Journal of the Marine Biological Association of the United Kingdom
March 2008; Volume 88 (2) : Pages 375-379
http://dx.doi.org/10.1017/S0025315408000647

Female description of the hydrothermal vent cephalopod *Vulcanoctopus hydrothermalis*

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Abstract:

During biological sampling of hydrothermal vents on the East Pacific Rise, the manned submersible 'Nautile' caught the first female of the endemic cephalopod Vulcanoctopus hydrothermalis. The specimen caught at the vent site Gromit (21°33 66′S, 114°17 98′W at 2832 m depth) is described here in detail and an amended diagnosis of the species proposed. The external morphology, measurements and internal structure resemble that of males of this species. One of the most remarkable characters is the lack of spermathecae and the absence of apical filaments in the oocytes to provide a site for sperm storage. It is suggested that some species of the genera Benthoctopus and Bathypolypus would be the most suitable octopod ancestor of V. hydrothermalis.

Keywords: hydrothermal vent; cephalopods; Vulcanoctopus hydrothermalis; female description

INTRODUCTION

The study of chemosynthetic ecosystems in the deep sea represents a challenging issue due to the difficulty of sampling, which involves the use of modern technologies such as manned submersibles. Vent animals were useful as flux indicators of hydrothermal activity for geologists prospecting for vents, who appreciated the need for an explanation of the large biomass of animals and of the means by which species could be maintained at vents in the face of local extinctions (Desbruyères et al., 2006). Since the first studies accounted back in the 1970s, many charismatic vent organisms have been discovered in these chemosynthetic environments. Thus, dozens of new species of clams, mussels, limpets, crabs or cephalopods, among others, have been identified and later classified, constituting a remarkable source of information regarding these extreme environments.

Based on the samples obtained in one of these studies, a new Octopoda genus and species *Vulcanoctopus hydrothermalis* González & Guerra, 1998 was erected. This classification was made based on two male specimens. These individuals were caught during the French cruise HOT 96, two meters from the main black smoker of *Genesis* site, which is located at 12º48.43'N-103º56.41'W, at 2600 m depth on the East Pacific Rise (González et al., 1998). The possibility of raising a new sub-family (Vulcanoctopodinae) within the Octopodidae was then discussed for the first time. The parasites found in these two individuals allowed us to erect also a new genus and species of copepod, *Genesis vulcanoctopusi* López et al., 2000.

In these hydrothermal vents, only benthic incirrate octopuses of *Graneledone* and *Benthoctopus* and benthopelagic cirrate octopus species of *Cirrotheuthis* and *Grimpoteuthis* have been reported, and only *V. hydrothermalis* have been recognised as endemic of these sites (Desbruyères et al., 2006). This benthic species has characters that represent either adaptations to the deep-sea (absence of ink sac, loss of the anal flaps, eye without iris and optic chiasma) or to a hydrothermal vent habitat (eyes are covered by a thin semi-translucent skin, high concentrations of metals and presence of amoebocytes clots in the venous system and in the renal sacs). It inhabits an isolated extreme environment among colonies of tube worms *Riftia pachyptila* and Alvinellidae polychaetes, or mussels *Bathymodiolus thermophilus*, very close to the base of the chimneys where hot water gushes out of the vents in the sea bed at temperatures 265ºC forming a dense plume of black "smoke" made up of minute particles of metal sulphides (Campbell et al., 1988), and also observed on the pillow lava at several meters from the active areas (González et al., 1998).

From a total of 30’18’’ of sequences involving twenty-five specimens of *V. hydrotermalis* filmed between 2600 and 2650 m depth in the East Pacific Rise (EPR) during the French cruise HOPE 99, the following behaviour patterns were described for the first time in this species: a) crawling, b) take off, c) tactile feeding, d) mounting and e) distance mating (Rocha et al., 2002). Afterwards, the capture of seventeen new males of *V. hydrotermalis* during HOPE 99 allowed us to describe the main morphological variations of this species. This information completed and improved the knowledge of the species (González et al., 2002). The analysis of the stomach contents showed that *V. hydrothermalis* preys mainly on Crustacea Decapoda (probably galatheid crabs) and bathypelagic species like *Halice hesmonectes* (Rocha et al., 2002; Voight, 2005)

An intriguing aspect of the samples used in these papers was the absence of females, which suggested the existence of a spatial segregation by sex (González et al., 2002).

The objective of the French cruise BIOSPEEDO (Biologie-Sud Pacifique Est-Etude de la Dorsale Océanique) using the submersible *Nautile*, onboard the R/V *L’Atalante,* was the study of the biodiversity and geodiversity of the hydrothermal vents along the east Pacific Rise between 7°24’S and 21°33’S. During this cruise, the first female specimen of *V. hydrothermalis* was collected at the hydrothermal vent site *Gromit* in April 2004 (Jollivet et al., 2004).

The aim of this paper is to describe, for the first time, the female of *Vulcanoctopus hydrothermalis*, which allow completing its diagnosis and description.

MATERIAL AND METHODS

A female *Vulcanoctopus hydrothermalis* of 34.9 mm mantle length (ML) was caught during the dive PL 1578 (Figure 1), by the robotized arm grab of the manned submersible *Nautile,* on the site Gromit, (EPR, 21°33 66’S, 114º17 98’W) at 2832 m depth in April 2004 (Jollivet et al., 2004).

The specimen was collected near by a high temperature hydrothermal vent, on a mussel bed of *Bathymodiolus thermophilus* and *Calyptogena magnifica*, crawling among stalked barnacles (*Neolepas* cf. *rapanui),* bythograeid crabs *Bythograea* spp., several fish species (Causse et al., 2005), and chiridotid holothurian *Chiridota* sp. The animal was caught in very good condition. It was fixed in formaldehyde (4% in sea water) and preserved in 70% ethanol.

Definition of counts, measurements and indices follow Roper & Voss (1983) and Clarke (1986).

The female was dissected and the digestive tract, reproductive tract, circulatory and excretory systems, and eyes were removed for detailed description. The reproductive apparatus was sectioned for analysing the maturity stage, number and size of oocytes and also to perform histological sections stained with haematoxylin and eosin. All oocytes in the ovary were measured, counted and photographed using the Image Analysis System Eclipsenet®. The specimen is deposited at the ECOBIOMAR cephalopod collection with the reference number VH06030.

RESULTS

Female external and internal description

The external morphology of the female is very similar to that of male (Figure 2). There were not, however, parasitic cysts in the skin as found in several males of EPR-13°N (López et al., 2000). Measurement, counts and indices after preservation are summarized in Table 1.

The mantle cavity and the digestive tract of female have similar characteristics to those of males (González et al., 1998). However, the dark swelling observed and described for males was not present in the female specimen. The digestive tract of female contained some food remains, which were impossible to identify. The circulatory, excretory and nervous systems are also similar to those of males.

The reproductive tract of the female comprises a pear shaped ovary and two oviducts (Figure 3). No external seminal receptacles were found. The posterior edge of the ovary fits into posterior part of the mantle. The proximal part of the oviducts is short (POLI = 17.2) and narrow in this specimen, which is in a maturing stage, with spermatophores attached to the external part of the ovary. The oviducal glands are almost spherical and of uniform white colour, its maximum diameter measuring 1.6 mm (OGDI = 4.6 %). The distal part of the oviducts is relatively long (DOLI = 46.7 %).

The histological structure of the oviducal glands is composed of an outer endothelial layer and an underlying connective tissue sheet, which appears profusely innervated and vascularized. In the inner part are the proximal and distal glands, both multilobulated and rounded by smooth muscular tissue covered by connective tissue and outer endothelium. The above glands are also innervated and vascularized, and they contain the lumen of the oviduct which is about 0.4-0.5 mm diameter. This lumen is surrounded by a thick wall composed of a smooth muscular coat formed by two layers of fibres lying in a particular orientation (the outer circular and the inner longitudinal). The wall of the lumen is covered by an endothelilal layer, which at regular intervals form expansions of this tissue. Therefore, each oviducal gland is structurally divided into two concentric glands around the oviduct and separated by muscular and connective tissue. Seminal receptacles (spermathecae) were absent (Figure 4).

The ovary wall is very slender and translucent. It was adhered to the internal side of the mantle. The ovary wall is 65μm thick (mean) and it is formed of three layers: the outer squamoush endothelial tissue, an intermediate lax connective tissue and the inner squamoush cell layer of endothelial tissue.

Eighty finger-like oocytes of different sizes, ranging from 0.15 to 4.0 mm maximum length and between 0.5 and 0.6 mm of maximum width, were counted in the ovary. Figure 5 shows the oocytes size distribution. The oocytes lack of peduncles and were fixed by their bases to the inner side of the ovary wall (Figure 6).

Amended diagnosis of the species including female characters

Body semi-translucent, with a muscular consistency; mantle pear-shaped and posteriorly mitre-like; presence of a large white body (which covers the eye, the optic nerves and the optic lobe), a crop, and a multilobulate digestive gland; absence of an ink sac. Arms 1.5-4.3 times ML. Two rows of suckers on each arm. Arm formula typically 1.2.4.3 or 1.2.3.4. No enlarged suckers. Maximum depth of the largest sector of the web about 22% of the longest arm. Gills with 7-8 lamellae per demibranch. Right arm III hectocotylized (HA 1.5-2.1 times ML) in males. Ligula short (8-10% of HA), lance-shaped and without transverse ridges. Calamus represents 30 to 50% of the ligula length in fully mature specimens. Spermatophore length 70-125% of ML. Finger-like oocytes (80) ranging from 0.15 to 4 mm maximum length lacks of peduncles. Oviducal glands without spermathecae.

DISCUSSION

One of the most remarkable character of *V. hydrothermalis* female is the lack of spermathecae. The absence of these reservoirs in the oviducal glands has been previously reported in four *Eledone* species (*E. cirrhosa*, *E. moschata*, *E. massyae* and *E. gaucha*), which suggest that this is a consistent characteristic of this uniserial arm sucker genus (Pérez et al., 1990). On the other hand, the inner structure of the oviducal glands of *V. hydrothermalis* is identical to that described in *Pteroctopus tethracirrhus* (Morales, 1973, Figs. 44 y 45), which indicated that this species also lacks of spermathecae. Therefore, this absence is not circumscribed to the subfamily Eledoninae, but also to some members of the Octopodinae such as *P. tetracirrhus*, and could be a not infrequent character among the Octopoda.

As described by Mann et al., (1970) in *Octopus dofleini*, after copulation, a ‘spermatophoric reaction’ takes place in which the sperm rope within the spermatophore is everted, together with a toothed ejaculatory apparatus. This complex process proceeds inside the mantle cavity of the female where are placed the sperm-free remnants of the spermatophores, dangling freely from the openings of the oviducts while the spermatangia are found within the oviduct of the female (Boyle, 1983). Sea water stimulates the motility of the spermatozoa, and they enter the lumen of the oviducal gland where they remain attached to the epithelium of the spermatheca (Froesch and Marthy, 1975). Spermatozoa are able to be viable there until the oocytes become mature. The duration of the sperm storage is variable, from several weeks in *E. cirrhosa* from the Mediterranean (Mangold et al., 1971), to ten months in *Bathypolypus arcticus* (O’Dor and Malacaster, 1983). Fecundation of the oocytes occurs in the lumina of the oviducal gland just before spawning. However, this is not the case of the four eledonid species neither of *V. hydrothermalis*, which lack of spermatechae. In the eledonid octopuses, the spermatophores penetrate in the oviducts and oviducal glands reaching the ovarian cavity, in which the spermatophoric reaction takes place. Spermatangia in form of bladders are found in the ovary of *E. cirrhosa* and *E. moschata* mated females and consequently fertilization is completely internal (Boyle, 1983; Mangold, 1983). However, in *E. massyae* and *E. gaucha* the apical filaments of the oocytes provide a site for sperm storage and fertilization mechanism. Modified follicular cells surrounding the sperm mass inside the filament are supposed to keep spermatozoa viable until oocytes are ripe (Pérez et al., 1990). The oocytes of *V. hydrothermalis* lack of apical filaments and not spermatangia in form of bladders were observed inside the ovary. Therefore, the fertilization mechanism remains unknown in this species.

Whatever is the fertilization mechanism, the existence of an Octopodinae such as *P. tetracirrhus* (a benthic deep sea octopod inhabiting waters of about 800 m depth) lacking spermathecae and with two rows of suckers in the arms, point out the possibility of the colonisation of extreme environments, such as hydrothermal vents, by a group of octopods with biserial suckers on the arms. Phylogenetic relationships of *V. hydrothermalis* are still unknown. However, considering structural, biogeographical and ecological reasons, some species of the genera *Benthoctopus* and *Bathypolypus* would be the most suitable octopod ancestor of *V. hydrothermalis*. Perhaps *Benthoctopus*, which lacks of an ink sac, would be the best candidate. Nevertheless, molecular analyses are necessary to test this hypothesis.

ACKNOWLEDGEMENTS

We thank Didier Jollivet (Chief scientist, Roscoff, France) and the team of the N/O L'*Atalante* and the submersible *Nautile*, for collecting the specimen studied here. We thank also Violaine Martin and Patrick Briand (IFREMER, Brest) for the Fig. 1 and the Fig. 2, respectively. The cruise was supported by the French programme DORSALE and the ECCHIS-GDR (CNRS & IFREMER).

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Table 1. Measurements (mm), counts and indices of *Vulcanoctopus hydrothermalis* female after preservation. DOLI (*Distal Oviduct Length Index* or length of the distal part of the oviduct as a percentage of ML), POLI (*Proximal Oviduct Length Index* or length of the proximal part of the oviduct as a percentage of ML) and OGDI (*Oviducal Gland Diameter Index* or diameter of the oviducal gland as a percentage of ML). (AF) Arm Formula: Comparative length of arms expressed numerically in decreasing order of arms numbered from dorsal to ventral; (ALI) Arm Length Index: oral surface from the lips to the tip of the arm as a percentage of mantle length; (ASC) Arm Suckers Counts: number of suckers per row on each arm; (CaLI) Calamus Length Index: length of the calamus measured from the last (distal most) sucker to its distal tip as a percentage of ligula length; (EDI) Eye Diameter Index: diameter of eye across bulbus as a percentage of ML; (FFuI) Free Funnel Index: the length of the funnel from the anterior funnel opening to the point of dorsal attachment to the head as a percentage of ML; (FuLI) Funnel Length Index: from the insertion point into the mantle to the tip as a percentage of ML; (GiLI) Gill Length Index: from the proximal filament to tip as a percentage of ML; (HWI) Head Width Index: across widest point between the eyes as a percentage of ML; (LCL) Lower Crest Length: length from the rostrum to the top of the two lateral walls of the lower beak; (LHL) Lower Hood Length: length from the tip of the rostrum to the posterior end of the hood of the lower beak; (LRL) Lower Rostral Length: length from the rostrum to the shoulder of the lower beak; (LiLI) Ligula Length Index: from distal most sucker to tip of arm as a percentage of hectocotylized arm; (ML) Mantle Length: posterior of body to point midway between the eyes; (MWI) Mantle Width Index: across widest point as a percentage of ML; (MS) Maturity Stage: Immature or mature; (PAI) Pallial Aperture Index: the measurement between the points to the attachment of the mantle to the head along the ventral margin of the mantle as a percentage of ML; (SDI) Sucker Diameter Index: the diameter measured across the aperture from outer rim to outer rim as a percentage of mantle length (ML); (SpLI) Spermatophore Length Index: total length of the spermatopohore as a percentage of ML; (TL) Total Length: from end of longest arm to posterior end of mantle; (UCL) Upper Crest Length: length from the rostrum to the top of the two lateral walls of the upper beak; (UHL) Upper Hood Length: length from the tip of the rostrum to the posterior end of the hood of the upper beak; (URL) Upper Rostral Length: length from the rostrum to the shoulder of the upper beak; (WDI) Web Depth Index: measurement of deepest sector of web measured from mouth to midpoint of sector between arms as a percentage of ML. (Web sector A, dorsal to dorsal arm; B, dorsal to dorso-lateral; C, dorso-lateral to ventro-lateral; D, ventro-lateral to ventral; E, ventral to ventral); (WF) Web Formula: Comparative depth of each web sector, alphabetically in decreasing order. (I) Immature; (M) mature and (D) damaged.

ML		37.9	SDI		5.8
MS		Maturing	DOLI		47.6
TL		165.5	POLI		17.2
MWI		69.9	OGDI		4.6
HWI		47.0	WDI	A	21.8
EDI		19.2		B	19.1
ALI	L1	376.5		C	17.4
	L2	310.6		D	15.4
	L3	291.4		E	13.9
	L4	261.3	WF	A>B>C>D>E	
	R1	D	PAI		40.9
	R2	118	GiLC		8
	R3	106	GiLI		34.1
	R4	90	FuLI		16.1
ASC	L1	126	FFuL		embedded in the skin
	L2	118	URL		0.67
	L3	100	UCL		5.62
	L4	90	UHL		2.02
	R1	D	LRL		0.31
	R2	118	LCL		4.25
	R3	106	LHL		1.79
	R4	90			

FIGURE LEGENDS

Figure 1. Map showing the locality where *Vulcanoctopus hydrothermalis* female has been collected (solid circle).

Figure 2. Photograph of the unique female *Vulcanoctopus hydrothermalis* collected to date

Figure 3. The reproductive system of the *V. hydrothermalis* female. DO: distal oviduct; EI: external view by transparency of the egg insertion; O: ovary; OG: oviducal gland; PO: proximal oviduct.

Figure 4. The histological structure of the oviducal glands of *V. hydrothermalis* showing the absence of spermatophores reservoir.

Figure 5. Size (maximum length in mm) egg distribution within the ovary of *V. hydrothermalis.*

Figure 6. The oocytes of *V. hydrothermalis* lack of peduncles and are fixed by their bases to the inner side of the ovary wall.

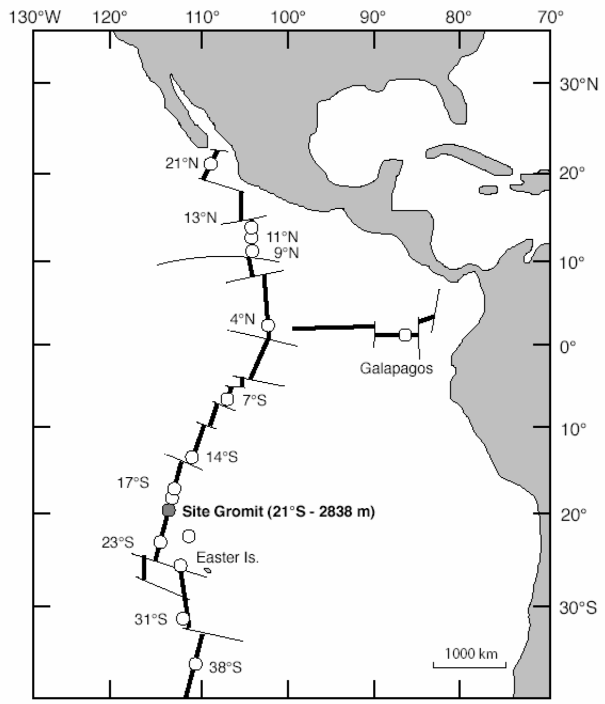

Figure 1. González et al.

Figure 2. González et al.

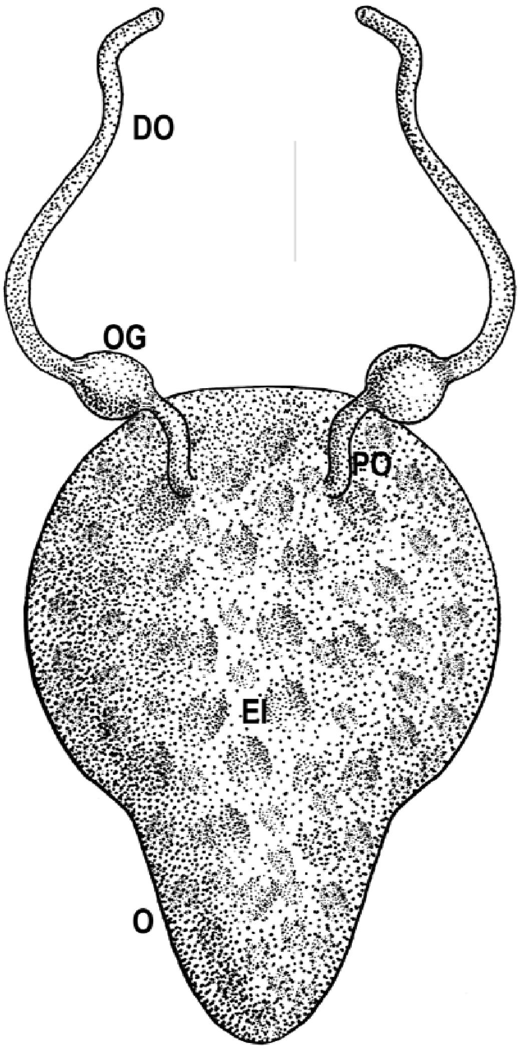

Figure 3. González et al.

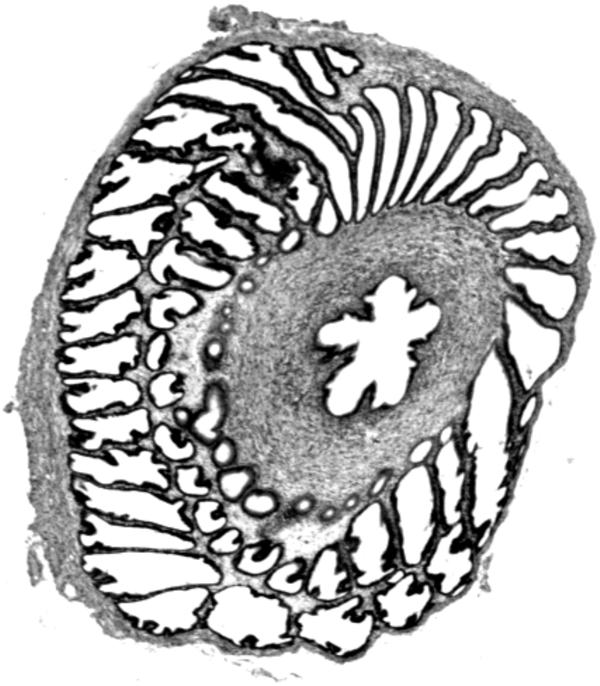

Figure 4. González et al.

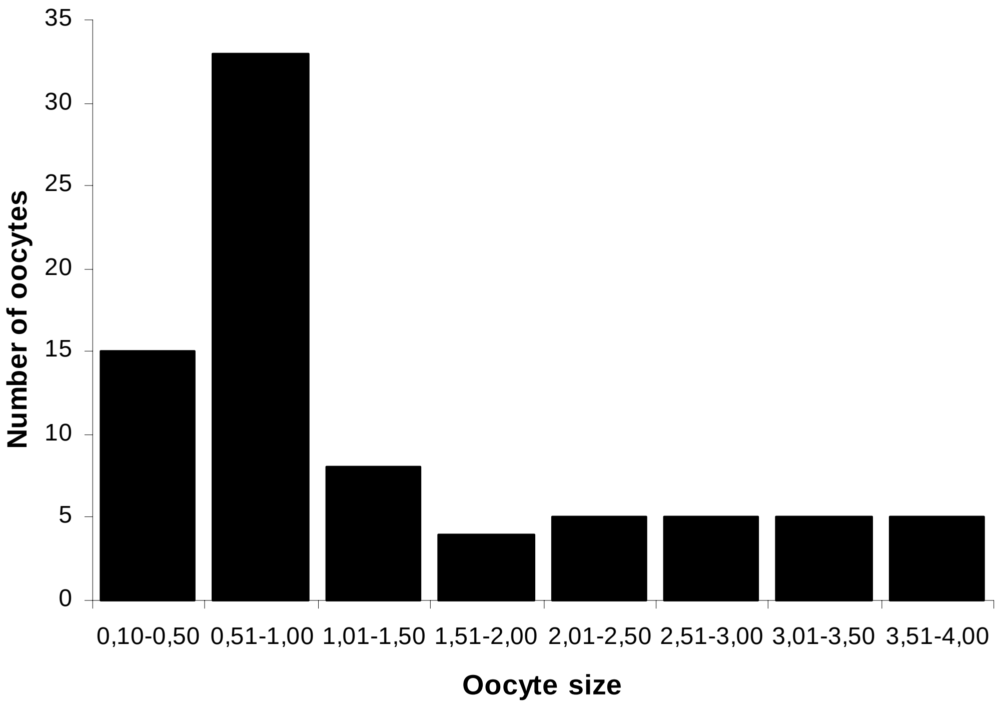

Figure 5. González et al.

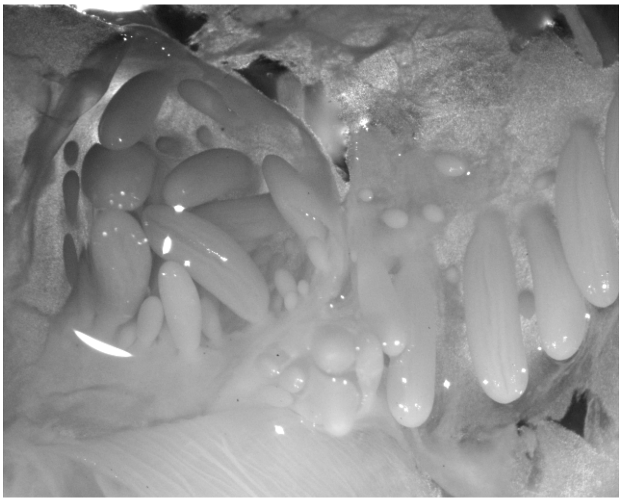

Figure 6. González et al.