. pachyptila* were aligned, and primers were designed in the exons to flank the intron to produce a 665bp fragment in 48 *Escarpia* and *Seepiophila* individuals.

PCR reactions and gel electrophoresis were performed as described in Miglietta et al. 2010, with annealing temperature for 1.5min at 50 °C (COI and 16S) or 52 °C (CYTB and HbB2i). Purified PCR products were submitted to the Penn State Genomics

Core Facility (University Park, PA) and run on 3730XL DNA sequencer.

Sequence Analyses

Gene sequences were assembled and edited using Geneious Pro v5.5.5 (Biomatters Ltd.), and then aligned using ClustalW (Thompson et al. 1994) and MUSCLE (Edgar, 2004) implemented through the Geneious and MEGA 5.05 interfaces (Tamura et al. 2011). Both ClustalW and MUSCLE gave identical alignments, and ClustalW alignments were used to generate trees. All alignments were edited and confirmed by eye to ensure that indel variation was scored consistently among taxa. Phylogenetic analyses of the alignments were conducted in MEGA using the Maximum Likelihood (ML) (Tamura and Nei 1993) methods with 1000 bootstrap replicates. Between species distances were computed in MEGA using the Maximum Composite Likelihood Method (Tamura et al. 2004) and are measured as the number of base substitutions per site. To test for the neutrality of the four genes, Tajima's D (Tajima 1989) statistic was estimated using Arlequin v3.5 (Schneider and Lischer 2009). Median-joining haplotype networks were created using the program Network v4.611 (Bandelt et al., 1999, available at www.fluxus-engineering.com).

Microsatellite Analyses

Primers and amplification conditions for the 28 polymorphic microsatellite loci developed for *E. laminata* and *E. southwardae* are listed and described in Cowart et al. 2012 (see supporting information). Different alleles at a microsatellite locus may differ by the repeat length; however, a variety of genotyping errors can lead to single base pair offsets in allele size determinations (Pompanon et al. 2005). To correct for motif indel

261 mutations, we calibrated the automated allele calls from fragment analysis software with
262 actual repeat lengths by sequencing a mean of five homozygote individuals per locus to
263 verify the length of alleles (GenBank Accessions KC900290 - KC900365). When the
264 apparent allele size was offset by less than one repeat, it was binned to the nearest
265 confirmed size. This dataset was then used for all downstream analyses. When a
266 heterozygote deficiency was observed, we tested for the presence of null alleles (which
267 can be particularly high in invertebrate populations, Chapuis and Estoup, 2007) using
268 INEst v.1.0 (Chybicki and Burczyk 2009), under the Individual Inbreeding Model.

269 Arlequin was used to test for significant departures from Hardy-Weinberg
270 Equilibrium (HWE) by computing observed and expected frequencies of heterozygosity.
271 To adjust p -values for multiple comparisons tests, the False Discovery Rate control
272 (FDR) (Benjamini and Yekutieli 2001) was implemented in QVALUE (Storey and
273 Tibshirani 2003) executed in the statistical software R version 2.10.1 (R Development
274 Core Team 2009). To estimate genetic differentiation, Wright's F -statistics were
275 computed with the aid of FSTAT v.2.9.3.2 (Wright 1951; Goudet 1995); these indices
276 describe the probability that individuals from different sub- and total populations will
277 share alleles identical by descent (Weir and Cockerham 1984). For the purposes of this
278 study, individual refers to a single tubeworm, subpopulations are all individuals from
279 specific seep location and total populations refer to all individuals from a particular
280 region.

281 To compute allelic and private allele richness for each population controlled for
282 sample size, the program HP-RARE (Kalinowski 2005) was used. To illustrate
283 population structure within and between the three regions, the programs STRUCTURE

v2.3.x (Pritchard et al. 2000; Pearse and Crandall 2004) and EDENetworks v.2.16 (Kivelä et al. *in prep*) were used. For STRUCTURE, independent allele frequencies and admixed populations were assumed; three replicate simulations were run using various values of K, with 100,000 Markov Chain Monte Carlo (MCMC) repetitions for each cluster and burn-in of 10,000.

To assign individuals to populations while detecting the presence of hybrids as linking agents between clusters, networks based on the shared allele distance were built with EDENetworks. EDENetworks illustrates the distribution of genetic distances (links) among agents (individuals or populations) of a system of populations, and infers the clustering of sets of individuals into distinct populations or species using percolation theory and without an *a priori* hypothesis based on their taxonomic identity or sampling location (Becheler et al. 2010; Moalic et al. 2011; Rozenfeld et al. 2007, Rozenfeld et al. 2008). The percolation theory allows for the splitting of a fully connected network into discrete clusters, and the critical threshold distance is known as the percolation threshold (Dpe), an inner property of the system (Stauffer and Aharony 1994; Watts, 2004).

Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was calculated with the aid of GenoDive v.2.0b22 (Meirmans and Hedrick 2011) based on population clusters identified by STRUCTURE, if applicable. To test for signatures of bottlenecks or recent population expansion, the program BOTTLENECK (Piry et al. 1999) was run using the Two-Phase Model and the Wilcoxon sign rank test at 1000 iterations.

Results

Mitochondrial and EPIC sequence analysis

307 The COI and 16S datasets included a total of 98 sequences, including 71
308 sequences of *Escarpia* not previously reported, and 16 sequences from Miglietta et al.
309 (2010). *Seepiophila jonesi* served as the outgroup taxon for both analyses. The complete
310 COI dataset consisted of a 658bp fragment amplified in 42 *Escarpia* and six *Seepiophila*
311 The complete 16S dataset consisted of a 435bp fragment amplified in 45 *Escarpia* and
312 five *Seepiophila*. All GenBank Accession numbers are located in the supporting
313 information (Table S2). As previously reported (McMullin et al. 2003; Miglietta et al
314 2010), COI and 16S phylogenies do not resolve the three described *Escarpia* spp.
315 (Figures S1 and S2) due to the extremely low number of haplotypes (nine haplotypes for
316 COI and only one for 16S). A haplotype analysis of COI is shown in Figure 4 and
317 identifies the most common haplotype as shared among the three *Escarpia* (n=22), with
318 rare haplotypes shared only within a group.

319 The CYTB dataset consisted of a 401bp fragment amplified in 46 sequences of
320 *Escarpia* and *S. jonesi*. The CYTB phylogeny identified two separate groups within
321 *Escarpia*: one moderately supported clade that included *E. laminata* and *E. spicata*, from
322 which *E. southwardae* is separated. *S. jonesi* individuals form a highly supported cluster
323 outside of the *Escarpia* group. The CYTB haplotype network identifies a shared
324 haplotype between *E. laminata* and *E. spicata* (Figure 4), as well as three unique
325 haplotypes for *E. spicata*. *E. southwardae* exhibited one distinct haplotype different from
326 *E. laminata*/*E. spicata* by only one mutation.

327 The HbB2i dataset included 48 sequences from *Escarpia* and *S. jonesi*, and
328 consisted of a 665bp fragment. The HbB2i phylogeny is in agreement with the separation
329 of the described species of *Escarpia*, although with only moderate bootstrap support and

low levels of nucleotide divergence (Figure S4). The HbB2i haplotype network also identifies geographically separated haplotypes (Figure 4). The rare haplotypes present at the three polymorphic genes are unique to a specific geographic region, with none of the rare haplotypes shared among *Escarpia* spp.

To provide information on the demographic history occurring among the *Escarpia*, we examined Tajima's *D* for the three polymorphic genes tested for each group separately. None of the Tajima's *D* tests showed values significantly departing from zero (Table S3). However, considering the low number of haplotypes and nucleotide divergence, one should cautiously accept the null hypothesis of neutrality and demographic stability given the lack of power provided by the dataset. Testing for population bottlenecks allowed us to detect of significant heterozygote excesses in all sites for *E. southwardae* and in one site for *E. laminata* (GC852) (see SI tables), supporting the occurrence of recent demographic expansions following establishment of these two populations.

Microsatellites identify three genetically different Escarpia groups

Mean summary statistics for the nine cross-species amplified microsatellite loci are detailed in Table 2. A total of 229 individuals were examined across all *Escarpia*; 222 alleles were detected across all loci with the largest number of alleles at locus EL454_9 (35), and the smallest at EL454_71 (8) (Table S4). For *E. spicata*, the mean number of alleles was eight, while average gene diversity spanned from 0.471 to 0.90, with a mean expected heterozygosity of 0.780. For *E. laminata*, the mean number of alleles was 13, while average gene diversity had a range of 0.343 to 0.921, with a mean expected

heterozygosity of 0.753. *E. southwardae* had a mean number of alleles of 12, and gene diversity had a minimum of 0.262 and a maximum of 0.907 and a mean expected heterozygosity of 0.753. Of the 27 sampling location vs. locus combinations, three departed significantly from HWE after FDR correction at 0.01.

F_{IS} values were calculated across all locations, as findings identified panmictic populations in each region (see results below). For *E. spicata*, the mean F_{IS} value across all loci was 0.194, for *E. laminata* F_{IS} is 0.149 and *E. southwardae* is 0.104 (Table 2). Mean allelic richness per site varied from 4.75 to 4.87, while private allelic richness ranged from 2.71 to 3.26. There was no significant difference in the average allelic and private allelic richness between the three *Escarpia* species ($p > 0.05$).

Both STRUCTURE and network analyses support the occurrence of three clusters of *Escarpia* (Figure 5), supporting the taxonomical hypothesis based on the morphological descriptions. For the network analyses, at $D_{pe} = 1$ and below, three clusters emerge with a significant clustering index ($p < 0.001$). Just above $D_{pe} = 1$, some individual nodes are isolated, despite the connection of three clusters. Although clustering reveals strong genetic structure among the three taxa/regions, we note that below the percolation threshold, several individual genotypes are more closely related to genotypes different from the morphological and geographical groups to which they were assigned (Figure 5). Network analyses also identify the GoM population as the central cluster, suggesting that colonization and diversification of the *Escarpia* occurred through the GoM. F_{ST} values also identified significant differentiation between the three geographically distinct populations of *Escarpia* (Table S4). Additionally, AMOVA

analyses support that the three *Escarpia* populations are significantly different from one another ($p = 0.001$, Table 3).

***Escarpia laminata* exhibits panmixia on a regional scale**

Eleven microsatellite loci, which include three additional species-specific loci, were examined in 129 *E. laminata* individuals from eight sites in the GoM. A total of 167 alleles were detected across all sites where the EL454_57 locus had the greatest number of alleles at 19 (Table S7). The MC294 site had the highest mean expected heterozygosity at 0.773, while WR269 exhibited the lowest at 0.658. While two sites at locus EL454_9 departed significantly from HWE, no locus significantly deviated from HWE across all sites. The global F_{IS} was 0.103, while the mean F_{IS} values for each site ranged from 0.083 at AT340 to 0.194 at West Florida Escarpment. Mean allelic richness per site spanned from 4.60 to 5.54, while private allelic richness ranged from 0.22 to 0.49 and there was no significant difference in the average allelic and private allelic richness between the eight sites ($p > 0.05$).

STRUCTURE analyses did not detect any genetic clustering on a regional scale among the eight sites in the GoM. Furthermore, none of the 28 pairwise population comparisons were significantly different after correction for multiple tests ($p > 0.05$). The global F_{ST} value was low (0.007) with a p -value of 0.053, suggesting very limited structure on the regional scale (Table S8). AMOVA testing identified a significant among individual component, for which 8.9% of the total variance is explained ($p = 0.001$) (Table 3). Maximum genetic differentiation was observed between AC601 and MC294, sites that are separated by a distance of 657km, although the maximum distance between

sites tested here is 980km. These findings indicate that gene flow is not correlated with geographic distance on the regional scale in the GoM.

***Escarpia southwardae* is panmixic at WACS**

Sixteen microsatellite loci were examined in 80 *E. southwardae* individuals from three sampling sites at WACS. A total of 223 alleles were detected across the three sites, and locus ES454_31 exhibited the highest number of alleles at 19 (Table S9). The Worm Hole site exhibited the highest mean expected heterozygosity at 0.709, while Regab had the lowest at 0.658. Three sites departed from HWE at loci ES454_32 and 45. The global F_{IS} was 0.104, while the mean F_{IS} values for each population ranged from 0.081 at Worm Hole to 0.149 at Baboon. Mean allelic richness per site spanned from 8.63 to 9.44, while private allelic richness ranged from 1.42 to 1.68, and there was no significant difference in the average allelic and private allelic richness among the three locations. None of the pairwise comparisons showed significant differentiation among populations; the global F_{ST} value was low (0.003) and we did not detect any difference between the three populations studied here ($p = 0.149$; Table S9).

As with the *E. laminata*, STRUCTURE analyses of *E. southwardae* did not detect any genetic clustering among the three sites at WACS. AMOVA testing identified a significant among individual component, for which as little as 0.49 % of the variance is explained ($p = 0.002$).

Discussion

Results obtained in this study show that despite the isolated and haphazard distribution of cold seep sites, there is panmixia over extensive regional scales. In contrast, we find only limited indications of gene flow among the three regions studied. These findings confirm the divergence of tubeworm populations between the three regions, and suggest the presence of mechanisms that prevent or severely limit gene flow across these spatial scales. However, in the absence of either experimental data confirming reproductive isolation or evidence of maintenance of genetic isolation in co-occurring populations, it is not possible to confirm that these differentiated populations represent biologically distinct species.

Barriers to gene flow across large scales

Although mitochondrial DNA is often used in the taxonomic identification of animals, neither the “barcoding marker” COI, nor the 16S rRNA gene, resolved the relationships among the three described species of *Escarpia*. Furthermore, the mtCYTB gene could only resolve the *Escarpia* into two groups, rather than three. The HbB2i locus, together with microsatellites, supports the existence of three differentiated groups, corresponding to the taxonomic hypothesis based on morphological and geographic criteria.

The fixed haplotypes and low diversities at mitochondrial and nuclear loci are an indication that strong demographic fluctuations occurred in the recent past and reduced the level of polymorphism. The lack of allele frequency variation in mtDNA among geographically separate populations could result from a number of factors. First, there may be insufficient time since the separation of populations. Second, as evolutionary rate

is influenced by species generation time, populations with longer generation times may accumulate mutations at a slower rate (Hartl and Clark, 2007). As seep vestimentiferans can live for hundreds of years (Bergquist et al. 2000), their long generation time could contribute to the lack of mutations seen in mtDNA. Third, the low mutation rates may be the result of vestimentiferans having efficient mutation repair systems. Additionally, the exclusively maternal transmission of mtDNA reflects on the distribution of maternal lineages rather than whole genome evolution, and possible differences between male and female effective population sizes may induce different rates of evolution of mitochondrial versus nuclear genomes. Finally, selective sweeps may interfere with the usual mutation-drift balance and influence mitochondrial evolution, as organelles have smaller effective population sizes and are more prone to genetic drift (Bazin et al. 2006; Galtier et al. 2009; Moalic et al. 2012). Whether these populations are the result of recent founder events followed by genetic drift and ultimately differentiation, or from a more ancient colonization that was followed by divergence among protospecies or species remains unknown. The lack of shared rare haplotypes at the mitochondrial loci is consistent with either. Regardless, gene flow is clearly very limited between the regions inhabited by the described *Escarpia* spp.

Vestimentiferans are thought to have arisen around 100 million years ago (Black et al. 1997). As recently as 3.5 million years ago, deep-water flows connecting the Pacific and Atlantic oceans were disrupted by the formation of the Isthmus of Panama as the North American, South American and Caribbean plates converged (Keigwin 1978; Knowlton 1993). Additionally, the deep water that flows from the Caribbean Sea into the GoM is trapped by geologic sills occurring below 800m in the Florida Straits and below

1900m in the Yucatan Channel, which may help to further isolate biological populations in the GoM from other parts of the Atlantic (Welsh and Inoue 2000; Rivas et al. 2005). All three *Escarpia* populations experience similar habitat conditions, including the high pressure that accompanies extreme depth, as well as elevated concentrations of hydrogen sulfide required by their chemoautotrophic symbionts (Childress and Fisher, 1992). Therefore, the genetic divergence and morphological variation reported here are likely not driven by habitat specialization, although, whether differences in morphology are influenced by phenotype plasticity or selective pressures remains to be investigated.

The relatively low levels of genetic differentiation reported, and the presence of rare haplotypes that are unshared between described species, supports the existence of a recent common ancestor, and possibly recent colonization events into different ocean basins. The genetic differentiation between the three *Escarpia* is possibly the result of a genetic drift and lack of gene exchange due to geographic and reproductive isolation. The analyses here indicate that contemporary migration between the three provinces, if occurring at all, is low. Species delimitation is difficult to demonstrate with geographically separated taxa (de Queiroz 2007). The best method to confirm the presence of reproductive barriers among potentially distinct species would be controlled crosses, which at this time are technically difficult to conduct with these deep sea taxa. However, based on the congruence of morphological differences with data from a nuclear gene and other polymorphic loci, as well as the large distances separating the described species, we suggest that the mitochondrial genetic data should not be used to suggest that the three described species are in fact a single species. We do caution that in the absence of data on reproductive isolation it is also possible that the three described species

represent either separate populations of the same species with scarce and/or highly sporadic gene flow or protospecies undergoing strict divergence and on the way to speciation.

On a regional scale, the evidence for panmixia suggests that despite the occurrence of isolated seep sites, *Escarpia* do have the ability to connect across geographic scales of nearly 1000k in the GoM and at least 120 km at WACS. The low divergence seen across sampling locations suggests that contemporary gene flow is occurring at the regional scale for both GoM and WACS.

The influence of deep currents on dispersal and recruitment of Escarpia

In addition to constraints imposed by larval biology, successful dispersal of marine larvae depends on movement with ocean currents (Scheltema 1968; Johnson and Black 1984). The moderately high dispersal potential of vestimentiferan larvae, coupled with deep currents, can account for the open exchange of migrants seen within the GoM and WACS regions. Currents below 1000m are often quite complex, moving in various directions and speeds (Hamilton, 1990). In the GoM, the Loop Current (LC) dominates the east, and while restricted to < 1000m depth, the LC sheds warm eddies that generate and influence deep Topographic Rossby Waves (TRW) at depths below 1000m (Hamilton 1990). Swirl speeds of deep eddies are estimated to range from 10 to 21 cm s⁻¹ at 2550m, increasing speed with more complex topography (Welsh and Inoue 2000), and TRW are estimated to occasionally move at speeds of 15 – 30cm/s⁻¹, having durations from 20 – 30 days and dominating the central and western GoM (Chelton and Shelax 1996; Oye and Lee 2002; Hamilton 2007).

512 Thermal tolerances of deep sea larvae also play an important role in dispersal, as
513 larvae moving into shallower waters and thus warmer temperatures can experience
514 changes in metabolism and feeding rates, influencing the amount of time they spend in
515 the water column (Young et al 1996; Young et al. 1998). McMullin et al. (2010) and
516 Young et al. (2012) found that most larvae of the shallower living GoM tubeworm,
517 *Lamellibrachia.luymesii*, are retained in the same general geographic location as the
518 adults, not dispersing > 300km from the source populations. Additionally, *L. luymesii* are
519 unable to tolerate the temperatures at depths above the thermocline, further restricting the
520 propagules to the region.

521 The speeds of deep currents, accompanied by prolonged larval duration, as well as
522 the lower temperatures needed for larval development, appear to be sufficient to transport
523 *Escarpi* migrants to habitats across the GoM sea floor at depths below 1000m.
524 Additionally, there are more than 50 known chemosynthetic sites distributed across the
525 GoM (Mineral Management Service, 2006). This along with model data of closely related
526 species suggests that GoM *Escarpi* do have the ability to colonize seeps across the
527 regional distance described in this study.

528 A major feature of the West African continental margin is the Congo deep-sea
529 river fan, which results from loads of sediment transported and deposited by the Congo
530 River (Khrapounoff et al. 2003; Sibuet and Vangriesheim 2009). The submarine fan
531 ranges in depth from 3400 to 4800m and contributed to the formation of the cold seep
532 habitats that are home to *E. southwardae* (Vangriesheim et al. 2009). Deep-sea
533 circulation at the Congo River fan is complex, and below 2000m, and currents have a
534 southeastward migration path (Stramma and England 1999). At Regab, current speeds

range from 8.9 to 12.3 cm s⁻¹, rarely exceeding 10 cm s⁻¹ (Vangriesheim et al. 2009).

Despite the relatively slow movement of currents in this region, the three sampling sites encompass a distance of less than 150 km, and if larval characteristics are comparable to other seep vestimentiferans, gene flow across these sites is realistic.

Conclusions

In this study, we used the analyses of several molecular genetic markers to support the occurrence of three genetically distinct entities that correspond to three *Escarpia* species described based on morphology from the Gulf of Mexico, the eastern Atlantic, and the eastern Pacific. This data suggests limitations to large-scale dispersal by *Escarpia* larvae and are in agreement with a scenario of ongoing divergence after relatively recent colonization in the three regions. However, the widespread genetic homogeneity observed within regions over hundreds of kilometers also support recurrent events of moderate scale dispersal. Finally, the lack of resolution seen in three mtDNA loci contrasts with the lineage and genotype sorting revealed by the nuclear datasets (one intron and several microsatellite loci). This suggests that EPIC and microsatellite markers can serve as tools to not only examine population structure within groups, but also to clarify boundaries and distributions of species with very low levels of divergence in classic bar coding genes.

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Authors Contributions

DAC: provided funding for research, performed research, analyzed data, wrote manuscript
COH: performed research, edits to manuscript
SAH: performed data analyses, edits to manuscript
SLC: performed data analyses, edits to manuscript
CRF: provided ideas for research, provided funding for research, edits to manuscript

SWS: provided laboratory equipment and space for research to be conducted,
interpretation of data, edits to manuscript

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Data Accessibility

DNA sequences: GenBank accessions KC357321-KC357424, KC870942-KC871002,
KC900290 - KC900365
Sequence alignments and phylogenetic trees: Tree Base submission numbers 14129,
14137, 14138, and 14139
Raw microsatellite genotypes and Metadata (cryovial ID, sample ID, date, region and site
of collection, deep sea vehicle, dive number, coordinates, depth, collection method,
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Figure Legends

Figure 1: A, Aggregation of *Escarpia southwardae*, at the Worm Hole site on the West
African coast (Photo complements of IFREMER, WACS campaign, dive 430).. B,
Anterior portion, dorsal view of *E. southwardae* tubeworm, reprinted with permission
from Andersen et al., 2004. AR: axial rod; BL: branchial lamellae; CG: ciliated groves
(males); DG: dorsal groove; DVM: dorsal vestimental wings; O: obturaculum; T: trunk;
V: vestimentum. NRC Research Press License # 2960880817425

Figure 2: Gulf of Mexico (GoM) cold seep sites sampled for this study. 1000 meter
contours from NASA-JPL Advanced Spaceborne Thermal Emission and Reflection
Radiometer (<http://www.geomapapp.org>).

Figure 3: West African cold seep sites sampled for this study. 1000 meter contours from NASA-JPL Advanced Spaceborne Thermal Emission and Reflection Radiometer (<http://www.geomapapp.org>).

Figure 4: Median-joining haplotype networks of the COI, CYTB and HbB2i genes. Colors represent *Escarpia* regions (described species) (red: *E. laminata* from the Gulf of Mexico, blue: *E. southwardae* from west coast of Africa and green: *E. spicata* from the Gulf of California. Sizes of haplotype circles and are proportional to the number of individuals possessing the same sequence and each line represents one mutational change separating two haplotypes.

Figure 5: Top: STRUCTURE results (admixture model, three replicate runs) for three putative species of *Escarpia* vestimentiferan tubeworms based on nine microsatellite markers. Each vertical bar represents an individual tubeworm. The y-axis is the proportion of each individual's genotype belonging to a distinct population cluster. Bottom: Network topologies of *E. laminata* (red), *E. spicata* (green) and *E. southwardae* (blue) individuals with the Shared Allele Distance (SAD) based on nine microsatellite markers. Only links with value smaller than or equal to the percolation distances are present. Nodes (circles) represent individuals. Three clusters are identified, one for each of the described *Escarpia* species and their respective geographic regions.

A

B

mtCOI

mtCYTB

HbB2i

Dpe = 1.15

Dpe = 1.00

Dpe = 0.99

Table 1: Locations of seep sites sampled for this study. *N* refers to the number of individuals collected and region refers to location from which the collections were made: Gulf of California (GoC), Gulf of Mexico (GoM), West African Cold Seeps (WACS).

Provisional Species	Seep Name	Latitude	Longitude	Depth (m)	<i>N</i>	Region
<i>Escarpia spicata</i> <i>n</i> = 20	TF4	27.357° N	111.290° W	1784	20	GoC
<i>Escarpia laminata</i> <i>n</i> = 129	AC601	26.392° N	94.514° W	2335	12	GoM
	AC818	26.185° N	94.574° W	2744	8	GoM
	WR269	26.677° N	91.665° W	1975	7	GoM
	GC852	27.095° N	91.265° W	1437	11	GoM
	AT340	27.644° N	88.365° W	2204	11	GoM
	MC294	28.675° N	88.481° W	1800	8	GoM
	DC673	28.516° N	87.518° W	2604	42	GoM
	West Florida Escarpment	26.040° N	84.915° W	3304	30	GoM
<i>Escarpia southwardae</i> <i>n</i> = 80	Worm Hole	4.760° S	9.941° E	3189	25	WACS
	Baboon	4.937° S	9.949° E	3128	28	WACS
	Regab	5.798° S	9.711° E	3153	27	WACS

Table 2: Summary statistics for 28 microsatellite loci amplified in three described *Escarpia* species.

<i>Escarpia</i> taxa (9 loci)	Summary Statistics (means across all loci)							
	N_A	H_O	H_E	F_{IS}	AR	AR^{SE}	P_{AR}	P_{AR}^{SE}
<i>E. spicata</i>	8	0.690	0.780	0.194	4.750	0.376	2.71	0.447
<i>E. laminata</i>	13	0.681	0.753	0.149	4.870	0.490	3.24	0.668
<i>E. southwardae</i>	12	0.702	0.753	0.104	4.780	0.445	3.26	0.700
<i>Escarpia laminata</i> (11 loci)	Summary Statistics (means across all loci)							
	N_A	H_O	H_E	F_{IS}	AR	AR^{SE}	P_{AR}	P_{AR}^{SE}
AC601	7.3	0.615	0.686	0.171	5.120	0.747	0.260	0.102
AC818	5.1	0.602	0.693	0.191	4.460	0.560	0.300	0.104
WR269	5.7	0.658	0.725	0.182	5.260	0.688	0.230	0.094
GC852	6.7	0.626	0.682	0.151	5.100	0.598	0.400	0.132
AT340	6.6	0.680	0.720	0.083	4.990	0.594	0.330	0.108
MC388	6.4	0.670	0.773	0.179	5.160	0.629	0.540	0.175
DC673	10.8	0.645	0.702	0.186	5.250	0.664	0.380	0.106
WFE	9.7	0.612	0.702	0.194	5.250	0.644	0.420	0.126
<i>Escarpia southwardae</i> (16 loci)	Summary Statistics (means across all loci)							
	N_A	H_O	H_E	F_{IS}	AR	AR^{SE}	P_{AR}	P_{AR}^{SE}
Regab	8.7	0.618	0.658	0.100	8.630	0.904	1.420	0.310
Wormhole	9.4	0.685	0.709	0.081	9.440	1.151	1.530	0.390
Baboon	9.2	0.636	0.664	0.149	8.930	1.101	1.680	0.520

Abbreviations: number of alleles observed across all loci (N_A), mean observed (H_O) and expected (H_E) heterozygosity, mean Wright's Inbreeding Coefficient (F_{IS}), mean rarified allelic richness (AR) and standard error (AR^{SE}); private allele richness (P_{AR}) and standard error (P_{AR}^{SE}). Rarefied over 20 samples and means are not significantly different ($P > 0.05$).

Table 3: Summary of Analysis of Molecular Variance (AMOVA) conducted for each study under the Infinite Allele Model, F_{ST} . Significance (*) was tested using 1000 permutations. Results for Stepwise Mutation Model (R_{ST}) are congruent with IAM. Abbreviations: d.f, degrees of freedom; SS, sum of squares.

Study	Source of Variation	d.f.	SS	Variation (%)	p-value
<i>Escarpia</i> Question (Three species)	Within individuals	229	718.50	80.60	---
	Among individuals	226	838.40	7.30	0.001*
	Among populations	2	126.80	12.10	0.001*
<i>Escarpia laminata</i>	Within individuals	129	499.00	90.70	---
	Among individuals	121	558.01	8.90	0.001*
	Among populations	7	37.12	0.05	0.053
<i>Escarpia southwardae</i>	Within individuals	80	411.50	94.80	---
	Among individuals	77	437.29	0.49	0.002*
	Among populations	2	12.82	0.03	0.149

Figure S1: COI maximum likelihood (ML) tree for 42 *Escarpia* and five *Seepiophila*, with fragment size of 658bp. 34 *Escarpia* sequences are newly isolated. *Seepiophila jonesi* is the outgroup taxon, and GenBank accession numbers follow the species name. Bootstrap support (1000 replicates, Tamura-Nei model) is located either above or below the node. Scale is measured as number of substitutions per nucleotide site. ML and NJ produced congruent trees for all genes, and only the ML trees are reported here.

Figure S2: 16S maximum likelihood (ML) tree for 45 *Escarpia* and five *Seepiophila*, with fragment size of 435bp. 38 *Escarpia* sequences are newly isolated. *Seepiophila jonesi* is the outgroup taxon, and GenBank Accession numbers follow the species name. Bootstrap support (1000 replicates, Tamura-Nei model) is located either above or below the node. Scale is measured as number of substitutions per nucleotide site.

Figure S3: CYTB maximum likelihood (ML) tree for 39 *Escarpia* and seven *Seepiophila*, with fragment size of 401bp. All sequences are newly isolated. *Seepiophila jonesi* is the outgroup taxon, and GenBank Accession numbers follow the species name. Bootstrap support (1000 replicates, Tamura-Nei model) is located either above or below the node. Scale is measured as number of substitutions per nucleotide site.

Figure S4: HbB2i maximum likelihood (ML) tree for 43 *Escarpia* and five *Seepiophila*, with fragment size of 665bp. All sequences are new isolated. *Seepiophila jonesi* is the outgroup taxon, and GenBank Accession number follow the species name. Bootstrap support (1000 replicates, Tamura-Nei model) is located either above or below the node. Scale is measured as number of substitutions per nucleotide site.

Table S1: Estimates of evolutionary divergence between species of *Escarpia* for three mitochondrial genes and one nuclear intron region. The numbers of base substitutions per site, averaging over all sequence pairs between groups are shown for each marker. Analyses were conducted using the Maximum Composite Likelihood model, and standard error estimates are shown above the diagonal.

Gene: <u>COI</u>	n = 42			
	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>
<i>E.laminata</i>		0.000	0.000	0.033
<i>E.southwardae</i>	0.001		0.000	0.033
<i>E.spicata</i>	0.001	0.001		0.033
<i>S.jonesi</i>	0.108	0.108	0.108	

Gene: <u>CYTB</u>	n = 39			
	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>
<i>E.laminata</i>		0.003	0.001	0.632
<i>E.southwardae</i>	0.005		0.003	0.687
<i>E.spicata</i>	0.002	0.007		3.246
<i>S.jonesi</i>	0.145	0.127	0.134	

Gene: <u>16S</u>	n = 45			
	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>
<i>E.laminata</i>		0.000	0.000	0.007
<i>E.southwardae</i>	0.000		0.000	0.007
<i>E.spicata</i>	0.000	0.000		0.007
<i>S.jonesi</i>	0.021	0.021	0.021	

Gene: <u>HbB2i</u>	n = 43			
	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>
<i>E.laminata</i>		0.001	0.002	0.006
<i>E.southwardae</i>	0.002		0.002	0.005
<i>E.spicata</i>	0.004	0.002		0.005
<i>S.jonesi</i>	0.016	0.014	0.013	

Table S2: Locations, number of individuals and GenBank IDs for all tubeworms tested in each of the four genes.

Gene: <u>COI</u>	n = 42		
Population	Location	# of individuals	GenBank IDs
<i>E. spicata</i>	Transform Fault	8	KC357329 - KC357333, KC870959-KC870961
<i>E. laminata</i>	DC673	7	KC357322 - KC357328
	AT340	2	GU059203, GU059207
	AC601	3	GU059211, KC357321
	WR269	2	GU059199, GU059197
	GC852	1	GU059193
	AC818	2	GU059163, GU059189
<i>E. southwardae</i>	Regab	4	KC357334 - KC357336, KC870957
	Worm Hole	3	KC357337 - KC357343, KC870955, KC870956, KC870958
	Baboon	10	KC357344, KC870953 - KC870954
	Gene: <u>16S</u>	n = 45	
Population	Location	# of individuals	GenBank IDs
<i>E. spicata</i>	Transform Fault	10	KC357364 - KC357371 KC870942 - KC870943
<i>E. laminata</i>	DC673	7	KC357347 - KC357351
	AT340	2	GU068183, GU068187
	AC601	2	GU068195, GU068193
	WR269	3	GU068181, GU068179, KC357346
	GC852	1	GU068177
	MC294	1	KC357345
<i>E. southwardae</i>	Regab	7	KC357354 - KC357356, KC357358, KC357362, KC870944, KC870949
	Worm Hole	1	KC357357
	Baboon	11	KC357359 - KC357361, KC357363, KC870945 - KC870948, KC870950 - KC870952

Gene: <u>CYTB</u>	n = 39		
Population	Location	# of individuals	GenBank IDs
<i>E. spicata</i>	Transform Fault	10	KC357377 - KC357382 KC870970, KC870972, KC870974
<i>E. laminata</i>	DC673	8	KC357384 - KC357387 KC357391 - KC357393
	AC601	2	KC870968-KC870969
	WR269	2	KC870964, KC870967
	GC852	2	KC870963, KC870965
	AC818	2	KC870966, KC870971
	MC294	1	KC870962, KC870973
<i>E. southwardae</i>	Regab	5	KC357385 - KC357389
	Worm Hole	4	KC357384, KC870975-KC870977
	Baboon	3	KC357383, KC870978-KC870979
Gene: <u>HbB2i</u>	n = 43		
Population	Location	# of individuals	GenBank IDs
<i>E. spicata</i>	Transform Fault	10	KC57398 - KC57402, KC870997- KC871001
<i>E. laminata</i>	DC673	8	KC57413 - KC57420
	AT340	2	KC870983, KC870984
	AC601	2	KC870985, KC870986
	WR269	1	KC870987
	AC818	1	KC870989
	MC294	3	KC870982, KC870990
<i>E. southwardae</i>	Regab	5	KC57408 - KC57412
	Worm Hole	3	KC57403 - KC57407, KC870991- KC870993
	Baboon	8	KC870994 - KC870997

Table S3: Summary statistics for Tajima's D test of neutrality organized by gene and species. *S* is number of sites with substitutions; *Pi* is the mean number of pairwise differences. Distance method used is pairwise difference, no Gamma correction, indels not taken into account.

Gene: COI	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>	Mean	s.d.
Sample Size	8	12	5	4	7.250	3.594
S	1	3	0	1	1.250	1.260
Pi	1.071	0.636	0.000	40.000	10.426	19.720
Tajima's D	1.166	-1.180	0.000	1.633	0.405	1.259
p-value	0.926	0.108	1.000	0.960	0.748	0.428
Gene: 16S	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>	Mean	s.d.
Sample Size	18	20	16	10	16	4.320
S	0	1	0	0	0.250	0.500
Pi	0.000	0.100	0.000	0.000	0.0250	0.0500
Tajima's D	0.000	-1.164	0.000	0.000	-0.291	-0.582
p-value	1.000	0.144	1.000	1.000	0.786	0.428
Gene: CYTB	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>	Mean	s.d.
Sample Size	8	7	6	5	6.500	1.291
S	0	0	3	0	0.750	1.500
Pi	0.000	0.000	1.400	0.000	0.350	0.700
Tajima's D	0.000	0.000	0.338	0.000	0.084	0.169
p-value	1.000	1.000	0.655	1.000	0.913	0.175
Gene: hbB2i	<i>E.laminata</i>	<i>E.southwardae</i>	<i>E.spicata</i>	<i>S.jonesi</i>	Mean	s.d.
Sample Size	16	20	10	8	13.5	5.507
S	0	0	2	0	0.500	1.000
Pi	0.000	0.000	1.088	0.000	0.435	0.871
Tajima's D	0.000	0.000	1.742	0.000	0.435	0.871
p-value	1.000	1.000	0.977	1.000	0.994	0.011

Table S4: Summary statistics for nine microsatellite loci amplified in three described *Escarpia* species

Population		EL454_2	EL454_5	EL454_9	EL454_21	EL454_25	EL454_52	EL454_64	EL454_70	EL454_71	Mean
<i>E. spicata</i>	N_A	9	7	7	6	8	9	12	11	3	8
$N = 20$	H_O	0.900	0.700	0.842	0.300	0.850	0.667	0.684	0.667	0.600	0.690
	H_E	0.830	0.765	0.816	0.680	0.827	0.860	0.900	0.871	0.476	0.780
	F_{IS}	-0.083	0.085	0.040	*0.560	-0.028	0.317	0.282	0.325	0.308	0.194
<i>E. laminata</i>	N_A	12	15	19	9	12	16	14	18	4	13
$N = 129$	H_O	0.700	0.730	0.631	0.752	0.610	0.821	0.853	0.872	0.162	0.681
	H_E	0.725	0.735	0.920	0.800	0.593	0.886	0.862	0.915	0.342	0.753
	F_{IS}	0.039	0.010	*0.396	0.060	-0.009	0.072	0.067	0.081	0.751	0.149
<i>E. southwardae</i>	N_A	15	11	10	9	7	18	17	10	4	12
$N = 80$	H_O	0.825	0.862	0.605	0.710	0.740	0.873	0.720	0.712	0.276	0.702
	H_E	0.870	0.876	0.874	0.780	0.741	0.906	0.746	0.726	0.262	0.753
	F_{IS}	0.049	0.015	*0.350	0.154	0.005	0.051	0.127	0.019	0.210	0.104

Abbreviations: number of alleles observed per locus (N_A), observed (H_O) and expected (H_E) heterozygosity, Wright's Inbreeding Coefficient (F_{IS}). Negative values scaled to zero; * **bold** asterisk indicates a significant difference after applying FDR correction = 0.01

Table S5: Pairwise genetic differentiation matrix. F_{ST} values (below diagonal) and Jost's D (above diagonal, calculated in GenoDive) between the three described species of *Escarpia*; species are significantly different at $p = 0.001$. Both measures quantify genetic diversity differently and both suffer from limitations, and therefore it has been suggested to use both measures in concert (Meirmans and Hedrick, 2011). F_{ST} measures the level of heterozygosity, while Jost's D quantifies genetic diversity in terms of effective number of alleles (Jost, 2008; Ryman and Leimar, 2009). In this case, calculations of both metrics show similar relationships and patterns of differentiation between groups.

	<i>Escarpia laminata</i>	<i>Escarpia spicata</i>	<i>Escarpia southwardae</i>
<i>Escarpia laminata</i>	---	0.422	0.455
<i>Escarpia spicata</i>	0.115	---	0.501
<i>Escarpia southwardae</i>	0.130	0.134	---

Table S6: Primers used in this study

Gene	Primers	Forward (5' – 3')	Reverse (5' – 3')	Reference
COI	LCO HCO	GGT CAA CAA ATC ATA AAG ATA TT GG	TAA ACT TCA GGG TGA CCA AAA AA TCA	Folmer et al., 1994
16S	16sar 16sbr	CGC CTG TTT ATC AAA AAA CAT	ACG TGA TCT GAG TTC AGA CCG G	Palumbi, 1996
Cytb	CytbF CytbR	GGW TAY GTW YTW CCW TGR GGW CAR AT	GCR TAW GCR AAW ARR AAR TAY CAY TCW GG	Boore and Brown, 2000
CYTB	CYTBF CYTBR	AAA TCC TCC CTC ACA GTC ATT	CCA GCC CCG ATG AAT CTT TC	This study
hbB2 intron (<i>Riftia/Ridgeia</i>)	hbB2F hbB2R	CAG TTC ACT GGA CGT CGT CT	CTA TGC AGG CAG ACC ATC G	S. Carney, 2005 (dissertation)
hbB2i (<i>Escarpia</i>)	hbB2i_F hbB2i_R	TCC ATC GCC CCA GGC TGT CTT C	GCC TTG AAT TCG TTG CTG TT	This study

Table S7: Summary statistics for eleven microsatellite loci amplified in eight populations of *Escarpia laminata* (GoM).

Population		EL454_2	EL454_5	EL454_6	EL454_9	EL454_21	EL454_25	EL454_52	EL454_54	EL454_67	EL454_70	EL454_71	Mean
AC601	N_A	6	8	3	12	5	5	11	11	2	13	2	7.3
$N = 12$	H_O	0.583	0.833	0.454	0.750	0.750	0.420	0.750	0.920	0.111	0.920	0.000	0.615
	H_E	0.710	0.793	0.627	0.931	0.822	0.492	0.884	0.891	0.111	0.920	0.160	0.686
	F_{IS}	0.179	-0.050	0.392	0.195	0.088	0.154	0.152	-0.028	0.816	0.000	1.000	0.171
AC818	N_A	5	5	3	8	6	4	9	6	2	4	2	5.1
$N = 8$	H_O	0.625	0.750	0.500	0.750	0.625	0.625	0.750	0.500	0.375	0.750	0.143	0.602
	H_E	0.742	0.792	0.425	0.883	0.820	0.575	0.883	0.818	0.325	0.708	0.494	0.693
	F_{IS}	0.157	0.053	-0.176	0.151	0.235	-0	0.151	0.559	-0.154	-0.059	0.795	0.191
WR269	N_A	5	6	4	8	4	4	9	6	2	10	3	5.7
$N = 7$	H_O	0.571	0.857	0.333	1.000	0.714	1.000	0.571	0.800	0.285	1.000	0.167	0.658
	H_E	0.593	0.791	0.454	0.924	0.703	0.692	0.912	0.890	0.264	0.945	0.621	0.725
	F_{IS}	0.037	-0.083	0.519	0.082	0.016	-0.444	0.373	0.350	-0.083	-0.058	0.800	0.182
GC852	N_A	7	8	3	6	7	5	9	10	3	10	3	6.7
$N = 11$	H_O	0.545	0.818	0.400	0.500	0.730	0.600	1.000	0.700	0.222	1.000	0.100	0.626
	H_E	0.714	0.814	0.360	0.803	0.840	0.600	0.900	0.890	0.216	0.895	0.280	0.682
	F_{IS}	0.152	0.062	0.229	0.247	0.067	0.160	-0.009	0.160	0.171	0.171	0.250	0.151
AT340	N_A	6	7	5	11	5	4	6	8	4	10	2	6.6
$N = 11$	H_O	0.730	0.636	0.545	0.890	0.818	0.636	0.730	0.800	0.454	0.730	0.300	0.680
	H_E	0.684	0.805	0.467	0.930	0.775	0.671	0.818	0.895	0.400	0.880	0.395	0.720
	F_{IS}	-0.018	0.071	0.067	0.071	0.087	0.164	0.087	0.079	0.152	0.25	-0.004	0.083
MC294	N_A	6	6	1	8	7	6	8	7	3	8	3	6.4
$N = 8$	H_O	0.875	0.750	---	0.500	0.875	0.750	0.875	0.625	0.428	0.857	0.125	0.670
	H_E	0.833	0.620	---	0.910	0.883	0.683	0.920	0.883	0.384	0.890	0.610	0.773
	F_{IS}	-0.050	-0.216	1	0.450	0.009	-0.098	0.045	0.292	0.286	0.174	0.795	0.179
DC673	N_A	11	11	7	14	8	8	11	17	3	18	3	10.8
$N = 42$	H_O	0.833	0.762	0.440	0.641	0.714	0.571	0.890	0.750	0.187	0.880	0.286	0.645
	H_E	0.736	0.747	0.520	0.920	0.791	0.600	0.880	0.902	0.180	0.920	0.304	0.702
	F_{IS}	0.244	0.246	0.133	*0.151	0.127	0.223	0.202	0.169	0.197	0.158	0.105	0.186
WFE	N_A	9	15	6	13	7	8	12	13	3	17	3	9.7
$N = 30$	H_O	0.600	0.600	0.433	0.423	0.800	0.600	0.800	0.821	0.520	0.862	0.083	0.612
	H_E	0.724	0.639	0.450	0.910	0.821	0.580	0.883	0.910	0.427	0.930	0.230	0.702
	F_{IS}	0.248	0.249	0.189	*0.146	0.283	0.117	0.213	0.119	0.177	0.113	0.324	0.194

Abbreviations: number of alleles observed per locus (N_A), observed (H_O) and expected (H_E) heterozygosity, Wright's Inbreeding Coefficient (F_{IS}).

* **bold** asterisk indicates a significant difference after applying FDR correction = 0.01

Table S8: Pairwise genetic differentiation matrix. F_{ST} values (below diagonal) and Jost's D (above diagonal) between the eight populations of *Escarpia laminata* (GoM); populations are ordered from west to east and are not significantly different ($p > 0.05$). Both measures quantify genetic diversity differently and both suffer from limitations, and therefore it has been suggested to use both measures in concert (Meirmans and Hedrick, 2011). F_{ST} measures the level of heterozygosity, while Jost's D quantifies genetic diversity in terms of effective number of alleles (Jost, 2008; Ryman and Leimar, 2009). In this case, calculations of both metrics show similar relationships and patterns of differentiation between groups.

	AC601	AC818	WR269	GC852	AT340	MC388	DC673	WFE
AC601	---	0.054	0.019	0.030	0.017	0.079	0.001	0.029
AC818	0.014	---	0.000	0.058	0.032	0.045	0.057	0.086
WR269	0.007	0.007	---	0.000	0.000	0.000	0.000	0.018
GC852	0.016	0.006	0.000	---	0.009	0.048	0.003	0.014
AT340	0.020	0.018	0.000	0.000	---	0.010	0.005	0.019
MC388	0.048	0.036	0.027	0.024	0.021	---	0.037	0.020
DC673	0.007	0.016	0.000	0.000	0.003	0.020	---	0.000
WFE	0.018	0.024	0.000	0.005	0.011	0.007	0.000	---

Table S9: Summary statistics for sixteen microsatellite loci amplified in three populations of *Escarpia southwardae* (WACS).

Locus	Population											
	Worm Hole (n = 25)				Baboon (n = 27)				Regab (n = 28)			
	N_A	H_O	H_E	F_{IS}	N_A	H_O	H_E	F_{IS}	N_A	H_O	H_E	F_{IS}
ES454_8	6	0.480	0.601	0.201	6	0.555	0.606	0.154	6	0.555	0.556	0.001
ES454_10	11	0.916	0.845	-0.027	15	0.821	0.848	0.031	13	0.815	0.877	0.072
ES454_13	9	0.400	0.420	0.049	7	0.444	0.422	0.073	8	0.423	0.408	0.086
ES454_25	18	0.880	0.938	0.063	15	0.926	0.902	0.017	16	0.923	0.924	0.043
ES454_28	7	0.791	0.791	0.058	7	0.785	0.777	-0.010	8	0.777	0.773	-0.006
ES454_31	19	0.800	0.918	0.129	14	0.852	0.901	0.095	11	0.730	0.872	0.201
ES454_32	4	0.160	0.405	*0.606	2	0.035	0.035	0.000	5	0.296	0.273	-0.084
ES454_34	6	0.791	0.720	-0.025	5	0.714	0.732	0.025	7	0.777	0.737	-0.054
ES454_44	5	0.680	0.627	-0.083	5	0.740	0.643	-0.070	6	0.654	0.670	0.093
ES454_45	10	0.200	0.452	*0.558	14	0.296	0.660	*0.582	11	0.192	0.466	0.633
ES454_50	11	0.904	0.859	0.135	7	0.571	0.685	0.167	9	0.880	0.820	0.032
ES454_57	9	0.840	0.841	0.001	13	0.852	0.880	0.075	10	0.815	0.852	0.044
ES454_60	9	0.720	0.624	-0.153	6	0.481	0.441	0.032	7	0.407	0.420	0.028
ES454_64	6	0.682	0.644	0.159	6	0.428	0.443	0.032	4	0.308	0.337	0.230
ES454_71	16	0.913	0.902	0.080	16	0.958	0.861	0.066	15	0.640	0.846	0.314
ES454_95	6	0.800	0.754	-0.061	7	0.714	0.784	1.00	5	0.704	0.708	0.006
Mean	9.4	0.685	0.709	0.081	9.2	0.636	0.664	0.149	8.7	0.618	0.658	0.100

Abbreviations: number of alleles observed per locus (N_A), observed (H_O) and expected (H_E) heterozygosity, Wright's Inbreeding Coefficient (F_{IS}). * **bold** asterisk indicates a significant difference after applying FDR correction = 0.01

Table S10: Pairwise genetic differentiation matrix. F_{ST} values (below diagonal) and Jost's D (above diagonal) between the three populations of *Escarpia southwardae*. Populations are not significantly different at $p > 0.05$. Both measures quantify genetic diversity differently and both suffer from limitations, and therefore it has been suggested to use both measures in concert (Meirmans and Hedrick, 2011). F_{ST} measures the level of heterozygosity, while Jost's D quantifies genetic diversity in terms of effective number of alleles (Jost, 2008; Ryman and Leimar, 2009). In this case, calculations of both metrics show similar relationships and patterns of differentiation between groups.

	Worm Hole	Baboon	Regab
Worm Hole	---	0.014	0.008
Baboon	0.007	---	0.001
Regab	0.004	0.000	---

Table S11: BOTTLENECK summary statistics for *E. laminata* sites. T.P.M: Two Phase Model (Wilcoxon test, 1000 replicates). N: sample size, ko: observed number of alleles, He: Hardy-Weinberg heterozygosity, Heq: expected heterozygosity measured, S.D: standard deviation of mutation drift equilibrium distribution of heterozygosity; Prob: probability of obtaining the measured He in a sample from an equilibrium population (Piry et al., 1999).

Population: AC601

Under the T.P.M.

locus	N	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	24	6	0.71	0.759	0.067	-0.736	0.185
EL454_5	24	8	0.793	0.84	0.042	-1.098	0.126
EL454_6	22	3	0.628	0.503	0.138	0.899	0.188
EL454_21	24	5	0.822	0.698	0.085	1.46	0.007
EL454_25	24	5	0.493	0.7	0.081	-2.559	0.031
EL454_52	24	11	0.884	0.904	0.022	-0.918	0.16
EL454_70	24	13	0.917	0.93	0.017	-0.789	0.167
EL454_54	24	11	0.891	0.905	0.023	-0.578	0.231
EL454_67	18	2	0.111	0.319	0.153	-1.358	0.237
EL454_71	24	2	0.159	0.288	0.155	-0.831	0.347
EL454_9	24	12	0.931	0.916	0.025	0.596	0.286

Population: AC818

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	16	5	0.742	0.743	0.072	-0.023	0.392
EL454_5	16	5	0.792	0.741	0.068	0.739	0.297
EL454_6	16	3	0.425	0.53	0.124	-0.848	0.232
EL454_21	16	6	0.817	0.801	0.053	0.293	0.51

EL454_25	16	4	0.575	0.649	0.098	-0.762	0.202
EL454_52	16	9	0.883	0.905	0.023	-0.946	0.169
EL454_70	16	4	0.708	0.661	0.091	0.52	0.385
EL454_54	12	6	0.818	0.842	0.04	-0.588	0.314
EL454_67	16	2	0.325	0.316	0.151	0.061	0.556
EL454_71	14	2	0.495	0.324	0.15	1.133	0.259
EL454_9	16	8	0.883	0.879	0.03	0.146	0.581

Population: WR269

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	14	5	0.593	0.761	0.064	-2.616	0.042
EL454_5	14	6	0.791	0.818	0.049	-0.549	0.278
EL454_6	12	4	0.455	0.696	0.082	-2.934	0.036
EL454_21	14	4	0.703	0.673	0.084	0.36	0.467
EL454_25	14	4	0.692	0.675	0.088	0.202	0.586
EL454_52	14	9	0.912	0.922	0.019	-0.506	0.335
EL454_70	14	10	0.945	0.943	0.013	0.152	0.779
EL454_54	10	6	0.889	0.872	0.031	0.544	0.596
EL454_67	14	2	0.264	0.328	0.146	-0.442	0.474
EL454_71	12	3	0.621	0.562	0.118	0.502	0.466
EL454_9	12	8	0.924	0.918	0.02	0.317	0.69

Population: GC852

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	22	7	0.714	0.809	0.052	-1.827	0.059
EL454_5	22	8	0.814	0.846	0.041	-0.776	0.173

EL454_6	20	3	0.358	0.51	0.133	-1.148	0.185
EL454_21	22	7	0.84	0.812	0.048	0.577	0.34
EL454_25	20	5	0.6	0.718	0.078	-1.517	0.087
EL454_52	22	9	0.896	0.873	0.032	0.746	0.252
EL454_70	20	10	0.895	0.903	0.023	-0.38	0.316
EL454_54	20	10	0.889	0.9	0.029	-0.36	0.264
EL454_67	18	3	0.216	0.522	0.129	-2.366	0.055
EL454_71	20	3	0.279	0.506	0.133	-1.7	0.117
EL454_9	12	6	0.803	0.841	0.042	-0.915	0.157

Population: AT340

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	22	6	0.684	0.768	0.062	-1.363	0.107
EL454_5	22	7	0.805	0.811	0.051	-0.107	0.389
EL454_6	22	5	0.468	0.709	0.082	-2.943	0.017
EL454_21	22	5	0.775	0.701	0.088	0.835	0.201
EL454_25	22	4	0.671	0.626	0.104	0.434	0.427
EL454_52	22	6	0.818	0.769	0.064	0.769	0.226
EL454_70	22	10	0.879	0.893	0.026	-0.57	0.257
EL454_54	20	8	0.895	0.855	0.038	1.063	0.101
EL454_67	22	4	0.398	0.628	0.099	-2.308	0.036
EL454_71	20	2	0.395	0.306	0.158	0.558	0.414
EL454_9	18	11	0.928	0.93	0.017	-0.119	0.444

Population: MC388

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
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EL454_2	16	6	0.833	0.801	0.052	0.614	0.357
EL454_5	16	6	0.617	0.801	0.052	-3.552	0.013
EL454_6	16	1	monomorphic				
EL454_21	16	7	0.883	0.844	0.043	0.922	0.189
EL454_25	16	6	0.683	0.801	0.05	-2.364	0.041
EL454_52	16	8	0.917	0.878	0.032	1.187	0.061
EL454_70	14	8	0.89	0.895	0.027	-0.193	0.41
EL454_54	16	7	0.883	0.844	0.04	0.972	0.172
EL454_67	14	3	0.385	0.551	0.117	-1.423	0.167
EL454_71	16	3	0.608	0.528	0.125	0.645	0.322
EL454_9	16	8	0.908	0.879	0.033	0.89	0.165

Population: DC673

Under the T.P.M.

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	84	11	0.736	0.837	0.04	-2.516	0.028
EL454_5	84	11	0.748	0.833	0.043	-1.964	0.054
EL454_6	82	7	0.52	0.727	0.079	-2.599	0.021
EL454_21	84	8	0.791	0.762	0.069	0.435	0.412
EL454_25	84	8	0.596	0.765	0.065	-2.58	0.026
EL454_52	84	11	0.879	0.839	0.037	1.093	0.087
EL454_70	82	18	0.921	0.912	0.019	0.448	0.393
EL454_54	80	17	0.902	0.906	0.019	-0.226	0.348
EL454_67	64	3	0.177	0.428	0.156	-1.611	0.109
EL454_71	56	3	0.305	0.439	0.149	-0.908	0.208
EL454_9	78	14	0.92	0.879	0.028	1.492	0.011

Population: WFE*Under the T.P.M.*

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
EL454_2	60	9	0.724	0.807	0.051	-1.609	0.068
EL454_5	60	9	0.64	0.809	0.05	-3.373	0.016
EL454_6	60	6	0.447	0.699	0.086	-2.913	0.017
EL454_21	60	7	0.821	0.744	0.069	1.118	0.072
EL454_25	60	8	0.577	0.781	0.058	-3.529	0.007
EL454_52	60	12	0.883	0.866	0.031	0.554	0.327
EL454_70	58	17	0.929	0.918	0.017	0.63	0.292
EL454_54	56	13	0.91	0.879	0.028	1.097	0.099
EL454_67	50	3	0.427	0.441	0.153	-0.092	0.386
EL454_71	48	3	0.23	0.443	0.153	-1.389	0.138
EL454_9	52	13	0.907	0.883	0.03	0.788	0.19
EL454_9	52	13	0.907	0.883	0.03	0.788	0.19

Table S12: BOTTLENECK summary statistics for *E. southwardae* sites Regab, Wormhole and Baboon. T.P.M: Two Phase Model (Wilcoxon test, 1000 replicates). N: sample size, ko: observed number of alleles, He: Hardy-Weinberg heterozygosity, Heq: expected heterozygosity measured, S.D: standard deviation of mutation drift equilibrium distribution of heterozygosity; Prob: probability of obtaining the measured He in a sample from an equilibrium population (Piry et al., 1999).

REGAB

Under the T.P.M.							
locus	n	ko	He	Heq	S.D.	DH/sd	Prob
ES454_8	54	6	0.556	0.7	0.085	-1.696	0.064
ES454_10	54	13	0.878	0.882	0.028	-0.171	0.346
ES454_13	52	7	0.403	0.753	0.067	-5.226	0.001
ES454_25	52	16	0.924	0.91	0.031	0.443	0.337
ES454_28	54	8	0.773	0.785	0.06	-0.202	0.359
ES454_31	52	11	0.873	0.855	0.037	0.469	0.382
ES454_32	54	5	0.273	0.638	0.103	-3.558	0.006
ES454_34	54	7	0.738	0.75	0.073	-0.166	0.338
ES454_44	52	6	0.67	0.7	0.086	-0.345	0.293
ES454_45	52	11	0.467	0.855	0.037	-10.564	0
ES454_50	50	9	0.82	0.816	0.05	0.069	0.445
ES454_57	54	10	0.853	0.834	0.044	0.431	0.386
ES454_60	54	7	0.419	0.745	0.073	-4.488	0
ES454_64	52	4	0.337	0.556	0.129	-1.699	0.082
ES454_71	50	15	0.847	0.908	0.019	-3.242	0.01
ES454_95	54	5	0.708	0.64	0.111	0.608	0.303

WORMHOLE*Under the T.P.M.*

locus	n	ko	He	Heq	S.D.	DH/sd	Prob
ES454_8	50	6	0.601	0.704	0.086	-1.209	0.108
ES454_10	48	11	0.846	0.86	0.033	-0.44	0.262
ES454_13	50	8	0.42	0.784	0.063	-5.815	0.001
ES454_25	50	18	0.939	0.93	0.015	0.614	0.331
ES454_28	48	7	0.791	0.756	0.067	0.521	0.364
ES454_31	50	19	0.918	0.936	0.013	-1.347	0.099
ES454_32	50	4	0.406	0.559	0.13	-1.177	0.14
ES454_34	48	6	0.72	0.71	0.082	0.116	0.464
ES454_44	50	5	0.628	0.65	0.102	-0.215	0.329
ES454_45	50	10	0.452	0.838	0.043	-8.976	0
ES454_50	42	11	0.859	0.867	0.032	-0.233	0.324
ES454_57	50	9	0.841	0.817	0.046	0.511	0.371
ES454_60	50	9	0.624	0.816	0.048	-3.988	0.005
ES454_64	44	6	0.645	0.716	0.08	-0.894	0.16
ES454_71	46	16	0.902	0.919	0.019	-0.837	0.148
ES454_95	50	6	0.754	0.707	0.082	0.58	0.323

BABOON*Under the T.P.M.*

Locus	n	ko	He	Heq	S.D.	DH/sd	Prob
ES454_8	54	6	0.606	0.705	0.086	-1.146	0.128
ES454_10	56	15	0.848	0.9	0.024	-2.205	0.045

ES454_13	54	9	0.422	0.812	0.049	-8.013	0
ES454_25	54	15	0.902	0.904	0.021	-0.11	0.379
ES454_28	56	7	0.778	0.75	0.066	0.418	0.408
ES454_31	54	14	0.901	0.895	0.024	0.274	0.487
ES454_32	56	2	0.036	0.241	0.166	-1.236	0.164
ES454_34	56	5	0.732	0.636	0.105	0.919	0.157
ES454_44	54	5	0.643	0.642	0.11	0.012	0.414
ES454_45	54	14	0.66	0.893	0.028	-8.48	0.002
ES454_50	56	7	0.686	0.745	0.073	-0.818	0.181
ES454_57	54	13	0.88	0.883	0.028	-0.099	0.376
ES454_60	54	6	0.441	0.701	0.089	-2.922	0.02
ES454_64	56	6	0.443	0.702	0.087	-2.997	0.015
ES454_71	48	16	0.861	0.916	0.019	-2.985	0.01
ES454_95	56	7	0.784	0.746	0.072	0.521	0.356

Table S13: Summary of null allele frequencies for 28 loci across three described species of *Escarpia*. N_F refers to the null allele frequency and SE refers to the standard error. The Individual Inbreeding Model (IIM) was implemented in INEst v1.0 (Chybicki and Burczyk, 2009)

<i>Escarpia</i> taxa (9 loci)	Summary Statistics for null alleles (means across all loci)		
	N_A	N_F	SE
<i>E. spicata</i>	8	0.123	0.067
<i>E. laminata</i>	13	0.086	0.022
<i>E. southwardae</i>	12	0.058	0.028
<i>Escarpia laminata</i> (11 loci)	Summary Statistics for null alleles (means across all loci)		
	N_A	N_F	SE
AC601	7.3	0.153	0.082
AC818	5.1	0.179	0.103
WR269	5.7	0.203	0.111
GC852	6.7	0.132	0.076
AT340	6.6	0.093	0.059
MC294	6.4	0.173	0.101
DC673	10.8	0.151	0.041
WFE	9.7	0.117	0.046
<i>Escarpia southwardae</i> (16 loci)	Summary Statistics for null alleles (means across all loci)		
	N_A	N_F	SE
Regab	8.7	0.085	0.016
Wormhole	9.4	0.076	0.017
Baboon	9.2	0.100	0.016

From Cowart et al. 2012 (Table 2): Characteristics of 28 microsatellite loci developed for *Escarpia laminata* (EL) and *Escarpia southwardae* (ES)

Locus	Primers (5' – 3')	Repeat Motif	Size Range (bp)	T_m (°C)	$N_A N_A^3$	$H_o H_e$	$F_{IS} F_{IS}^3$
EL454_2*	F: GAGACACCGTACGAATGACAGA R: ACTGGAACCTATGGCACGGAC	(TGTGCGCG) ₁₉	135 - 297	56	12 31	0.670 0.720	0.037** 0.031
EL454_5*	F: TGCATGTATACGTGGAGTGAGA R: CCCTGCATTCTATAATTGCTT	(TACATGCA) ₁₈	204 - 288	53	15 30	0.751 0.750	0.007 0.018
EL454_6	F: TGTGCTTCTCAAACCACC R: CAAAAGGCTTTCTCGTCCAC	(CCACCAT) ₂₀	209 - 254	53	8	0.388 0.415	0.085
EL454_9*	F: TTATGTTCCCGTTGAAAGCC R: GTGGGCGCTATGTCTGATTT	(CACCACA) ₁₃	83 - 185	54	19 35	0.682 0.914	0.314** 0.284
EL454_21*	F: CTCAGCAGGAGGGTCAGTTC R: AACACACTGCTCTCTCGCAA	(GGAGGC) ₁₆	115 - 176	57	9 19	0.753 0.809	0.070** 0.108
EL454_25*	F: GTGCACTGTTGCATTATGGG R: CCATACATTCCATACCTTCG	(AGGGT) ₁₆	153 - 213	52	12 12	0.650 0.608	-0.021 -0.016
EL454_52*	F: CGATGATGCCAACTAAATAGGG R: GACGACATTTAAGACAGGCG	(TGTGTGGC) ₁₇	179 - 313	53	16 34	0.794 0.888	0.069 0.072
EL454_54	F: CGCCCTAAGCACTGTATTCC R: TATAGACGCCAGGTGGAACG	(CACACG) ₁₄	139 - 223	56	20	0.739 0.894	0.151
EL454_64*	F: AACACCAAACTGTGTCTCTCG R: ATGCGTGGAATGAAGGAGAC	(CATA) ₂₄	185 - 261	55	--- 20	0.750 0.835	--- 0.039
EL454_67	F: TGCAGCAGTTTCACGAATC R: ACGCTTCGTAGCACTGACCT	(TAT) ₁₅	92 - 125	57	4	0.323 0.286	-0.143
EL454_70*	F: GTGCCGACACTTGAGCACTA R: AGGCTAACCATGCAGTCACA	(TGC) ₁₆	100 - 185	57	26 33	0.874 0.886	0.035 0.054
EL454_71*	F: AATGCCAGATGTTTTCCCAA R: TGCAATGGCACACCTGTTAC	(GTT) ₁₉	115 - 199	54	4 8	0.150 0.397	0.518 0.254
ES454_8	F: CACTGGGAAAACGGTGAGTT R: CGCCTAGCTCTTTCTGAGGTT	(CATCACTA) ₂₃	125 - 198	56	9	0.530 0.589	0.096
ES454_10	F: AAGAACACGGACACGGACAC R: CGTCATCATCCTCACATTG	(CACACGCA) ₁₆	112 - 192	54	18	0.851 0.857	0.011
ES454_13	F: ATTCACCCACGCATTCTCTC R: GAGTGGGTGAGTGCACGAG	(ACACACGC) ₂₁	136 - 208	56	10	0.423 0.415	-0.020
ES454_25	F: GTGGCGTCAGATCCTCAGA R: CAATGGCAGTACTTCGGGAT	(GCACAC) ₁₃	109 - 193	56	21	0.910 0.922	0.012

ES454_28	F: ATGAATGGACTCATCCACGC R: CATGTTTTACCCTCTTATTACGTTT	(GTGCAT) ₂₄	114 - 162	53	11	0.785 0.780	-0.006
ES454_31	F: AATGAATTTCCCTCCCAAGG R: TTCGATTGATGTCATGTCCG	(ACACGC) ₂₈	131 - 207	53	22	0.794 0.900	0.116
ES454_32	F: GCGTGAGATTCCACCAGAAT R: CTCACGTGATGTATCGTCCG	(CACACG) ₁₄	108 - 168	55	5	0.164 0.240	0.302**
ES454_34	F: TATCTGTCTGCCTTCCTGCC R: GACAACCGTGGCTTTTGAAT	(CACGCA) ₂₃	109 - 133	55	7	0.761 0.729	-0.040
ES454_44	F: GCCCATTTGTTTATCGTGCT R: CGGGTCTACATAAAAGCTTG	(TGATGG) ₁₃	72 - 126	52	7	0.692 0.646	-0.071
ES454_45	F: GGGATGATAACAAGAAACGC R: TGTCTGTCTGTACGTCTGTTTG	(CAGAGG) ₁₃	140 - 251	53	23	0.230 0.532	0.569**
ES454_50	F: ACTGCACAAGATACGATGCG R: CTAAGTCTCGTTTTCGGCGT	(GCGAT) ₂₀	95 - 325	55	13	0.785 0.788	0.130
ES454_57	F: ACGCACTCACTCACGCAG R: GGGTTGGTGTTTGTGGAA	(GTGC) ₂₈	118 - 202	56	16	0.836 0.858	0.027
ES454_60	F: TCCTCGTCAGACAATCCAAA R: CCCAACCACGGGACACTAC	(TCCA) ₃₄	152 - 200	56	10	0.536 0.494	-0.083
ES454_64	F: CACACAAATGACATCCTAGCG R: GCGCATGTGTGCGTGTAT	(AGAC) ₂₀	104 - 137	55	8	0.473 0.475	0.010
ES454_71	F: AATGGGTACACAATACCGCC R: AGGCAGATGAAACGGAGTGT	(TGTC) ₂₅	127 - 199	56	24	0.837 0.871	0.042
ES454_95	F: CGCGTTCCTCACTATCACAA R: CTGGTGAGCAGATGAAAGCA	(TTA) ₁₉	124 - 148	55	8	0.739 0.749	0.016

*Loci amplified across three *Escarpia* species, T_m = Annealing temperature, N_A = number of observed alleles

N_A^3 = number of observed alleles across three species, H_o = observed heterozygosity, H_e = expected heterozygosity

F_{IS}^3 = F_{IS} values across three species

**Significant deviation from HWE after FDR correction = 0.01