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Short Communication

Arms regeneration in the squid *Lolliguncula panamensis* (Mollusca: Cephalopoda)

Sairi Saraí León Guzmán[1] & María del Carmen Alejo-Plata[2]
[1]Posgrado en Ecología Marina, Universidad del Mar Campus Puerto Ángel, Oaxaca, México
[2]Instituto de Recursos, Universidad del Mar Campus Puerto Ángel, Oaxaca, México
Corresponding author: María del Carmen Alejo-Plata (plata@angel.umar.mx)

ABSTRACT. During two sampling trips carried out over the continental platform of Gulf of Tehuantepec, a total of 101 *Lolliguncula panamensis* Berry, 1911 were caught. Thirty mature specimens (19 females and 11 males) were found to have unusually short arms. Dorsal mantle length, arm's length, and body weight were measured from the fresh specimens. Evidence of regeneration was observed at different points along arms; these may have been the result of partial autonomies. In the injured arms, the surface of the arm's tip was wholly covered with skin. Fracture planes were found in our histological sections; the autotomized arms exhibited constricted muscle fibers in the longitudinal sections indicative of wound closing. The arms of the specimens observed were very similar to the regenerating arms of other squids. This study represents the first to report arms regeneration and hectocotylus in this squid wild-caught. These results suggest that *Lolliguncula panamensis* exhibit partial autotomy, and the ability to regenerate the arms. Additionally, each of the eight arms can do so, presumably during defensive interaction or mating.

Keywords: *Lolliguncula panamensis*; Loliginidae; arm regeneration; autotomy; Gulf of Tehuantepec

Lolliguncula panamensis Berry, 2011 is distributed in the Eastern Pacific from the Gulf of California, Mexico to Peru (Roper *et al.*, 1995), and principally inhabits depths of less than 45 m (Sánchez, 2003). This species is a highly opportunistic predator foraging primarily in coastal and epipelagic waters (Arizmendi-Rodríguez *et al.*, 2011). In Mexico, this species is recurrent in the bycatch of the shrimp trawl fishery (Alejo-Plata *et al.*, 2001); however biological information for this species is scarce.

Two sampling trips were carried out during November-December 2017 and May 2018 to the Gulf of Tehuantepec between Salina Cruz (16º08'29"N, 95º10'50"W) and Barra de Suchiate (16º13'00"N, 92º14'30"W) (Fig. 1). A total of 55 bottom trawls were carried out at a depth of 14.7 to 42.8 m using trawl nets with a 52.5 mm mesh size. A total of 101 *L. panamensis* were caught.

Thirty mature specimens (19 females and 11 males) were found to have unusually short arms. Dorsal mantle length (ML), arm's length (AL), and body weight (BW) were measured from the fresh specimens (Roper & Voss, 1983). Stages of arm regeneration were determined according to the criteria of Tressler *et al.* (2014): frayed edge (I), smooth edge (II), growth bud appearance (III), tip emergence (IV), and tip elongation (V). Whole individuals were frozen and later fixed in 10% neutral buffered formalin in seawater tallowed by preservation in 70% ethanol. Ten arms were dehydrated in a graded alcohol series, cleared with citrosol and embedded in paraplast. Longitudinal sections of six micros thick were made a Leica rotating microtome and stained with hematoxylin-eosin and with Massons trichrome (Bancroft *et al.*, 1990).

The proportion of regeneration was of 19% females and 10% males. Nineteen females had a total of 50 arms in regeneration (33%) and 11 males with 48 arms in regeneration (60%) (Table 1). Arm regeneration in ale may be important because the arms play an important role in grasping the female during mating in this species.

In females, L4 (left arm 4 length) and R4 (right arm 4 length) were most frequently lost; in males, L2 and R3 were most frequently lost while L4 (hectocotylus) and R4 were least frequently missing (Fig. 2). An accident likely relates the unusual shortness of the arms

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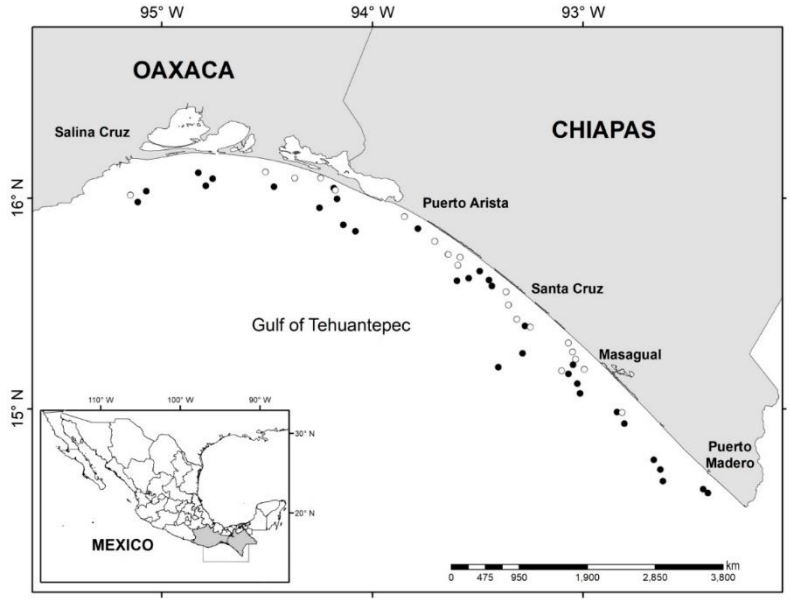

Figure 1. Study area with locations of 58 bottoms trawls (black dots). White dots indicate records of *Lolliguncula panamensis.*

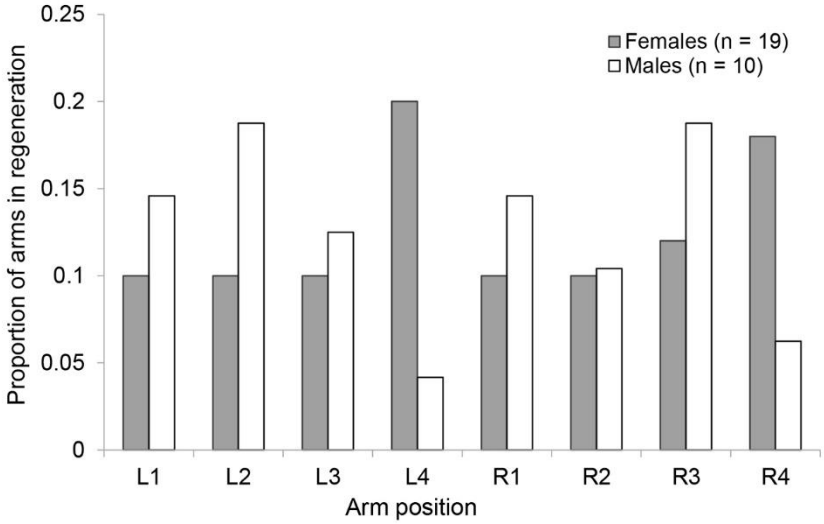

Figure 2. *Lolliguncula panamensis*. Arm positions (L1-L4, R1-R4) that are most often autotomized (regeneration arm stub) in the Gulf of Tehuantepec. L: left; R: right.

(*e.g.*, feeding, fighting, jigging) (Ikeda *et al.*, 2004) or with predator avoidance (Bush, 2012). The hectocotylized arm was less susceptible to injury in comparison to other arms (Fig. 2) as was observed by Bello (1995) for bobtail squids; apparently, only the tip of the hectocotylus was involved in the traumatic loss and subsequent regeneration process. Besides, protection and more rapid regeneration of this specialized arm to be due to its importance in mating (Wada, 2017).

Evidence of regeneration was observed at different points along arms from *L. panamensis*; these may have been the result of partial autotomies. In the injured arms, the surface of the arm's tip was completely covered with skin (Fig. 3). Fracture planes were observed; the autotomized arms exhibited constricted muscle fibers in the longitudinal sections, indicative of wound closing, and cells with dark pink nuclei concentrated at the edges of both ends of arm tissue were observed (Fig. 4). This study represents the first to report for arms regeneration and hectocotylus in *L. panamensis* wild-caught. The arms of the specimens observed were very similar to the regenerating arms of other squids (Bello, 1995; Bush, 2012).

Table 1. Measurements of arms *Lolliguncula Panamensis* caught at the Gulf of Tehuantepec on November-December 2017 and May 2018. Dorsal mantle length (DML), total weight (W, g), arm 1 length (A1), arm 2 length (A2), arm 3 length (A3), arm 4 length (A4), left (L), right (R), regenerating arm length (RAL), stage, and length (mm).

Sex	Date	DML	W	A1 L	RAL	A1 R	RAL	A2 L	RAL	A2 R	RAL	A3 L	RAL	A3 R	RAL	A4 L	RAL	A4 R	RAL
1 F	Nov/17	62.9	20	28.1		21.5	(II)	31.4		25.7	(II)	39.1		30.6	II	37.8		39	
2 F	Nov/17	67.1	20	19.3		18.1		25.8		25.5		27.5		30.1		26.7	(II)	34.1	
3 F	Nov/17	98	50	18.2	2.7 (V)	21.6		32.3		18.8	(II)	17.3	1.9 (III)	33.7	2.2 (IV)	40		32.6	(II)
4 F	May/18	105	81.3	17		17.5		19.4	(II)	36.7		44.8		30.7	3.8 (IV)	28		28.3	
5 F	May/18	90	46	12		11.7		11.22	(II)	26.21		15.5	4.4 (IV)	40		14	6.7 (III)	21.9	6 (III)
6 F	May/18	62	14.2	12.1		10.3	(II)	32		33		26		27		27.2		28	
7 F	May/18	109	75.2	19		18.8		20		20.2		39.6		30.2	9.7 (III)	29		29.3	
8 F	May/18	96.9	52	30		31		47.4		33.3	(II)	34		35		56.3		52	
9 F	May/18	97.5	85.6	11.3	(II)	10.24	(II)	11.25	(II)	11.23	(II)	13.2	(II)	15.45	(II)	34.6	(II)	11.83	(II)
10 F	May/18	110	72.5	30		21.3	2.5 (III)	22		22.1		39		38.9		28.2		28	
11 F	May/18	104	46.8	27.7		20.9	0.9 (III)	18		18		45		44.7		16.2	2.2 (III)	21.5	1.3 (III)
12 F	May/18	98.6	52.4	33		32.9		46.1	(II)	47.3		36		35.9		55		54.9	
13 F	May/18	92.9	48.4	12.4		12		20.1		22		17.8	2 (III)	17.9		47		47.3	
14 F	May/18	104	54.8	17		17.7		19.3		19		21.5	1.5 (III)	20.8	4 (III)	16.4	2.7 (III)	23.6	1.5 (III)
15 F	May/18	91	40.1	22.1	(II)	30		20		21		37		37.9		38.2		37	
16 F	May/18	103	66	18		19		19.3		19		23		23.4		27.5	(II)	16.5	(II)
17 F	May/18	100	47	18.8	(II)	21	8.5 (V)	22	2 (IV)	20.8		19.9	(II)	22.8	(II)	21.2	(II)	22.8	(II)
18 F	May/18	92.3	55	13		13.4		22		22.3		42		43		31.5	(II)	32	(II)
19 F	May/18	84.9	38.6	16.9	(II)	17	(II)	18	2.9 (V)	13.9	2 (IV)	15	(II)	20	(III)	16	1.9 (V)	12	2 (IV)
1 M	Dec/17	28.9	1.6	3.9		5.7		6.3		5.0	(II)	7.6		7.1		4.6	(II)	7.5	
2 M	Dec/17	41.1	4.7	13.5		6.8	1.6 (IV)	11	(II)	24.1		25		24.9		10.8		10.05	
3 M	May/18	73.6	22.8	20		19		15		14.8		32		35		35.5		26.9	1.8 (IV)
4 M	May/18	115	41	19.4	(II)	30		16.8	1.2 (IV)	20.1	1.8 (IV)	32		23.2	3 (IV)	53.4		54	
5 M	May/18	65.1	17.8	14.2	(II)	15.7		16		22		15.9	(II)	22.8	(II)	13.4	(II)	7.8	(II)
6 M	May/18	107	75	15.7	(II)	27		35		36		32		32.5		52		51.3	
7 M	May/18	104	66.3	17	(II)	27.5	(II)	41.5	(II)	55.2	(II)	33		14.2	(II)	36	(II)	52	
8 M	May/18	87.3	36.5	19	(II)	15.7	(II)	40		40.6		34		31		33		33.2	
9 M	May/18	99.3	51.9	15.4	(II)	15.3	(II)	44.3	(II)	22.8	(II)	35		32	(II)	35		34.9	
10 M	May/18	99.7	66.7	26		16.9	(II)	36.7	(II)	35.4	(II)	42		39.4	(II)	33	(II)	34	
11 M	May/18	82.4	33.3	20.8		13.6	(II)	13	(II)	42.8		43		32.9	(II)	29		28.3	

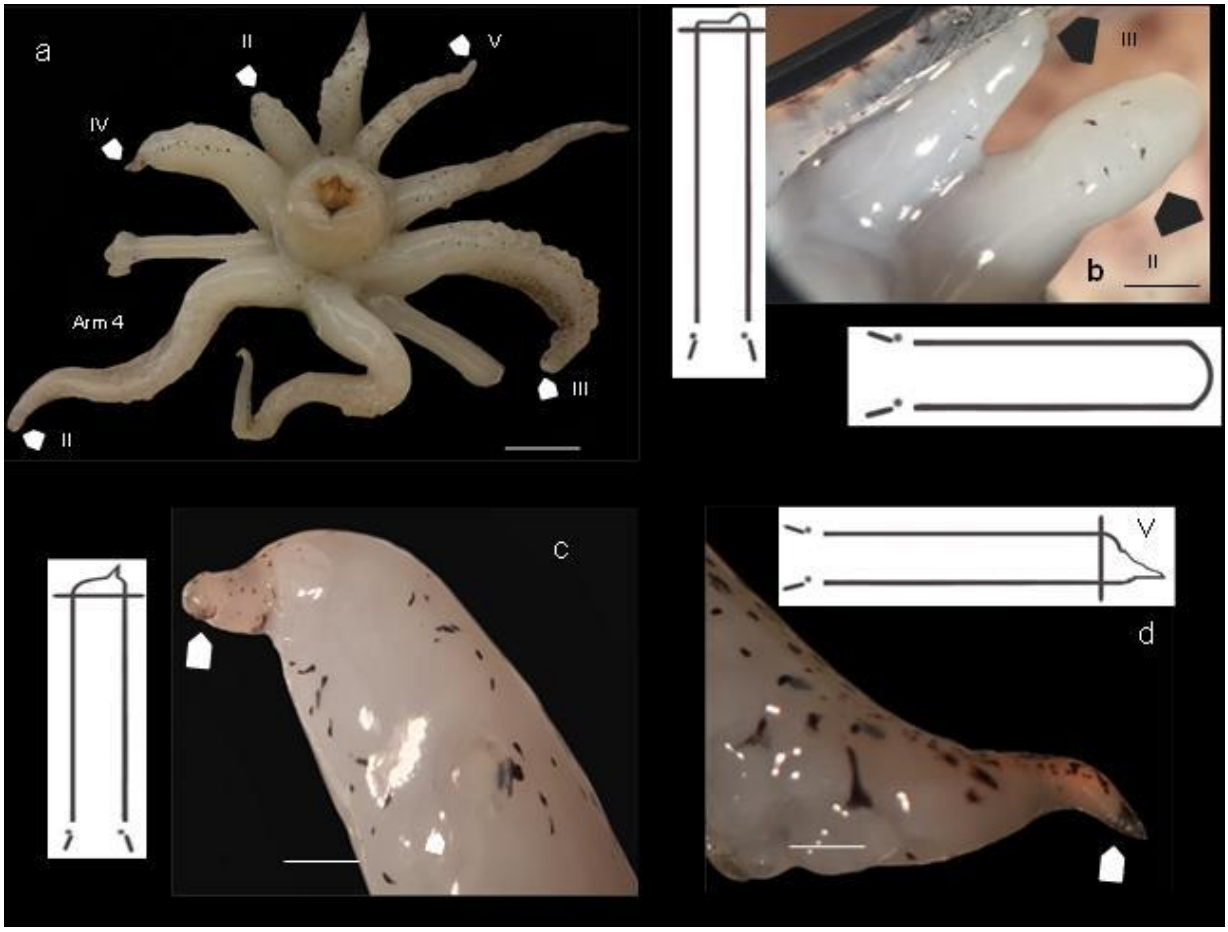

Figure 3. Arms regeneration stages in *Lolliguncula panamensis*. a) Oral view of arms in female 98 mm DML; b) Stage II: the leading edge of the regeneration arm exhibits a smooth hemispherical appearance, and suction cups first appear during this stage; stage III: a growth bud emerges at the start of this stage; d) Stage IV: elongated tip extends from the growth bud; e) Stage V: elongated tip takes on a tapered appearance closely resembling the tip of an intact arm. Scale bar = 27 mm a) and d); 12 mm c) and b); 51 mm e).

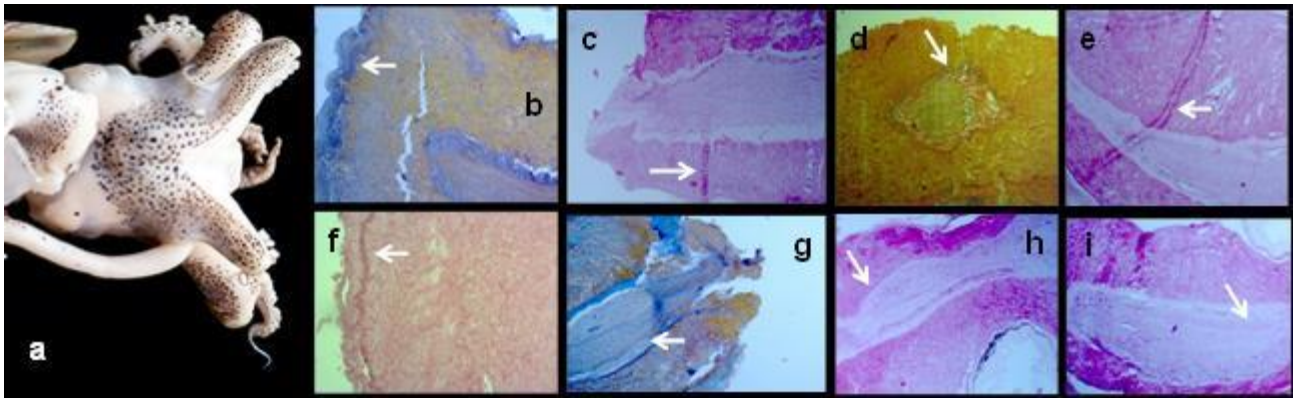

Figure 4. *Lolliguncula panamensis*. a) Female with arms autotomized (LM = 84.9 mm), b) autotomized end of the first (L1, II), fracture plane where the arm autotomized (arrow). Longitudinal sections through the regeneration zone, arrow showing the plane where autotomy occurs: c) arm L2 (V); d) arm R2 (IV); e) arm R4 (IV); f) autotomized end of the third (R3, III) lost arm. Damage in the arm, possibly from partial autotomy, that has been regenerated (arrow); g) arm R2 (IV); h) arm L4 (V). Male with arms autotomized, (LM = 115 mm), i) longitudinal sections through the regeneration zone arm R2 (IV). Histological sections stained with hematoxylin-eosin (c, e, g, i), and Massons trichrome (b, d, f, h) 10x.

Cephalopods are soft-bodied invertebrates; in them, the regeneration is common (Imperadore & Fiorito, 2018 summarize the information on regeneration). They have even evolved the ability to autotomize and to regenerate the lost appendages subsequently (Wada, 2017). Autotomy is typically a last resort defense in predator-prey interactions since it involves the loss (Fleming *et al.*, 2007). Some shallow-water octopuses autotomize and regenerate arms (Norman, 1992; Wada, 2017); other octopuses autotomize at a preformed fracture plane or also have a specific section along the arm (Norman, 1992). The squid *Octopoteuthis deletron* is the first cephalopod species reported to be capable of exhibit 'economy of autotomy'; the ability to vary the detachment site according to the amount of appendage lost to the predator. For example, has numerous places where an arm can sever; arms breakage always occurred immediately proximal to the point of interaction, minimizing tissue loss (Bush, 2012), and thereby reducing the associated costs of this defense, while still allowing escape (Fleming *et al.*, 2007).

Our results suggest that *Lolliguncula panamensis* exhibit partial autotomy, and the ability to regenerate the arms. Additionally, each of the eight arms can do so, presumably during defensive interaction or mating.

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