Spatial Distribution of *Eudactylina* sp. (Copepoda: Siphonostomatoida: Eudactylinidae) Infecting Gill of Angel Sharks (*Squatina* sp.) Captured in the Northeastern Gulf of Mexico

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ABSTRACT

Parasite spatial distribution studies shed light on host-parasite relationships especially in regards to host and site specificity. This is especially true for parasitic copepods that infect demersal fishes. The objective of this study was to examine the relationship between the angel shark, *Squatina* sp. within the Gulf of Mexico and an unnamed species of parasitic copepod (*Eudactlyina* sp.) that infects its gills. Infection prevalence was 88.5% and the population of copepods is overdispersed (aggregated) in its host and not uniformly distributed between male and female sharks. Copepods were found to infect particular hemibranchs and exhibited regional attachment across horizontal and longitudinal positions on hemibranchs and across vertical positions of the gill lamellae. Additionally, copepods were attached perpendicular to or facing water flow. The number of eggs per copepod was similar across all attachment regions and hemibranch positions. In summary, my results indicate a high level of microhabitat site specificity by copepods and copepod orientation relative to respiratory water flow is similar to that reported for other parasitic copepods inhabiting sharks.

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INTRODUCTION

Copepoda is one of the most species-rich taxa within Crustacea (Brusca and Brusca, 2003) and collectively displays extensive morphological, ecological, and life-history diversity (Boxshall and Halsey, 2004). Copepods are common in marine and freshwater ecosystems, where they play important ecological roles as food for small fishes, consumers of small organisms, parasites and pathogens of aquatic invertebrates and vertebrates, and intermediate hosts for parasites, some of which are associated with human disease (Kabata, 1979; Ho, 2001; Piasecki et al., 2004).

Siphonostomatoida (Copepoda) includes about 1,400 species (Boxshall, 2015) and together its representatives infect a wide variety of marine invertebrates and vertebrates (Kabata, 1981; Ho, 2001) as well as a small number of freshwater fishes (Boxshall and Halsey, 2004). On fishes, most siphonostomes (Siphonostomatoida) are ectoparasites that can infect virtually all parts of a fish (general body surface and fins, buccal chamber, branchial chambers and gills, olfactory chambers, and cloaca), wherein they can display appreciable levels of host and site specificity (Kabata, 1970; Benz, 1986; Benz and Bullard, 2004; Caira and Healy, 2004).

Host and site specificity are two important phenomena displayed by parasites. Host specificity is the predilection of a parasite species to infect specific hosts while site specificity is the predilection of a parasite species to infect particular regions on or in a host (Bush et al., 1997). These two phenomena are often considered ecological characteristics but each likely is determined by a complex set of factors that together operate from molecular to ecosystem levels (Bush et al., 2001). Aside from a few model

pathogen-host systems, especially including sea lice (Caligidae) and salmonid (Salmonidae) hosts, we know strikingly little about how the vast majority of parasitic copepods interact with their hosts in nature.

Site specificity studies treating parasitic copepods have primarily focused on parasites that infect the body surfaces and gill of teleosts (Teleostei) (e.g., van den Broek, 1979; Voorhees and Schwartz, 1979; Bron et al., 1991; Jaworski and Holm, 1992; Hallett and Roubal, 1995; Tirard et al., 1996). Regarding elasmobranchs (Elasmobranchii), such studies have primarily focused on copepods that infect the gill (Benz, 1980, 1986; Benz and Dupre, 1987; Benz and Adamson, 1990; Dippenaar et al., 2008, 2009), with Benz (1986) and McElwain et al. (2010) providing the only quantitative reports on infections of the body surfaces and olfactory sacs, respectively. Results of the aforementioned studies revealed various levels of site specificity and, in one instance (McElwain et al., 2010), facilitated the formulation of hypotheses regarding host colonization, parasite development, and reproduction. Although pattern-based studies of ecological processes are generally steeped in assumptions (Cale et al., 1989), reports such as those mentioned above for elasmobranchs are important because they pertain to large and vagile species and processes that are currently impractical to study directly in the open ocean and unlikely to be investigated in captive environments due to financial constraints.

Eudactylina van Beneden, 1853 (Siphonostomatoida: Eudactylinidae) currently comprises 45 species (36 were named in peer-reviewed works, 9 were named in an unpublished dissertation of Gregory Deets [see Deets, 1994]), all of which exclusively infect the gills of elasmobranchs (Boxshall and Halsey, 2004; Izawa, 2011). Little is known about the infection patterns of these parasites; however, Dippenaar et al. (2009)

found an uneven infection intensity across and about hemibranchs, and a predominately upstream orientation with respect to the putative pattern of respiratory water flow in *E. pusilla* Cressey, 1967, a species that infects tiger sharks, *Galeocerdo cuvier* Péron & Lesueur, 1822 (Carcharhinidae, Carcharhiniformes).

This study investigated the host-parasite relationship between an angel shark species (*Squatina* sp.) residing in the Gulf of Mexico and its *Eudactylina* species, primarily focusing on:

1) identification of the parasite infecting the gills of an angel (*Squatina* sp.) shark in the Gulf of Mexico;

2) assessment of possible relationships between infection prevalence, abundance, and intensity (considering each of six copepod groups: all copepods, all females, ovigerous females, non-ovigerous females, males, and larvae) and each of the following: host sex, host size (total length, mass), body side of shark (left vs. right), hemibranch position, attachment location about a hemibranch, and attachment location regarding functionally distinct gill filament regions;

3) assessment of copepod body orientation (regarding the six copepod groups noted above) relative to the theoretical water flow over the gills at parasite attachment locations, and;

4) assessment of possible relationships between the number of eggs per *Eudactylina* sp. ovisac (right, left, right and left combined) regarding hemibranch position and parasite attachment locations about a hemibranch.

In addition, to facilitate the aforementioned analyses, assessments were carried out regarding possible relationships between the number of gill filaments and estimated mean

gill filament length and each of the following: shark sex, shark size (total length, mass), body side of shark (left vs. right), hemibranch position, and regions about a hemibranch.

MATERIALS AND METHODS

For the copepod distribution analyses, 26 angel sharks were captured from 2010 through 2012 in the Gulf of Mexico at depths of 104−436 meters using a bottom trawl (Table 1, Figure 1A). After assessing the copepod distribution, the heads were discarded. Later, additional angel sharks captured from the same geographic region (Table 2; cf. Figure 1A and 1B) were obtained for tooth counts because they were inconsistent with the diagnosis of *Squantina dumeril* Lesueur, 1818, the angel shark species reported at this depth in the Gulf of Mexico. In the field, sharks were sexed, measured (total length, nearest mm), weighed (nearest kg if sharks were not weighed, mass was estimated using simple linear regression), humanely killed if not already dead, decapitated; heads were tagged and preserved in 10% buffered formalin until further processing (Table 1).

To assess copepod attachment locations, left and right gill arches were individually removed and their hemibranchs were processed (dorsal to ventral) using a stereomicroscope as follows. Each gill filament was sequentially numbered and its length (distance from its origin on the gill arch to its free distal tip) was traced on a hemibranch specific outline made with the help of a drawing tube. As copepods were collected each was assigned an identification number and the location of its cephalothorax (attachment location) along the gill filament was recorded on the hemibranch map. Calibration of hemibranch maps facilitated the length of gill filaments to be estimated (nearest mm) as well as the distance between the gill filament origin and each copepod's attachment location (nearest mm) (estimates were made from scanned

Table 1. Data for 26 angel sharks, *Squatina* sp., (used for copepod distribution analyses) captured in the Gulf of Mexico between 2010 and 2012 using a bottom trawl; locations pertain to position where trawl operations commenced; -- denotes missing data.

Capture date	Location[a]	Depth (m)	Fork length (cm)	Mass (kg)	Sex
18 November 2010	13	130	39	--	♀
			46	--	♀
	11	177	37.5	0.3	♂
			41.4	0.4	♂
			39	--	♀
21 October 2011	5	156	107.5	8.1	♂
	2	147	103.7	7.7	♂
23 October 2011	7	149	51.3	1.9	♀
			53.4	2.12	♂
31 October 2011	8	255	85	5.1	♀
			91.5	6.2	♂
			92	6.1	♀
			94	6.5	♂
			95	6.1	♂
3 November 2011	12	161	50.5	1.1	♀
			99	7.9	♂
6 November 2011	14	225	100	7.5	♂
8 November 2011	15	206	97.8	7.1	♂
9 November 2011	16	144	36.8	0.4	♂
13 October 2012	1	430	64.2	1.9	♀
			78.4	2.4	♀
20 October 2012	4	104	47.7	0.8	♂
22 October 2012	3	118	60.8	1.6	♀
	6	128	63.5	1.9	♂
26 October 2012	10	436	91.4	6.3	♀
	9	380	98.6	7.9	♀

[a] Locations (latitude, longitude) correspond to dots (from left to right) on Figure 1 and coordinates are as follows: location 1 (26º29’89” N, 96º16’81” W), location 2 (27º50’05” N, 93º35’83” W), location 3 (27º57’27” N, 93º24’30” W), location 4 (27º59’75” N, 93º09’20” W), location 5 (27º56’45” N, 92º43’07” W), location 6 (28º02’61” N, 91º35’40” W), location 7 (28º00’65” N, 90º57’62” W), location 8 (29º38’40” N, 87º13’00” W), location 9 (29º29’91” N, 87º12’31” W), location 10 (29º29’08” N, 86º53’43” W), location 11 (28º56’57” N, 85º42’11” W), location 12 (28º58’44” N, 85º37’55” W), location 13 (28º56’48” N, 85º29’98” W), location 14 (27º27’30” N, 84º45’33” W), location 15 (26º41’70” N, 84º10’30” W), location 16 (26º27’44” N, 84º06’19”).

Table 2. Data for 34 angel sharks, *Squatina* sp., (used for tooth counts) captured in the Gulf of Mexico between 2010 and 2012 using a bottom trawl; locations pertain to position where trawl operations commenced; -- denotes missing data.

Capture date	Location[a]	Depth (m)	Total length (cm)	Mass (kg)	Sex	Upper jaw	Lower jaw
17 November 2010	16	75	46	--	♀	10–10	10–10
			54	--	♀	10–10	10–10
			97	--	♀	10–10	10–10
			100	--	♀	10–10	10–10
			101	--	♀	10–10	10–10
11 October 2011	4	90	45	1.28	♀	10–10	10–10
			48	1.5	♀	10–10	10–10
15 October 2011	9	203	974	7	♀	10–10	10–10
18 October 2011	5	95	937	6.6	♀	10–10	10–10
			956	6.8	♀	10–10	10–10
20 October 2011	6	288	534	2.1	♀	10–10	10–10
23 October 2011	11	144	102	7.5	♀	10–10	10–10
30 October 2011	12	129	91	6.3	♂	10–10	10–10
31 October 2011	13	78	96	7.2	♀	10–10	10–10
	14	255	97	7.3	♂	10–10	10–10
			97	7.3	♂	10–10	10–10
3 November 2011	17	198	750	3.7	♀	10–10	10–10
			960	7.7	♀	10–10	10–10
	18	198	92	6.4	♀	10–10	10–10
	19	163	47	0.9	♀	10–10	10–10
	20	157	42	0.6	♀	10–10	10–10
18 October 2012	1	120	41	0.5	♀	10–10	10–10
			49	0.9	♀	10–10	10–10
20 October 2012	7	59	--	--	--	10–10	10–10
	8	104	422	0.5	♀	10–10	10–10
			439	0.7	♀	9–9	10–10
			445	0.7	♂	9–9	10–10
			477	0.8	♂	9–9	10–10
22 October 2012	2	128	475	--	♀	9–9	10–10
			635	--	♂	10–10	10–10
	10	102	35	0.4	♂	9–9	10–10
27 October 2012	3	227	617	1.7	♀	10–10	10–10
			856	4.7	♀	10–10	10–10
	15	161	995	6.6	♂	10–10	10–10

[a] Locations (latitude, longitude) correspond to dots (from left to right) on Figure 2 and coordinates are as follows: location 1 (27º53’97”, 9º43’294”); location 22º80’26”, 91º35’40”); location 3 (29º38’23”, 86º49’96”); location 4 (27º01’19”, 96º38’67”); location 5 (27º42’26”, 95º48’32”); location 6 (27º48’35”, 94º09’17”); location 7 (28º14’60”, 93º16’56”); location 8 (27º59’75”, 93º09’20”); location 9 (27º53’15”, 91º45’18”); location 10 (28º05’17”, 91º37’96”); location 11 (27º58’83”, 91º13’52”); location 12 (29º19’37”, 87º57’29”); location 13 (29º48’37”, 87º18’68”); location 14 (29º38’40”, 87º13’00”); location 15 (29º53’65”, 86º54’38”); location 16 (29º48’65”, 86º17’74”); location 17 (29º06’94”, 85º54’30”); location 18 (29º16’49”, 85º45’13”); location 19 (29º04’50”, 85º42’94”); location 20 (28º59’90”, 85º37’78”).

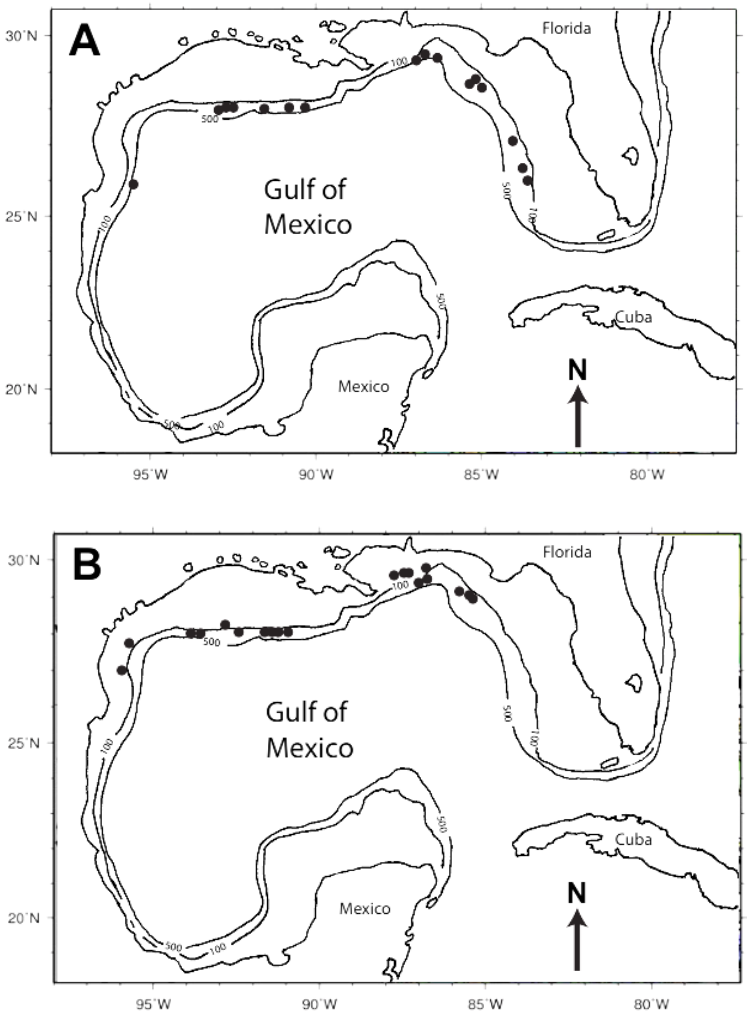

Figure 1. Gulf of Mexico bathymetric maps (depth in meters) showing sample locations (dots) where angel sharks, *Squatina* sp., were captured (between 2010 and 2012) using a bottom trawl. A. Sharks used for parasite distribution study. B. Sharks used for tooth counts. Tables 1 and 2 provide latitude and longitude coordinates of sample locations.

hemibranch maps using NIH ImageJ, National Institute Health, Bethesda, MD). The attachment locations of copepods were assessed vertically, longitudinally, and horizontally. To assess the vertical attachment location of copepods, gill filaments were divided into seven distinctive regions differing by function: 1) capping tissue (which surrounds the efferent arteriole), 2) upper half of gill lamella proximal surface (i.e., surface facing the gill arch), 3) lower half of gill lamella proximal surface, 4) upper half of gill lamella distal surface (i.e., surface facing the free distal gill filament tip), 5) lower half of gill filament distal surface, 6) tissue surrounding the corpus cavernosum between adjacent gill lamellae on the same gill filament, and 7) the excurrent water channel (non-respiratory surface between two gill filaments) (Figure 2A). Accordingly, copepod vertical attachment locations (location of the copepod's maxillipeds to a gill lamella) were recorded along with the longitudinal body axis orientation relative to theoretical respiratory water flow (see Benz, 1984). To assess where copepods attached along the longitudinal axis of the gill filament, gill filaments were divided into quadrants based on their lengths from their gill arch origin to their free distal tip (Figure 2B). As copepods were encountered, they were assigned to a percentile calculated by attachment distance from the gill filament origin divided by gill filament length. Another set of quadrants, based on the number of gill filaments (dorsal to ventral) per hemibranch (Figure 2C), was used to assess horizontal attachment locations. All copepods were sexed and female copepods were noted as being ovigerous or non-ovigerous. If ovigerous, the number of eggs in the left and right ovisacs was recorded.

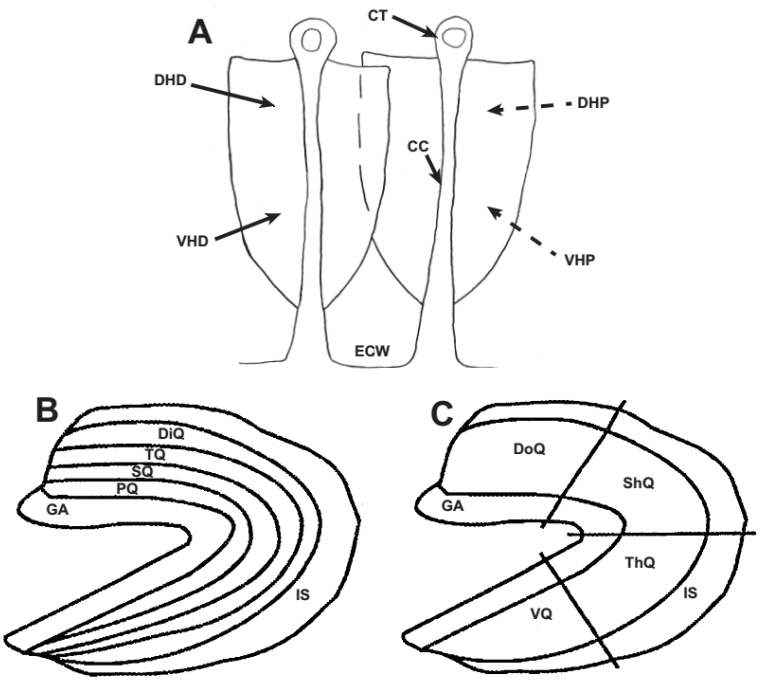

Figure 2. Conventions used in assessing the vertical, longitudinal, and horizontal distributions of *Eudactylina* sp. on the gills of *Squatina* sp. A. Cross section through two adjacent gill filaments showing the seven potential regions of parasite attachment used in the vertical distribution analysis. B. Lateral view of hemibranch showing divisions used to analyze longitudinal attachment distribution. C. Lateral view of hemibranch showing divisions used to analyze horizontal attachment distribution. CC = tissue surrounding the corpus cavernosum between adjacent gill lamellae on the same gill filament, CT = capping tissue surrounding the efferent arteriole, DHD = upper half of gill lamella distal surface (i.e., surface facing the free distal gill filament tip), DHP = upper half of gill lamella proximal surface (i.e., surface facing the gill arch), DiQ = distal gill filament quadrant, DoQ = dorsal hemibranch quadrant, EWC = non-respiratory tissue forming the excurrent water channel between two gill filaments, GA = gill arch, IS = interbranchial septum, PQ = proximal gill filament quadrant, ShQ = second hemibranch quadrant, SQ = second gill filament quadrant, ThQ = third hemibranch quadrant, TQ = third gill filament quadrant, , VHD = lower half of gill lamella distal surface, VHP = lower half of gill lamella proximal surface, VQ = ventral hemibranch quadrant. Figure 1A modified from Benz (1984). Figure 1B and 1C modified from Dippenaar et al. 2009.

Taxonomic Analysis

Copepods were removed from the gill, preserved in 70% ethanol, and compared (using stereo and compound microscopes) with descriptions of species of *Eudactylina* by Pearse, (1950), Deets (1994), Diebakate and Raibaut (2000), and Izawa (2011). Select copepods were stained, cleared in a solution of lignin pink and lactic acid, dissected, and studied using the wooden slide technique of Humes and Gooding (1964). Copepod illustrations were made with the assistance of a drawing tube.

Statistical Analyses

Statistical analyses were performed using Microsoft Excel for Student's *t*-tests, Chi-square goodness of fit, and simple linear regression analyses and GraphPad (GraphPad Software, Inc., La Jolla, CA) for ANOVA and Tukey's HSD test.

Infection Parameter Analyses

Infection prevalence, the percentage of hosts infected by a particular parasite species (Bush et al., 1997), was calculated for three host groups (all sharks, female sharks, male sharks) regarding each of the following six copepod groups: all copepods, all females, ovigerous females, non-ovigerous females, all males, and larvae as:

$$P_{ia} = x_{ia} / n_{ia} \times 100$$

where:

P_{ia} = prevalence of parasite group a in host i,

x_{ia} = number of sharks in host group i infected by parasite group a, and

n_{ia} = number of sharks in host group i examined for parasite group a.

Abundance of infection (abundance; Bush et al., 1997), the number of parasite individuals (regarding each of the aforementioned six copepod groups) in the sample of sharks (considering each of the aforementioned shark groups), was calculated as:

$$A_{ia} = \Sigma\, x_{1\text{-}n}$$

where:

A_{ia} = abundance of host group *i* regarding parasite group *a*,

$\Sigma\, x_{1\text{-}n}$ = number of individuals of parasite group *a* infecting individual shark $x_{1\text{-}n}$ in the sample population, and

n = number of sharks in the sample population.

Intensity of infection (intensity; Bush et al., 1997), the number of parasite individuals (regarding each of the aforementioned copepod groups) observed in the sample of infected sharks (considering each of the aforementioned shark groups), was calculated as:

$$I_{ia} = \Sigma\, x_{1\text{-}n}$$

where:

I_{ia} = intensity of host group *i* regarding parasite group *a*,

$\Sigma\, x_{1\text{-}n}$ = number of individuals in parasite group *a* infecting individual shark x_{1-n} in the sample of infected sharks, and

n = number of infected sharks in the sample population.

Chi-square goodness of fit tests were used to assess the possible aggregation in infection abundance and intensity and host sex, left vs. right hemibranchs, hemibranch position, and vertical, longitudinal and horizontal attachment locations. Simple linear regression analyses (SLRA) were used to assess possible relationships between copepod abundance and intensity and host size. Confidence intervals for copepod prevalence, intensity, and

abundance were calculated using algorithms of Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005).

Copepod Orientation Relative to Water Flow

A Chi-square goodness of fit test was used to assess relationships between copepod body orientations relative to the theoretical flow of water over the gills at the parasite attachment locations.

Copepod Fecundity Analyses

Chi-square goodness of fit tests were used to assess the frequencies between egg numbers per ovigerous copepod (left vs. right ovisacs and left and right ovisacs combined) and copepod attachment across hemibranch position and horizontal, longitudinal, and vertical attachment locations.

Gill Filament Measurement and Meristic Analyses

To determine the estimated total length of all gill filaments for each hemibranch, gill filaments were sorted into bins of ten or less filaments. The first and last gill filaments of a hemibranch as well as the middle gill filament of each bin were measured. The middle gill filament measurements were multiplied by the number of gill filaments in their respective bins to arrive at a total length estimate of the gill filaments in each bin. The total of all such estimates per hemibranch was used as an estimate of total length of all gill filaments per hemibranch. Chi-squared tests were used to assess possible relationships between the number of gill filaments and shark sex and body side of shark (left vs right) as well as possible relationships between the numbers of gill filaments per hemibranch. Student's *t*-tests were used to assess possible relationships between the estimated total gill filament length per hemibranch and shark sex and body side of shark.

Simple linear regression analysis was used to assess possible relationships between the number of gill filaments, estimated total gill filament length per hemibranch and shark size. Analysis of variance (ANOVA) was used to assess possible differences between estimated total gill filament length per hemibranch and hemibranch position as well as the estimated total gill filament length of hemibranch quadrants.

RESULTS

Taxonomic Analysis

All copepods were identified as an unknown *Eudactylina* sp. (an unnamed species, new to science) based upon the following morphological criteria. A description of the adult female follows (based on 3 individuals; Figures 3 & 4). Body (Figure 3A) about 1.4 mm long (including caudal rami). Cephalothorax longer than wide, dorsally covered with cuticular flaps and with lateral notches to accommodate the movement of maxillae. Four well-defined thoracic somites dorsally covered with cuticular flaps. Somites 2 and 3 each slightly longer than prior somite. Somite 4 shorter than somite 3. Genital complex and abdomen ventrally covered with cuticular flaps; abdomen 2 segments, caudal rami issue from apex of terminal segment lateral to anal indentation. Cadual rami (Figure 4G) ventrally covered with cuticular flaps and bearing 4 setae; apical setae somewhat more robust, lateral and medial setae thin. Ovisacs containing uniserate egg arrangement.

Antennule (Figure 3B) distinctly 4 segments; all setae spiniform or otherwise naked; proximal segment with short seta; second segment with 4 short setae plus long seta and stout claw-like seta; penultimate segment with 5 thin and 1 thick setae; apical segment with 14 setae. Antenna (Figure 3C) 5 segments; all setae naked; proximal segment small, unarmed; second segment bearing small spine; third segment with cuticular flaps and 2 thin setae extending from stout spine; penultimate segment bearing seta at midlength; apical segment with 2 setae and terminal claw with stout seta. Mouth tube typical for genus. Maxillule (Figure 3D) biramous; endopod with long denticulated seta and short naked seta; exopod with long seta bearing 2 small setae. Maxilla (Figures 3E & 3F)

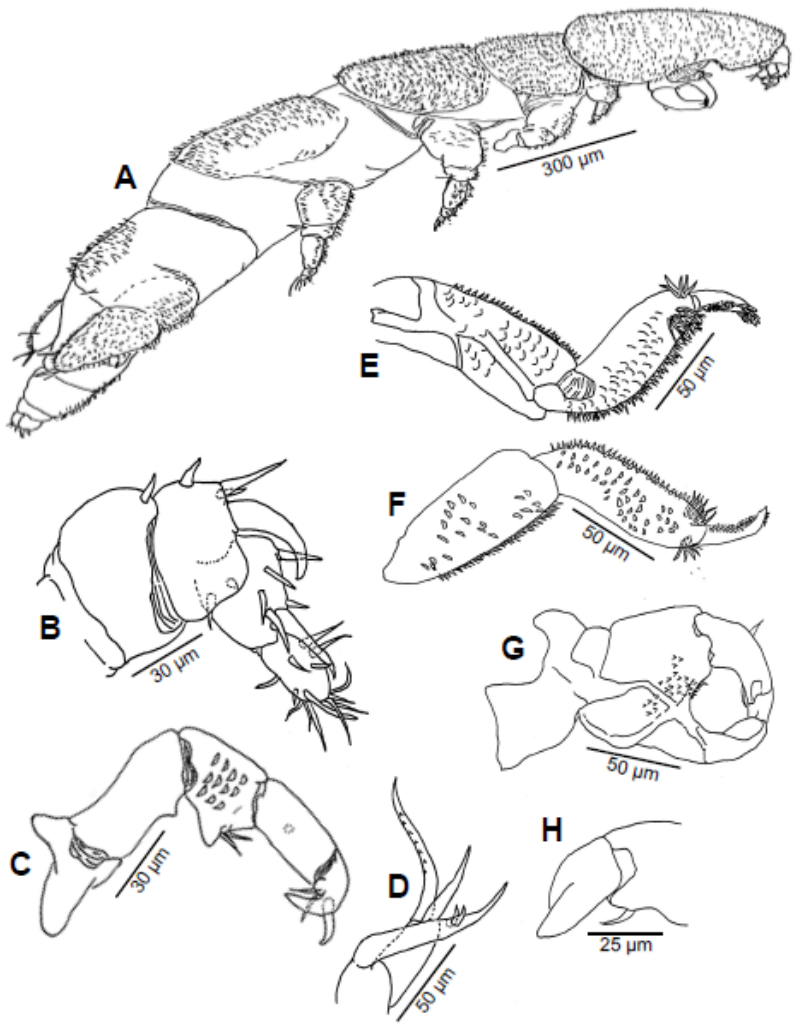

Figure 3. *Eudactlyina* sp., female; A. general habitus, right lateral view; B. antennule, anterodorsal view; C. antenna, anterodorsal view; D. maxillule, left lateral view; E. maxilla, right lateral view; F. maxilla, left lateral view; G. maxilliped, lateral view; H. maxilliped, tip of claw. Illustrations modified from G.W. Benz (unpublished).

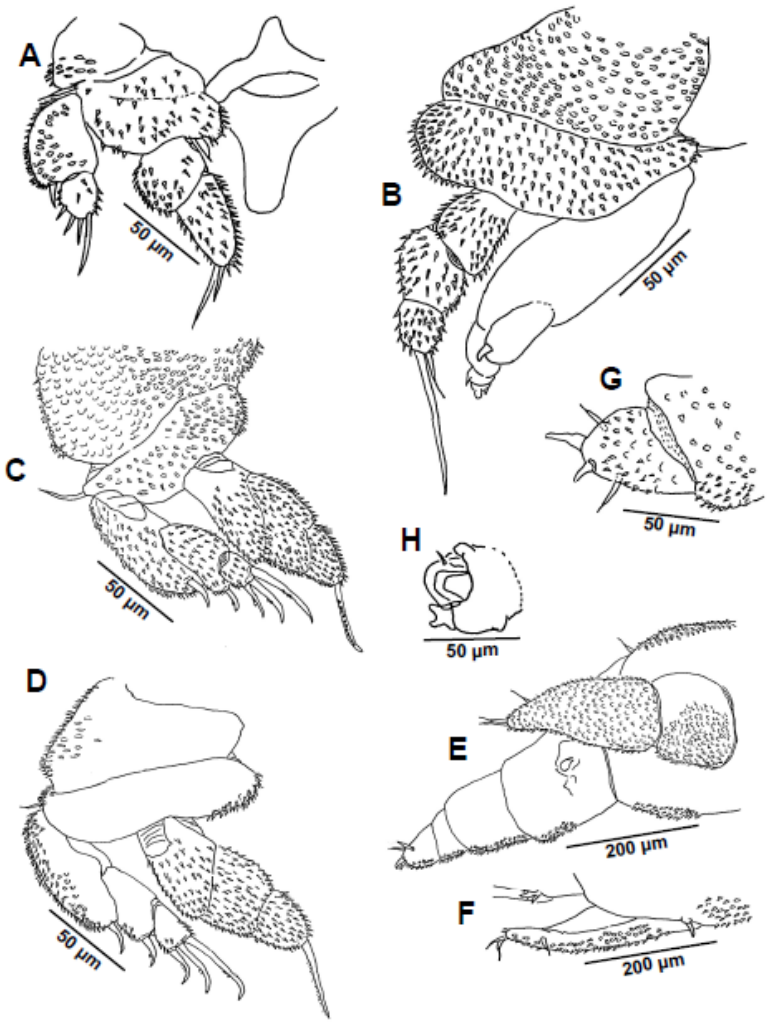

Figure 4. *Eudactylina* sp., female; A. leg 1, anterior face ventral view; B. leg 2, anterior face ventral view; C. leg 3, anterior face ventral view; D. leg 4, anterior face ventral view; E. body posterior, right lateral view; F. leg 5, dorsal view; G. caudal ramus, ventral view; H. leg 6, oviduct orifice region. Illustrations modified from original drawings by G.W. Benz.

brachiform; lacertus bearing lateral cuticular flaps and dorsal spinules; brachium bearing ventral spinules, lateral cuticular flaps, and 2 tufts of setules; calamus denticulated distally. Maxilliped (Figure 3G) chelate; corpus with spinules; myxal region elongated with spoon-shaped tip; shaft hook-shaped with 2 setae (Figures 3G & 3H).

Legs 1–4 (Figures 4A–4D) with sympods each comprised by a coxa and basis; coxa of legs 1 and 4 partially covered with ventral cuticular flaps and lateral spinules; coxa of legs 2 and 3 densely covered with ventral cuticular flaps and lateral spinules; leg 1 basis with ventral cuticular spinules, one lateral and one medial setae; basis of legs 2–3 densely covered with ventral cuticular flaps and lateral spinules and bearing single lateral seta; leg 4 basis with lateral cuticular spinules and lateral seta. Leg 1 (Figure 4A) biramous, bimerous, all segments with cuticular spinules; legs 2–4 (Figures 4B–4D) biramous, trimerous. Leg 1 exopod (Figure 4A) with naked seta on first segment and 3 short and 1 long setae on second segment. Leg 1 endopod (Figure 4A) first segment without seta, second segment with 2 setae of dissimilar lengths. Leg 2 exopod (Figure 4B) lacking cuticular spinules, first segment long, robust, bearing distolateral process with apical spine, second segment short and unarmed, apical segment complex, bearing three naked elements. Leg 2 endopod (Figure 4B) segments with cuticular spinules, first and second segments without seta, apical segment with 2 setae of dissimilar lengths. Leg 3 (Figure 4C) segments with cuticular spinules; exopod first segment with distolateral seta, second segment with denticulated seta, apical segment small, with 3 denticulated setae; first and second endopod segments lacking seta, apical endopod segment with long denticulated seta. Leg 4 (Figure 4D) exopod and endopod similar to those of leg 3. Leg 5 (Figures 4E & 4F) comprised of 2 segments, both laterally covered with cuticular flaps, first segment

lacking seta, second segment with 2 apical and 1 subapical spiniform setae. Leg 6 (Figure 4H) defines oviducal orifice, with small seta and several blunt projections.

Gill Filament Measurements and Meristic Analyses

There was no difference in shark size (length analysis $t = 1.001$, $df = 24$, $P = 0.32$; mass analysis $t = 1.29$, $df = 24$, $P = 0.21$), estimated total length of gill filaments per shark ($t = 0.29$, $df = 24$, $P = 0.78$), or number of gill filaments per shark ($t = -0.86$, $df = 24$, $P = 0.4$) between male and female sharks. Shark length and mass were positively related as longer sharks had more mass ($r^2 = 0.98$, $n = 26$, $P < 0.001$; Figure 5). Shark size was positively related to the estimated total gill filament length per shark as larger sharks had longer gill filaments (shark length vs. gill filament length analysis $r^2 = 0.91$, n = 26, $P < 0.001$; shark mass vs. gill filament length analysis $r^2 = 0.87$, $n = 26$, $P < 0.001$; Figure 6). However, the number of gill filaments per shark was variable and not related to shark size (shark length vs. number of gill filaments per shark analysis $r^2 = 0.31$, $n = 26$, $P = 0.73$; shark mass vs. number of gill filaments per shark analysis $r^2 = 0.24$, $n = 26$, $P = 0.15$). Left and right hemibranchs had the same number of gill filaments (number of gill filaments analysis $t = -1.71$, $df = 25$, $P = 0.17$) and gill filaments were the same length (estimated total length of gill filaments analysis $t = -0.36$, $df = 25$, $P = 0.73$). The estimated total length of gill filaments per hemibranch ($F = 5.35$, $df = 8$, $P < 0.001$) and the number of gill filaments per hemibranch ($F = 39.73$, $df = 8$, $P < 0.001$; Figure 7) was not the same considering hemibranch position. Tukey's HSD analyses defined groups of similar hemibranchs regarding the two last mentioned variables (Figure 7, Appendix 1). Lastly, the estimated mean length of gill filaments among horizontal quadrants was the

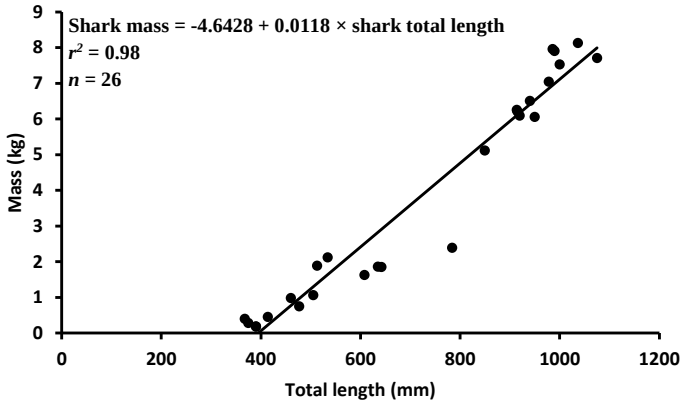

Figure 5. Simple linear regression analysis of shark total length versus shark mass for 26 angel sharks, *Squatina* sp., captured in the Gulf of Mexico.

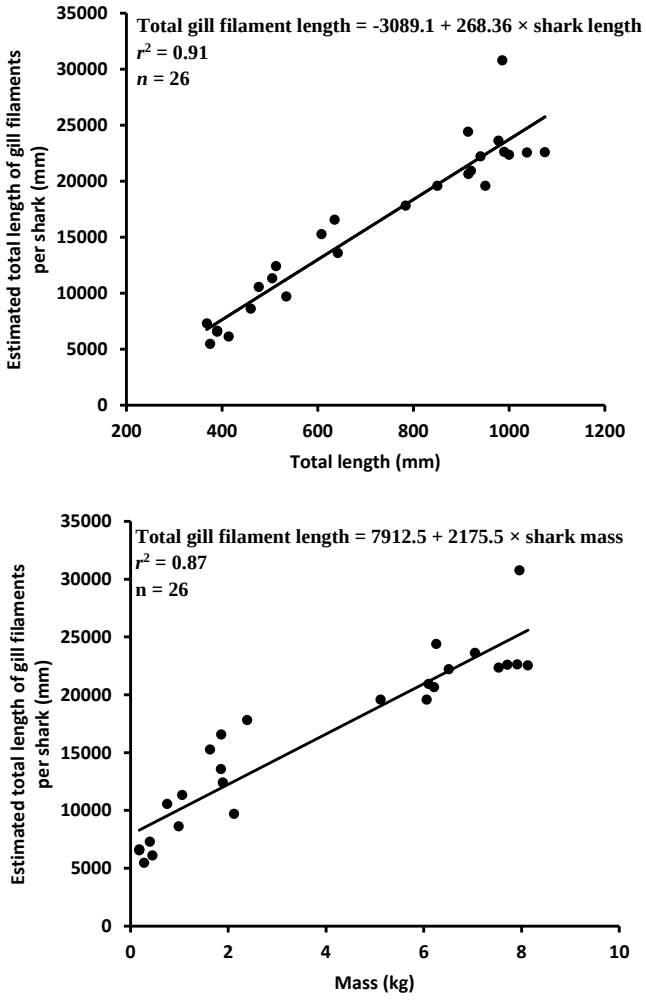

Figure 6. Simple linear regression analysis of estimated total length of gill filaments per shark versus shark total length (top) and shark mass (bottom) for 26 angel sharks, *Squatina* sp., captured in the Gulf of Mexico.

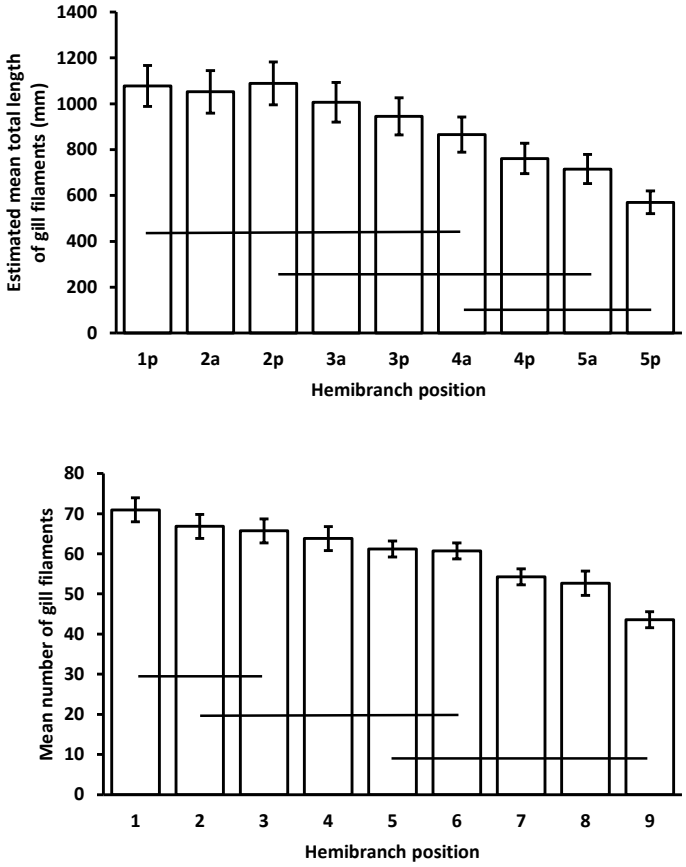

Figure 7. Estimated total mean length (±$SE\bar{x}$) of gill filaments per hemibranch position (top) and mean number of gill filaments (±$SE\bar{x}$) per hemibranch position (bottom) for 26 angel sharks, *Squatina* sp., collected in the Gulf of Mexico. Horizontal lines indicate statistically similar groups revealed by Tukey's HSD pairwise analyses.

same (F = 2.48, df = 3, P = 0.07; Figure 8). However, a trend indicated gill filaments in ventral quadrants may be shorter than those in dorsal, second, and third quadrants.

Infection Parameter Analyses

A total of 233 specimens of *Eudactylina* sp. (76 males, 46 ovigerous females, 104 non-ovigerous females, 7 larval females) were attached to the gill lamellae of 26 angel sharks. No attached specimens were observed elsewhere. An additional 92 specimens were observed as dislodged specimens, not attached to the host.

Female copepods attached by grasping the lateral edges of gill lamellae and typically spanned the lateral edges of several lamellae. Males typically infected the interlamellar water channels by grasping the central region of a lamella. In all cases, larval females attached by grasping the lateral edges of gill lamellae as described above for non-larval females and a male always grasped the larva dorsally in what appeared to be a precopulatory embrace (Benz and Adamson, 1990). Total copepod prevalence was 88.46%, female copepod prevalence was 80.77%, and male copepod prevalence was 76.92% (Table 3).

Considering female versus male sharks, copepod prevalence values were greater on female sharks regarding all copepods, female copepods, non-ovigerous copepods, and male copepods; however, on male sharks the prevalence of ovigerous and larval copepods was over twice that for female sharks (Table 4).

The number of copepods per shark was not dependent on shark size (shark total length vs. total copepod abundance r^2 = 0.34, n = 26, P = 0.11; shark mass vs. total copepod abundance r^2 = 0.37, n = 26, P = 0.77; shark total length vs. total copepod intensity r^2 = 0.36, n = 23, P = 0.12; shark mass vs. total copepod intensity r^2 = 0.38, n =

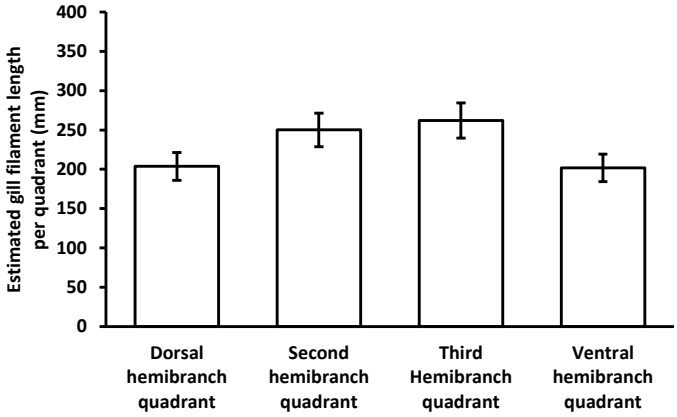

Figure 8. Estimated mean gill filament length ($\pm SE\bar{x}$) per horizontal hemibranch quadrant for 26 collected angel sharks, *Squatina* sp., collected in the Gulf of Mexico.

Table 3. Prevalence, mean intensity, and mean abundance of *Eudactylina* sp. (males, ovigerous females, non-ovigerous females, larval females, total females, and all copepods) collected from gill lamellae of 26 angel sharks, *Squatina* sp., captured in the Gulf of Mexico. CI = 95% confidence intervals for each measurement.

	Prevalence (CI)	Mean intensity (CI)	Mean abundance (CI)
Males	76.92 (0.56–0.91)	3.3 (2.4–7.0)	2.92 (1.77–5.73)
Ovigerous females	46.15 (0.27–0.67)	2.3 (2.58–7.50)	2.04 (1.04–3.85)
Non-ovigerous females	76.92 (0.56–0.91)	4.52 (3.6–8.05)	4.0 (2.54–6.35)
Larval stage females	26.92 (0.12–0.48)	0.39 (1.0–1.57)	0.35 (0.12–0.58)
Total females	80.77 (0.61–0.93)	7.17 (5.19–12.48)	6.35 (4.0–10.46)
All copepods	88.46 (0.69–0.98)	10.22 (6.52–15.74)	9.04 (5.69–14.19)

Table 4. Prevalence and mean intensity of *Eudactlyina* sp. (males, ovigerous females, non-ovigerous females, larval stage females, total females, and total copepods) collected from gill lamellae of 14 male and 12 female angel sharks, *Squatina* sp., in the Gulf of Mexico. CI = 95% confidence interval for measurements.

	Male sharks		Female sharks	
	Prevalence (CI)	Mean intensity (CI)	Prevalence (CI)	Mean intensity (CI)
Males	71.43 (0.42–0.92)	4.42 (2.8–11.6)	83.33 (0.52–0.98)	2.09 (1.3–3.3)
Ovigerous females	64.29 (0.35–0.87)	3.5 (2.33–8.44)	25.0 (0.5–0.57)	0.91 (2.0–4.67)
Non-ovigerous females	71.43 (0.42–0.92)	5.33 (3.7–11.6)	83.33 (0.51–0.98)	3.64 (2.5–7.2)
Larval stage females	35.71 (0.13–0.65)	0.58 (1.0–1.6)	16.67 (0.02–0.48)	0.18 (0.0–0.0)
Total females	78.57 (0.49–0.95)	9.42 (5.73–17.18)	83.33 (0.52–0.98)	4.73 (3.2–10.8)
Total copepods	85.71 (0.57–0.98)	13.33 (7.33–22.75)	91.67 (0.62–0.99)	6.82 (4.45–12.64)

23, $P = 0.7$), the estimated total length of gill filaments per shark ($r^2 = 0.26$, $n = 26$, $P = 0.45$), or total number of gill filaments per shark ($r^2 = 0.07$, $n = 26$, $P = 0.18$).

Excluding larval females, frequencies of copepods were unequal among male and female sharks (male copepod analysis $\chi^2 = 11.84$, $df = 1$, $P < 0.001$; ovigerous copepod analysis $\chi^2 = 20.55$, $df = 1$, $P < 0.001$; non-ovigerous copepod analysis $\chi^2 = 5.54$, $df = 1$, $P < 0.018$; female copepod analysis $\chi^2 = 22.55$, $df = 1$, $P < 0.001$; total copepod analysis $\chi^2 = 30.74$, $df = 1$, $P < 0.001$; Table 4). Male sharks hosted nearly twice as many copepods as female sharks based on the total copepod and all copepod sub-groups except ovigerous females and larval females. Considering the latter two sub-groups, male sharks hosted almost three times more copepods than female sharks.

Copepods infecting sites on corresponding left versus right hemibranchs showed no preference to side (Table 5). However, copepods did show preference considering hemibranch position (Figures 9–13). Regarding vertical distributions on gill lamellae, female copepods preferred infection sites on upper regions but male copepods showed no regional preference for infection sites (Table 6). Regarding horizontal and longitudinal distributions, male and female copepods preferred infection sites among horizontal quadrants (Table 7) and male copepods preferred infection sites among longitudinal quadrants (Table 8). Also, copepods preferred longitudinal positions along the length of gill filaments (Table 9) and Tukey's HSD of copepod mean attachment distances revealed significant differences within all copepod groups (Appendix 2). Male and female copepods showed different preferences regarding infection sites along the length of gill filaments as male copepods were located more distally located from the gill arch

Table 5. Observed frequencies of *Eudactylina* sp. for left, right, and combined left and right hemibranchs and Chi-square goodness of fit statistic values (and corresponding *df* and *P* values) for left and right hemibranch intensity comparisons.

	Left	Right	Left and Right	χ^2	*df*	*P*
1st posterior	9	6	15	0.6	1	0.44
2nd anterior	14	5	19	4.26	1	0.05
2nd posterior	20	13	33	1.48	1	0.22
3rd anterior	23	17	40	0.9	1	0.34
3rd posterior	14	9	23	1.09	1	0.3
4th anterior	23	24	47	0.02	1	0.88
4th posterior	13	11	24	0.17	1	0.68
5th anterior	11	9	20	0.2	1	0.66
5th posterior	5	7	12	0.33	1	0.56

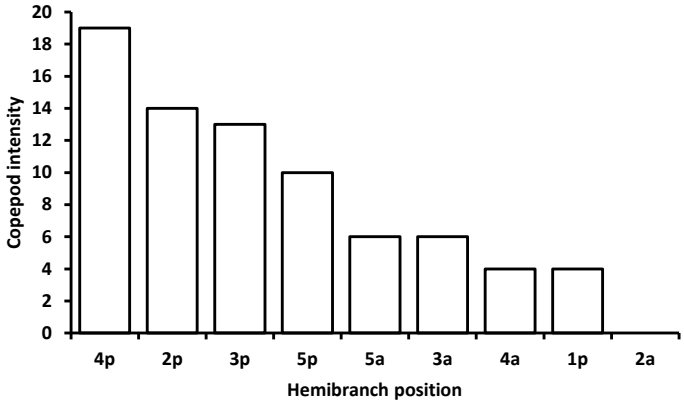

Figure 9. Observed frequencies of male *Eudactylina* sp. per hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test (χ^2 = 34.13, df = 8, P < 0.001).

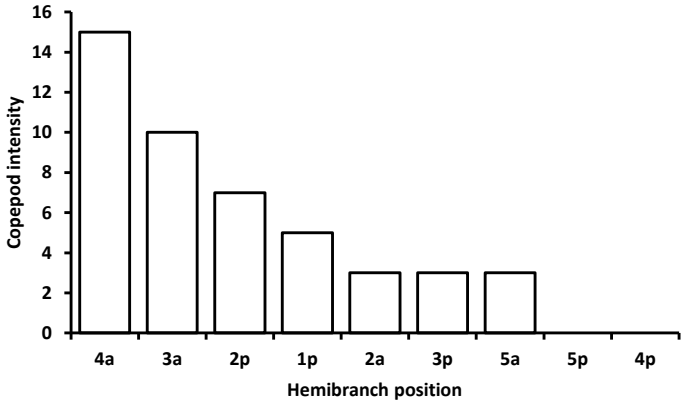

Figure 10. Observed frequencies of ovigerous female *Eudactylina* sp. per hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test ($\chi^2 = 37.35$, $df = 8$, $P < 0.001$).

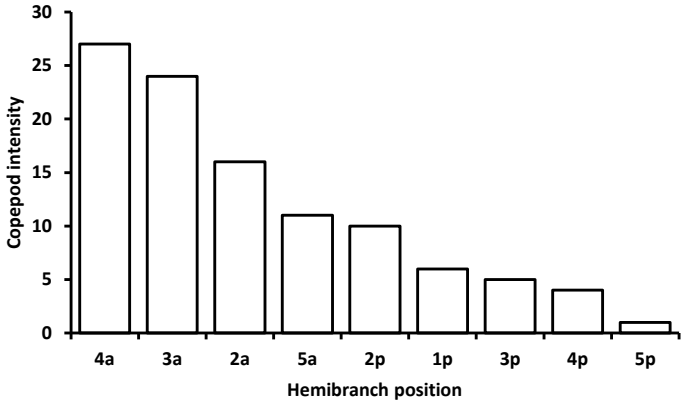

Figure 11. Observed frequencies of non-ovigerous female *Eudactylina* sp. per hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test ($\chi^2 = 57.0$, $df = 8$, $P < 0.001$).

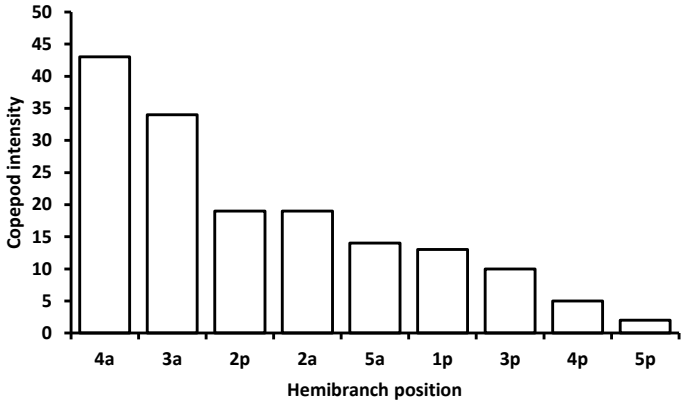

Figure 12. Observed frequencies of total female *Eudactylina* sp. per hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test ($\chi^2 = 79.92$, $df = 8$, $P < 0.001$).

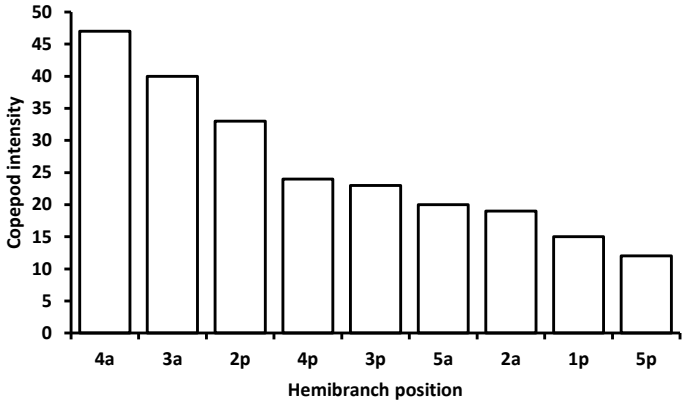

Figure 13. Observed frequencies of total *Eudactylina* sp. per hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test ($\chi^2 = 40.65$, $df = 8$, $P < 0.001$).

Table 6. Observed vertical frequencies of *Eudactylina* sp. (males, females, and total copepods) on gill lamellae of angel sharks, *Squatina* sp., and Chi-square goodness of fit statistic values (and corresponding *df* and *P* values) for intensity comparisons among various gill lamellae regions. Gill lamellae regions as follows: upper half of gill lamella proximal surface (i.e., surface facing the gill arch), lower half of gill lamella proximal surface, upper half of gill lamella distal surface (i.e., surface facing the free distal gill filament tip), and lower half of gill filament distal surface.

	Region of gill lamellae				χ^2	*P*
	Upper half proximal	Upper half distal	Lower half proximal	Lower half distal		
Males	21	24	14	16	3.32	0.35
Females	71	58	11	17	68.03	< 0.001
Total copepods	93	82	25	33	60.34	< 0.001

Table 7. Observed frequencies of *Eudactylina* sp. (males, females, and total copepods) per horizontal quadrant (dorsal, second, third, and ventral) and Chi-square goodness of fit statistic values (and corresponding *df* and *P* values) for quadrant intensity comparisons.

	Hemibranch quadrants				χ^2	*df*	*P*
	Dorsal	Second	Third	Ventral			
Males	5	18	22	31	18.42	3	< 0.001
Females	30	46	57	24	17.29	3	< 0.001
Total copepods	35	64	79	55	17.42	3	< 0.001

Table 8. Observed frequencies of *Eudactylina* sp. (males, females, and total copepods) per longitudinal quadrant (proximal, second, third, and distal) and Chi-square goodness of fit statistic values (and corresponding *df* and *P* values) for quadrant intensity comparisons.

	Hemibranch quadrants				χ^2	*df*	*P*
	Proximal	Second	Third	Distal			
Males	2	26	27	21	21.37	3	< 0.001
Females	34	51	43	29	7.25	3	0.06
Total copepods	36	77	70	50	18.07	3	< 0.001

Table 9. Mean (±*SD*) *Eudactylina* sp. (males, ovigerous females, non-ovigerous females, total females, and total copepods) attachment distances (mm) along longitudinal hemibranch quadrants (proximal, second, third, and distal) and one-way ANOVA statistic values (and associated *df* and *P* values).

	Hemibranch quadrants				*F*	*df*	*P*
	Proximal	Second	Third	Distal			
Males	3.73 (1.91)	6.59 (2.14)	11.36 (3.29)	14.07 (4.54)	22.85	75	< 0.001
Ovigerous females	3.73 (1.55)	7.81 (2.82)	11.48 (3.83)	16.00 (4.52)	21.21	43	< 0.001
Non-ovigerous females	2.57 (1.58)	6.47 (2.16)	10.96 (3.36)	14.42 (5.14)	67.6	112	< 0.001
Total females	2.81 (1.62)	6.81 (2.39)	11.18 (3.43)	15.49 (4.86)	96.73	156	< 0.001
Total copepods	2.86 (1.56)	6.89 (2.19)	11.20 (3.37)	14.81 (4.63)	128.01	232	< 0.001

than females (male copepod mean attachment distance = 10.27 (± 4.6), female copepod mean attachment distance = 8.65 (± 5.27), $t = 2.29$, $df = 231$, $P = 0.02$).

Copepod Orientation to Water Flow

A significant majority of total copepods, total female copepods, and male copepods were attached to gill lamellae in positions perpendicular to or facing the putative flow of water over the gills (Table 10).

Copepod Fecundity Analyses

Left and right ovisacs contained the same number of eggs ($\chi^2 = 0.03$, $df = 1$, $P = 0.86$). Copepod fecundity did not differ considering vertical ($\chi^2 = 11.0$, $df = 3$, $P = 0.99$), horizontal ($\chi^2 = 1.12$, $df = 3$, $P = 0.77$), or longitudinal ($\chi^2 = 3.49$, $df = 3$, $P = 0.32$) attachment locations (APPENDIX C). Although the frequencies of eggs per ovigerous copepod were unequal across all hemibranch positions (i.e., infected and uninfected hemibranchs; $\chi^2 = 59.52$, $df = 8$, $P < 0.001$), copepod fecundity did not differ among hemibranch positions (Figure 14).

Table 10. Observed frequencies of *Eudactylina* sp. (males, females, and total copepods) regarding attachment orientation (positive, negative, and perpendicular)[a] on gill lamellae of angel sharks, *Squatina* sp., relative to the theoretical flow of water over the gills and Chi-square goodness of fit statistic values (and corresponding *df* and *P* values) for attachment orientation analyses.

	Attachment orientation			χ^2	*df*	*P*
	Positive	Negative	Perpendicular			
Male	23	13	40	14.7	2	0.002
Female	63	32	62	11.9	2	0.008
Total copepod	86	45	102	22.3	2	< 0.001

[a] Attachment orientation as follows: positive indicates a copepod's cephalothorax facing towards, negative indicates a copepod's cephalothorax facing away from, and perpendicular indicates a copepod's cephalothorax is facing neither toward or away from the putative flow of water over the gills.

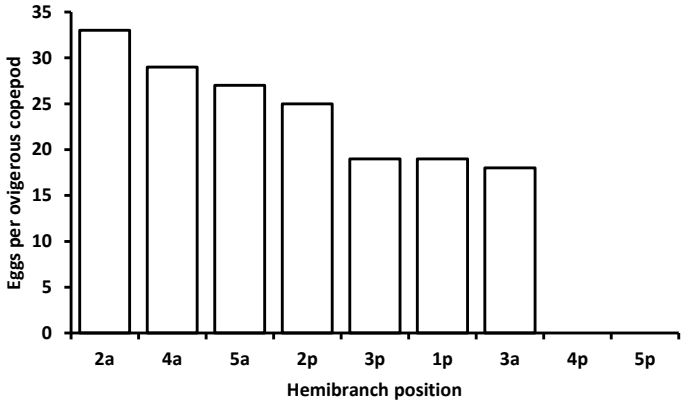

Figure 14. Eggs per ovigerous *Eudactylina* sp. based on hemibranch position of angel sharks, *Squatina* sp., captured in the Gulf of Mexico. Unequal frequencies of copepod intensity among hemibranch positions indicated by Chi-square goodness of fit test (χ^2 = 59.52, df = 8, P < 0.001).

DISCUSSION

This is the first report of new collections of *Eudactlyina* copepods from angel sharks (*Squatina* sp.) in North American waters since Pearse (1950) originally described *Eudactylina spinula* from female specimens collected from the Atlantic angel shark, *S. dumeril*, captured off the coast of North Carolina. Morphological characteristics of the female copepod described herein do not precisely match those of Pearse's description of *E. spinula* or any other *Eudactylina* spp. (cf. description provided above with information contained in Pearse (1950), Deets (1994), Diebakate and Raibaut (2000), and Izawa (2011)). The female copepod described herein can be distinguished from the female *E. spinula* by the number of antennule segments and bimerous condition of its leg 1. The bimerous condition of leg 1 is shared with five other female *Eudactylina* species: *E. dasyati* Izawa, 2011 hosted by the red stingray, *Dasyatis akajei* (Müller et Henle, 1841), from the Pacific ocean off the coast of Japan; "*E. epaktolampter*" Deets, 1994 hosted by the smooth lanternshark, *Etmopterus pusillus* Lowe, 1839, from the Gulf of Mexico; *E. gymnuri* Izawa, 2011 hosted by the Japanese butterfly ray, *Gymnnura japonica* (Temminck et Schlegel, 1850), from the Pacific ocean off the coast of Japan; "*E. turgipes*" Deets, 1994 hosted by an unidentified butterfly ray, *Gymnura* sp., from the Gulf of Mexico; and *"E. urolophi*" Deets, 1994 hosted by the round stingray, *Urolophus halleri* Cooper, 1863, from the Atlantic ocean off the coast of California. The female *Eudactlyina* described herein differs from *E. dasyati* and "*E. epaktolampter*" by having a four-segmented antennule; it differs from "*E. urolophi*" regarding the armature of the antennule segments, possession of a distolateral process on the proximal exopodal

segment of leg 2, absence of a lateral protuberance on the proximal endopodal segment of leg 2, absence of cuticular spinules on the endopod of leg 2, and the armature of the caudal rami; it differs from *E. gymnuri* regarding the armature of the antennule segments, modified claw-like condition of the distal antennule segment, modified condition of the proximal exopod of leg 2, armature of the exopodal segments of leg 2, absence of cuticular spinules on the endopod of leg 2, and armature of the caudal rami; and it differs from "*E. turgipes*" regarding the armature of the antennule, elongated condition of the terminal endopodal segment of leg 1, modified condition of the proximal exopodal segment of leg 2, and armature of the caudal rami. Based on these differences, it is proposed that the copepod described herein is new to science. However, given that the descriptive work of Pearse (1950) lacked detail along with the fact that the type material deposited as *E. spinula* has been noted as being relatively useless for taxonomic purposes (Deets, 1994), it is felt that new material collected from *S. dumeril* from the type location should be examined before making a decision to proceed with the erection of a new *Eudactylina* sp. based on the presently studied copepods from the Gulf of Mexico. Another matter prompting that decision involves the identity of the sample sharks in the present study, as the tooth counts indicate a diagnosis of the Mexican angel shark (*S. mexicana* Castro-Aguirre, Espinosa Pérez et Campos, 2006). No *Eudactylina* sp. has been described from the Mexican angel shark and *Eudactylina* species have been shown to display high degrees of host specificity and rarely infect more than a single host species (Deets, 1994). That said, others have proposed that *S. mexicana* does not occur in the Gulf of Mexico and that *S. dumeril* is the only species that comprises the resident

population of angel sharks (Stelbrink et al., 2010; Ebert et al., 2013). For this reason, *Squatina* sp. has been used throughout this report for the host species of *Eudactylina* sp.

In the present study, the estimated total gill filament length per shark was strongly and positively related to both shark total length and mass as larger sharks had longer gill filaments. Those results corroborate those of Hughes et al. (1986) regarding the nursehound (= larger spotted dogfish), *Scyliorhinus stellaris* (Piiper et Schumann, 1967) and Emery and Szczepanski (1986) regarding several pelagic and coastal sharks; white shark, *Carcharodon carcharias* (L.), thresher shark, *Alopias vulpinus* Bonnaterre, 1788, shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, blue shark, *Prionace glauca* (L.), dusky shark, *Carcharhinus obscurus* (Lesueur, 1818), and sandbar shark, *C. plumbeus* (Nardo, 1827). No relationships were found between the total number of gill filaments and shark total length or mass, a finding consistent with those of Schwartz et al. (1993) regarding the smooth dogfish, *Mustelus canis* (Mitchill, 1815) and Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson, 1836). The mean number of gill filaments and estimated mean total length of gill filaments were unequal regarding hemibranch position, results which corroborate Benz and Dupre (1986) concerning the blue shark, Dippenaar (2009) regarding the tiger shark, and Duncan et al. (2011) regarding freshwater stingrays (*Potamotrygon* spp.). In the present study, the estimated length of gill filaments was not found to differ among horizontal hemibranch quadrants, a similar pattern to that found Benz and Dupre (1987) in the blue shark. The number of gill filaments and gill filament length are two factors positively related to gill surface area (Hughes, 1966) and in the present study, the number of gill filaments and estimated mean gill filament length per hemibranch showed a pattern of reduction posteriorly regarding

hemibranch position (Figure 7). Assuming there is a positive relationship between hemibranch surface area and the rate of respiratory water flow associated with various hemibranchs, the aforementioned reduction may be indicative of a lessening of respiratory water flow from the anterior to posterior hemibranchs in *Squatina* sp.

The high infection prevalence of the copepod reported herein indicate that this is a common parasite of angel sharks in the Gulf of Mexico. Corresponding data has been reported by (Dippenaar, 2009) for *E. pusilla* infecting tiger sharks off the coast of South Africa and together these results suggest that other *Eudactylina* species may display similar infection levels. Also, the results of copepod intensity reported herein for are not that dissimilar from those reported by Dippenaar (2009) for *E. pusilla* with estimates of mean intensity = 20 copepods per shark; estimated infection range = 1–37 individuals per shark. In the present study, no relationship between copepod intensity and shark size was discovered. A result possibly explained by the roughly similar infection ranges of *Eudactylina* sp. and *E. pusilla* found on angel sharks in the present study and on tiger sharks by Dippenaar (2009), respectively, though said angel sharks were considerably smaller than the tiger sharks. While parasite intensity can be affected by many environmental, physiological, and morphological factors (Bush et al., 1997), the above results suggest that the infection intensity of *Eudactylina* spp. could be affected by the amount of water flow through the gills and branchial region, as it might affect gill colonization. Also, as copepods did not exhibit different frequencies between the left and right body sides of sharks, it seems that the determinant factors affecting copepod intensity do so equally considering the side of the shark body. On the other hand, the lack of any relationship among this *Eudactylina* sp. intensity and the number of gill

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filaments per shark or the estimated total length of gill filaments per shark would seem to indicate that colonization and infection intensity may not be linked to the volume of water flowing over the gills. In the absence of this relationship, an active role by the parasite would be expected rather than passive establishment by respiratory water flow.

The observed frequencies of this copepod regarding hemibranch position were not homogeneous, with copepod intensity seemingly showing no relationship to gill filament length or number of gill filaments per hemibranch. Those results are unlike those for the copepod *Nemesis lamna* Risso 1826 (Eudactylinidae) infecting white sharks and *E. pusilla* infecting tiger sharks (Dippenaar et al., 2008; 2009) and yet, the author advances no explanation for these patterns.

The differences noted between female and male vertical attachment (females prefer upper regions and males show no preference) could indicate that females require greater oxygen than males because the regions where females attached should be associated with higher oxygen concentrations in respiratory water. The abdomen and genital complex of ovigerous females *in situ* appear reddish, indicating they may be at least partially hematophagus. The attachment of females near to the outer marginal blood channel of a gill lamella (see Benz, 1984) could provide opportunity to tightly grasp the lamella while accessing blood. Males differ from their corresponding females in that they are smaller and possess small subchelate rather than large chelate maxillipeds (see Kabata, 1979). The small size of males seem to accommodate their propensity to attach between adjacent gill lamellae, where they may be somewhat more shielded from non-respiratory water flow about the gills. Unlike their corresponding females, males do not appear reddish *in*

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situ, suggesting that they do not feed once they are mature or that they feed on materials other than host blood (e.g., mucous or epithelial cells or both).

The horizontal and longitudinal distributions of total copepods on ventral and central regions, respectively, of hemibranchs are reminiscent of the distributions of *E. pusilla* infecting tiger sharks (Dippenaar et al., 2009) and *K. carchariaeglauci* infecting blue sharks (Benz and Dupre, 1987). These patterns could result from differential water flow rates across hemibranch regions. They could also be influenced by the ability of female *Eudactylina* sp. to attach along the length of a gill filament. Benz (1984) proposed that elasmobranch gill lamellae possess marginal lamellar projections that may increase in number per lamella distally and possibly hinder the grasping ability of females.

The orientation of copepods relative to the direction of the putative water flow is consistent with *K. carchariaeglauci* infecting the gills of blue sharks (Benz and Dupre, 1987), *N. lamna* infecting the gills of white sharks, *E. pusilla* infecting the gills of tiger sharks (Dippennaar et al., 2008; 2009), and *Kroeyerina elongata* Wilson, 1932, infecting the olfactory sacs of blue sharks (McElwain et al., 2010). Eudactylinids (Eudactylinidae), kroyerids (Kroyeridae), and some other copepods infecting elasmobranch gills or olfactory sacs appear streamlined in a manner that seemingly would reduce drag and facilitate an energy savings regarding attachment.

The distribution of parasites within host populations and on individual hosts are two phenomena parasite distribution studies attempt to explain. The above results indicate that this copepod is overdispersed (aggregated) among the host population, typical of most parasites (Rhode, 1984). In addition, this copepod does not infect hemibranch positions, region of a hemibranch, and regions on gill lamellae uniformly.

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APPENDICES

APPENDIX A. Statistic values (*t* and *P* values) from Tukey's HSD of estimated gill filment length and number of gills filaments per hemibranch position for 26 angel sharks, *Squatina* sp., captured in the Gulf of Mexico.

	Gill filament length		Number of gill filaments	
	t	*P* < 0.05	*t*	*P* < 0.05
1st posterior vs. 2nd anterior	0.230	no	2.099	no
1st posterior vs. 2nd posterior	0.096	no	2.887	no
1st posterior vs. 3rd anterior	0.637	no	3.674	yes
1st posterior vs. 3rd posterior	1.186	no	5.248	yes
1st posterior vs 4th anterior	1.899	no	5.248	yes
1st posterior vs. 4th posterior	2.833	no	8.660	yes
1st posterior vs. 5th anterior	3.244	yes	9.709	yes
1st posterior vs. 5th posterior	4.547	yes	14.433	yes
2nd anterior vs. 2nd posterior	0.326	no	0.787	no
2nd anterior vs. 3rd anterior	0.407	no	1.574	no
2nd anterior vs. 3rd posterior	0.956	no	3.149	no
2nd anterior vs. 4th anterior	1.669	no	3.149	no
2nd anterior vs. 4th posterior	2.603	no	6.560	yes
2nd anterior vs. 5th anterior	3.014	no	7.610	yes
2nd anterior vs. 5th posterior	4.317	yes	12.334	yes
2nd posterior vs. 3rd anterior	0.733	no	0.787	no
2nd posterior vs. 3rd posterior	1.282	no	2.362	no
2nd posterior vs. 4th anterior	1.995	no	2.362	no
2nd posterior vs. 4th posterior	2.928	no	5.773	yes
2nd posterior vs. 5th anterior	3.340	yes	6.823	yes
2nd posterior vs. 5th posterior	4.643	yes	11.546	yes
3rd anterior vs. 3rd posterior	0.549	no	1.574	no
3rd anterior vs. 4th anterior	1.261	no	1.574	no
3rd anterior vs. 4th posterior	2.195	no	4.986	yes
3rd anterior vs. 5th anterior	2.607	no	6.036	yes
3rd anterior vs. 5th posterior	3.910	yes	10.759	yes
3rd posterior vs. 4th anterior	0.713	no	0.000	no
3rd posterior vs. 4th posterior	1.646	no	0.000	no
3rd posterior vs. 5th anterior	2.058	no	0.000	no
3rd posterior vs. 5th posterior	3.361	yes	0.000	no
4th anterior vs. 4th posterior	0.934	no	3.411	yes
4th anterior vs. 5th anterior	1.346	no	4.461	yes
4th anterior vs. 5th posterior	2.648	no	9.185	yes
4th posterior vs. 5th anterior	0.412	no	1.050	no
4th posterior vs. 5th posterior	1.714	no	5.773	yes
5th anterior vs. 5th posterior	1.303	no	4.723	yes

APPENDIX B. Tukey's HSD values of mean copepod (males, ovigerous females, non-ovigerous females, total females, and total copepods) attachement distances along longitudinal hemibranch quadrants (proximal, second, third, and distal).

	t	$P < 0.05$
Males		
Proximal vs. Second	1.16	no
Proximal vs. Third	3.09	no
Proximal vs. Distal	4.16	no
Second vs. Third	5.16	no
Second vs. Distal	7.58	yes
Third vs. Distal	2.77	no
Ovigerous females		
Proximal vs. Second	2.47	no
Proximal vs. Third	4.63	no
Proximal vs. Distal	7.33	yes
Second vs. Third	2.60	no
Second vs. Distal	5.81	no
Third vs. Distal	3.14	no
Non-ovigerous females		
Proximal vs. Second	5.11	no
Proximal vs. Third	10.51	yes
Proximal vs. Distal	12.62	yes
Second vs. Third	6.12	no
Second vs. Distal	8.98	yes
Third vs. Distal	3.78	no
Total females		
Proximal vs. Second	5.81	no
Proximal vs. Third	11.59	yes
Proximal vs. Distal	15.64	yes
Second vs. Third	6.69	yes
Second vs. Distal	11.58	yes
Third vs. Distal	5.53	no
Total copepods		
Proximal vs. Second	6.49	yes
Proximal vs. Third	13.29	yes
Proximal vs. Distal	17.73	yes
Second vs. Third	8.26	yes
Second vs. Distal	13.72	yes
Third vs. Distal	6.17	no

APPENDIX C. Vertical, horizontal, and longitudinal distributions of eggs per ovigerous *Eudactylina* sp. attached to gill lamellae of angel sharks, *Squatina* sp., from the Gulf of Mexico.

	Eggs per ovigerous copepod
Vertical distribution (gill lamellae)	
Dorsal-half proximal	25
Dorsal-half distal	25
Ventral-half proximal	23
Ventral-half distal	24
Horizontal distribution (hemibranch)	
Dorsal quadrant	27
Second quadrant	24
Third quadrant	23
Ventral quadrant	25
Longitudinal distribution (hemibranch)	
Proximal quadrant	30
Second quadrant	26
Third quadrant	25
Distal quadrant	20