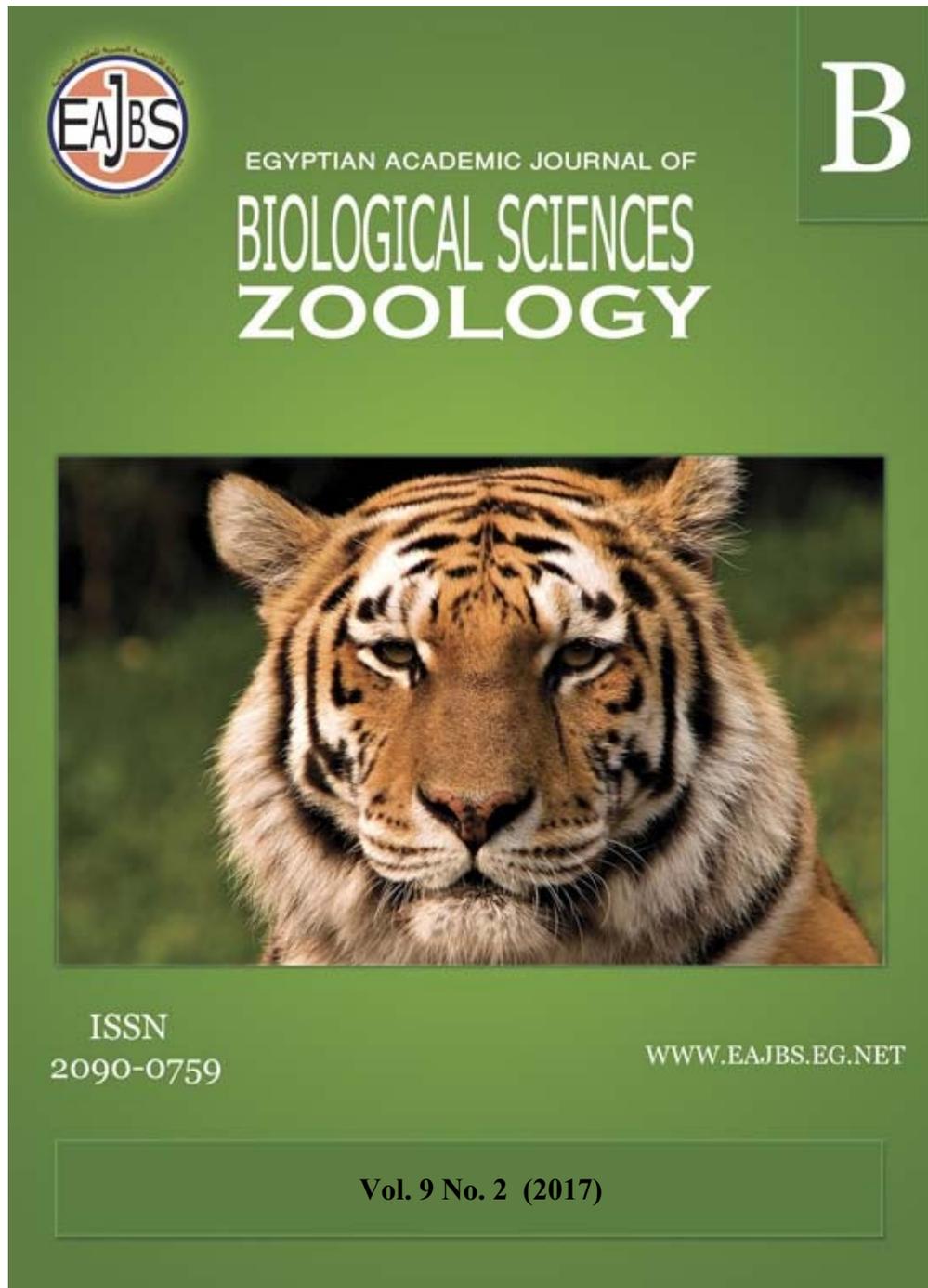


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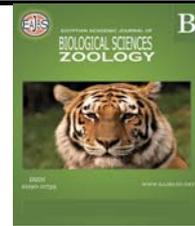


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Laboratory Observations of the Veronicellid Slug Biology *Laevicaulis stuhlmanni aegypti* Ali & Robinson, A new Subspecies and Record in Egyptian Agro-system

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ABSTRACT

The veronicellid slug *Laevicaulis stuhlmanni aegypti* Ali & Robinson (Family: Veronicellidae) is a new subspecies and record that had been discovered for the first time in garden in El Zamalek at Cairo governorate, Egypt. The main biological aspects of the veronicellid slug had been recorded i.e., incubation period, hatchability percentage, life cycle, oviposition, generation period as well as life span. These observations were recorded under laboratory conditions during slug's activity season starting from March till early December. Biology of veronicellid slug had not adequate study of their life cycle and behavior, especially for new subspecies record in the Egyptian environment. Self-fertilization is the normal breeding system for laying eggs; with big number of egg clutches, that makes the slug is a dangerous invader pest with population for attacking any new environment in the future.

INTRODUCTION

First time finding the veronicellid slug *Laevicaulis stuhlmanni aegypti* Ali & Robinson as new subspecies was recorded in Egyptian environment, which is considered as a serious agro-horticultural pest and introduced. This invasive species remains the possibility of being introduced further in other closer sites with threat to agriculture or to the environment.

Veronicellids are mainly tropical and subtropical terrestrial slugs (Order: Systellommatophora), they are known for attacking number of ornamental plants including flower beds, as a serious garden pest that causing big damage to seedlings and plants with significant agricultural, environmental, and garden impacts with significant losses to numerous crops (Raut, 1999; Robinson and Hollingsworth, 2005; Gomes *et al.*, 2013).

This family is widely distributed in tropical and subtropical regions of America, southern Asia, Africa, Madagascar, and Indian Ocean Islands (Herbert and Kilburn, 2004) in addition to the Pacific and across the Fiji Islands. (Barker *et al.*, 2005)

Veronicellids are known for nocturnal activity which prefer late hours at nighttime spreading mainly in backyards, irrigated gardens, natural habitats to agricultural, and cultivated areas. The economic plant hosts are different including tobacco (Godan, 1983), Indian-spinach, black mustard, lettuce, and spinach (Raut and Panigrahi, 1990)

The veronicellid slugs are large in size and can achieve high population densities that become so widely distributed and readily transported in association with human activities.

Studying the biology and life cycle of this group is considered as important aspect in determining an appropriate control program and its application time in future. Unfortunately, the biology of these family species is still insufficient (USDA, 2010) that needs further and more adequate studies; however, some studies have been conducted about the distribution and life histories of few economically important species, such as *Laevicaulis alte* (Nagabhushanam and Kulkarni, 1971a; 1971b; Cowie, 2000; Herbert and Kilburn, 2004; Brodie and Barker, 2012b; Ramakrishna *et al.*, 2014) and *Sarasinula plebeian* (Brodie and Barker, 2012a) in different countries and regions.

The present observations are preliminary study of this terrestrial gastropod as new record in the Egyptian environment contributing for understanding of biological aspects of dangerous pest and introduced or invasive species that could spread as emerging pests in agro-horticultural ecosystems.

MATERIALS AND METHODS

Laevicaulis stuhlmanni aegypti adult slugs were collected by hand during March and April 2016 under different types of ornamental plants from garden located in El Zamalek at Cairo governorate (30°03'27.4"N 31°13'28.1"E). Veronicellids slugs were safely transferred for further studies to Malacology laboratory of Zoology and Agricultural Nematology department, Faculty of Agriculture, Cairo University.

The slugs were kept in plastic boxes (27 x 27 x 25 cm) covered in bottom with moistened clay soil as humid environment but not wet to ten centimeters, which maintained high humidity and provided with fresh lettuce leaves (*Lactuca sativa* L.) as a food source and the remained leaves were removed, then covered with muslin cloth fixed with rubber band to prevent slugs from escaping and kept under laboratory conditions (Godan, 1983).

March and April were the beginning of activity season for the slug's reproduction, warm weather and high temperature degree in laboratory, which is different from the other terrestrial slugs in the Egyptian agro-system that prefer wintertime and cool weather for reproducing eggs such as *Limacus flavus* and *Deroceras laeve*. The rearing boxes were searched daily for clutches (egg-masses) and remoistened as required. The newly deposited clutches were transferred carefully to new prepared small box with nine cm diameter and five cm height. The time and the date of oviposition were recorded, while slug individuals were kept in the same old box.

The clutches were observed daily till hatching to determine the incubation period and hatchability.

The new hatch slugs of each slug species were housed individually in plastic cups, ten centimeters deep and six centimeters in diameter. Each cup contained two to three centimeters of moistened soil which maintained high humidity and adding fresh lettuce leaves used as a main source of food then each cup was covered with muslin

cloth with rubber band to prevent slugs from escaping and kept under laboratory conditions. The cups were examined daily and fresh lettuce leaves as well as water were added until new hatch slugs and juveniles reached maturity. The main biological aspects such as life span, reproductive behavior, and other biological aspects were recorded during this study.

Taxonomy of this species was based on the morphology of the hermaphroditic reproductive system made by David G. Robinson, USDA APHIS National Malacological Laboratory, and the vouchers were kept at the National Malacological Laboratory, Philadelphia, Pennsylvania, USA.

RESULTS

Description:

This species is rather flattened, becomes more elongate when the slug is crawling; have a narrow foot in addition to a posterior anus and eyes on contractile tentacles; the sensory tentacles are bilobed, the tentacles are hidden under the notum when the slug is inactive; has a large mantle covering the entire back, and have no shell (Runham and Hunter, 1970). The color is leveling from light to dark mottled brown.

The slugs adult were collected from field weighed 3.47 ± 0.81 g ranged from 2.03 to 5.94 g with averaged 44.81 ± 5.06 mm in length ranged from 34.93 to 50.99 mm and averaged 15.52 ± 2.05 mm in width ranged from 11.44 to 18.45 mm (n= 50).

Habitat and Behavior:

This group of slugs can damage plants by feeding on many ornamental and agricultural plants such as banana, cabbage, cauliflower, cassava, citrus, coffee, eggplant, mango, papaya, pepper, pumpkin, star fruit, sweet potato, taro, tomatoes, spinach, cucumbers, and yam (USDA, 2006; Kumari and Thakur, 2006; Brodie and Barker, 2012a; 2012b).

Veronicellidae group has big variation of slug species known as herbivorous molluscs, which are so voracious comparing to other terrestrial slugs such *Limacus flavus* in spite of the similar weight. It is considered an agricultural pest on numerous crops and can be extremely abundant (Cowie *et al.* 2009) preferring leaf tissues and soft stems.

Chewing and rasping damage to plants, presence of eggs, juveniles and adults, mucus and slime trails, in addition to ribbon like feces are characteristic signs of Veronicellidae infestation.

One of the main repeated characteristic behavior is burying their bodies inside the soil as source of humidity and avoiding losing water, which was similar to Garcia *et al.* 2007 stated that *Sarasinula plebeia* has been recorded that it bury themselves to depths around 25 to 100 cm during the dry season. Larger specimens can be active during cooler times of the day, but they also prefer nighttime activity (Brodie and Barker, 2012a).

In less humidity or drought conditions, the slugs are lying above each other or accumulate as aggregation in one place protecting their bodies from drought or losing water. Usually they were found near water bodies or moist soil similar to *Veronicella cubensis* behavior (von Ellenrieder, 2004).

Some species such as *Sarasinula plebeia* and *Laevicaulis alte* that belong to the same family are generally found in moist leaf litter, under decaying wood or organic material on the ground preferably under stones, grass, and ground crevices (Brodie and Barker, 2011a; 2012b), which is the same habitat with *Laevicaulis s. aegypti* and relatively close to human habitation. The species could be transferred with ornamental

plants and horticultural trade.

Laevicaulis stuhlmanni aegypti was co-occurring with other terrestrial malacofauna including slugs *Deroceras laeve* (O.F. Müller, 1774), *Ambigolimax valentianus* (d'Audebard de Férussac, 1821), *Limacus flavus* (Linnaeus, 1758) and snails *Oxychilus cf. cellarius* (O. F. Müller, 1774), *Eobania vermiculata* (O. F. Müller, 1774), *Cochlicella acuta* (O. F. Müller, 1774) and *Polygyra cereolus* (Megerle von Mühlfeld, 1816) in the same location.

Biology:

Laevicaulis stuhlmanni aegypti is hermaphrodite species that each one possessing both male and female sexual organs; thus self-fertilization is dominant occur in this species and no mating or cross-fertilization had been notice for this species under laboratory conditions, successive generations have been maintained by self-fertilization (South, 1992).

Self-fertilization may occur if the slug kept in isolation as well (Rueda *et al* 2002). Reproduction is higher during the rainy season with maturity reached at 2.5 months (Garcia *et al.* 2007). One individual slug could establish self-sustaining populations especially in new and environment such as the Egyptian agro-system.

The eggs are oval to Spherical elongate shape and clear translucent; its dimension is around $3.34 \pm 0.55 \times 5.07 \pm 0.33$ ranged from 2.17 - 4.14 X 4.51 - 5.87; the eggs are joined together by an thin interconnecting thread forming a string that the slugs shape into a spiral-like egg mass with special fecal pellets ribbon like on the top of the eggs that contain high concentrations of soil, which help maintain high humidity levels, more moisture, humid and protection. (Plate 1: A, B, C).

Herbert and Kilburn (2004) state that *L. alte* lays eggs in batches up to 100 eggs, while in *Laevicaulis stuhlmanni aegypti* could lays eggs in clutches up to 190 eggs in culture boxes.

The results below are review aimed at throwing some light on the main biological aspects of the new record subspecies of *Laevicaulis stuhlmanni aegypti* under laboratory conditions.

Incubation Period and Hatchability:

The reared slugs deposited egg batches, averaged 50.85 ± 23.79 ranged from 5 to 140 eggs per clutch (n= 85) with incubation period averaged 13.18 ± 2.62 days ranged from 10 to 19 days during May and June under laboratory conditions of $29.88 \pm 0.64^\circ\text{C}$ and $56.75 \pm 4.49\%$ R.H during activity season (Table 1).

Table 1: Observations of *Laevicaulis stuhlmanni aegypti*'s egg clutches during May and June under laboratory conditions.

	N	Egg/clutch	Number of new hatch born from each clutch	Hatchability Percentage %	Incubation period
Range	85	5-140	1-134	5-100	10-19
Average \pm SD	85	50.85 ± 23.79	44.25 ± 23.22	85.15 ± 16.60	13.18 ± 2.62

Under laboratory conditions $29.88 \pm 0.64^\circ\text{C}$; $29-31^\circ\text{C}$ and humidity $56.75 \pm 4.49\%$ R.H.; $52-64\%$ R.H.

The egg masses were observed daily till hatching to determine egg incubation period and hatchability. The hatchability ranged from 5 to 100% with an average of $85.15 \pm 16.60\%$ (Table 1).

Eggs of *Laevicaulis alte* are laid in clutches of up to 100 eggs (Herbert and Kilburn, 2004), with a general clutch size of 46 to 70 eggs (Herbert and Kilburn,

2004), while in this study *Laevicaulis stuhlmanni aegypti* clutch size ranged from five to 140 eggs.

Generally, the depositing eggs period started from middle of March till middle of November.

The deposited egg masses increase in number with shorten in incubation period due to higher temperature degree from late May till early September.

From our observation, the egg masses that deposited starting late July till early September, mainly in August, the incubation period were shorter averaged 10.86 ± 3.12 days ranged from 6-19 days (n= 35) with more eggs number in the clutches. (Table 2)

Table 2: Observations of *Laevicaulis stuhlmanni aegypti*'s egg clutches during August under laboratory conditions.

	N	Egg/clutch	Number of new hatch born from each clutch	Hatchability Percentage %	Incubation period
Range	35	50-145	13-126	18.57-124	6-19
Average \pm SD	35	75.86 \pm 20.66	59.86 \pm 25.81	78.16 \pm 22.61	10.86 \pm 3.11
Under laboratory conditions 30.38 \pm 0.89 °C; 29-32 °C and humidity 59.25 \pm 9.37% R.H.; 38-74 % R.H.					

It had noticed that the egg mass may be hatched completely within few days ranged from two to three days to full hatch then feed on the rest of eggs masses. The embryo can be clear visual after six to nine days inside the egg during the incubation period (Plate 1: D) becoming darker in color till be ready to hatch (Plate 1: E, F, G).

Life Cycle and Generation Periods:

After hatching, the new hatches were separated each in prepared plastic cups and observed daily till maturity and oviposition.

It was difficult to differentiate between immature and mature slugs by the external shape or size. Thus, the life cycle from hatching till juveniles reach maturity including the pre-oviposition period ranged from 53 to 115 days with an average of 86.50 ± 15.26 days (n= 23) at an average of 29.81 ± 1.12 °C ranged from 27-32 °C and 59.5 ± 7.57 % R.H ranged from 38-74 % R.H.

Thirty individual slugs were under observation till last to 23 slugs in the end of this study. The mortality ratio was high in juvenile period (about 23.33%).

The generation period lasted an average of 102.61 ± 15.23 days under the same mentioned laboratory conditions (Table 3). Hunter (1966) noticed that the period of life cycle differed according to slug species.

Table 3: Duration in days of different stages of *Laevicaulis stuhlmanni aegypti* Ali & Robinson under laboratory conditions

Period	Incubation period	Life cycle + Pre-oviposition period	Generation period	Oviposition	Post-oviposition	Life span
N	23	23	23	23	13	13
Range	11-19	53-115	69-127	1-74	5-20	127-188
Average \pm SD	15.13 \pm 2.8	86.50 \pm 15.26	102.61 \pm 15.2	46.83 \pm 23.0	13.92 \pm 5.33	155.08 \pm 21.16
	2		3	7		

Development time of slugs varies according to weather conditions developing faster and commences in warm conditions to reach maturity.

The new hatch of slugs measures 1-2 mm in length. Weight of 10 new hatch

individual together weighed 0.08 ± 0.31 g ranged from 0.03 to 0.16 g (n= 21), while the 20 new hatch individual weighed 0.17 ± 0.03 g ranged from 0.11 to 0.24 g (n= 15) (Plate 1: H, I).

Oviposition:

Under laboratory conditions of $28.55 \pm 2.27^\circ\text{C}$ ranged 22 - 32 °C and 62.36 ± 7.99 % R.H. ranged 38-76 % R.H., the oviposition period (from first to last deposited egg period) averaged 46.83 ± 23.07 days (Table 3) (n=23) during which the slug individual laid an average of 5.48 ± 2.98 clutches each with an average of 47.81 ± 22.21 eggs (Plate 2: A, B, C).

Days elapsed between deposited batch and another averaged 10.37 ± 6.57 days (Table 4). On the other hand, the total number of deposited eggs per individual averaged 266.09 ± 149.72 eggs during its oviposition period under laboratory conditions (Table 4).

Table 4: Fecundity of *Laevicaulis stuhlmanni aegypti* Ali & Robinson under laboratory conditions.

	No. of deposited clutches/ day	No of eggs/ clutch	No. of clutches/ slug	Period in days between each clutch	Total number of deposited eggs/ slug
N	114	128	23	93	23
Range	1-3	10-120	1-12	2-37	50-583
Average \pm SD	1.11 ± 0.36	47.81 ± 22.21	5.48 ± 2.98	10.37 ± 6.57	266.09 ± 149.72

Summer months are activity season, not winter like the other terrestrial slugs in Egyptian environment. The total number of eggs laid by slugs differs from species to species (Carrick, 1938).

Post-oviposition and Life Span Periods:

Post-oviposition period was considered from last laid egg till mortality. It durated an average of 13.92 ± 5.33 days (n= 13 out of 23) only 13 individual had been recorded the post-oviposition (Table 3) while ten individual slugs were still alive till the end of reproduction season 2016 to the new season 2017.

Life span period (from hatching till death) averaged 155.08 ± 21.16 days under laboratory conditions of $28.46 \pm 2.88^\circ\text{C}$ and 61.1 ± 7.59 % R.H. (n= 13) because not all the individual slugs recorded a post-oviposition period (Table 3) (Plate 2: D, E, F).

Maturity can be reached in less than three months in some individuals, but breeding only occurs during favorable conditions.

Growth and Development:

The weight was one of the parameters of growth indication that had gradually increased every month. The new juveniles after one month of their life cycle weighed 1.19 ± 0.39 g ranged from 0.48 to 1.97 g (n= 30) while the mature adult slugs, that deposited eggs after less than 3 months, weighed 4.66 ± 1.05 g ranged from 2.77 to 6.36 g (n= 14).

DISCUSSION

This slugs group is herbivores animals recording high infestation with high peaks of feeding on different host plants causing severe damage (Runham and Hunter, 1970; Raut and Panigrahi, 1990).

Few species had been studied their morphometric and biology i.e., *Laevicaulis alte* and *Sarasinula plebeian* (Herbert and Kilburn, 2004; Brodie and Barker, 2012a; 2012b; Das and Parida, 2015) that this group needs further studies about their biology and life history.

Life span of *Laevicaulis alte* was reportedly in seven months, while *Laevicaulis stuhlmanni aegypti* was around five to six months. In India, the breeding season for some Veronicellidae was observed from June to September and monsoon season, with the greatest reproductive activity occurring right after the period of greatest rainfall, while South African populations breed in the warm, rainy summer months (Herbert and Kilburn, 2004).

Incubation period is varying according to the temperature and conditions averaged around ten days in August, 13 to 15 days in the whole reproduction season, which was different of what Nagabhushanam and Kulkarni, 1970a stated that the incubation period could be around 17 days.

Hatching occurs around 1 to 3 weeks with newborn slugs (Herbert and Kilburn, 2004), which was the same with the results of this study, measuring around 7 to 8 mm in length.

Sexual Maturity can be reached in as soon as five months (Herbert and Kilburn, 2004), which were similar to the results of this study or seven months of age (Brodie and Barker, 2012a; 2012b), but breeding only occurs during favorable conditions and generally reproduction rate is high during the rainy season for some Veronicellid species (Garcia *et al.* 2007).

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Plate 1



Plate 1: (A, B, C). The egg masses of the *Laevicaulis stuhlmanni aegypti* Ali & Robinson species in laboratory culture. (D) The embryo visual clear inside the eggs after 6 days of depositing. (E, F, G) The eggs of *Laevicaulis stuhlmanni aegypti* Ali & Robinson start to become darker in color and ready to hatch in two days. (H, I) new hatches of the slug *Laevicaulis stuhlmanni aegypti* Ali & Robinson.

Plate 2



Plate 2: (A, B, C), the slug *Laevicaulis stuhlmanni aegypti* Ali & Robinson while depositing egg in spiral-egg mass. (D, E, F,) the slug *Laevicaulis stuhlmanni aegypti* Ali & Robinson in reproduction season depositing eggs recording to lie span period under laboratory conditions.

ARABIC SUMMERY

الملاحظات المختبرية لبيولوجيا البزاقة "ليفيكولس استهلماي ايجيبيتي " علي و روبنسون، تحت النوع الجديدة و المسجلة حديثا في النظام الزراعي المصري

رهام فتحي علي

قسم الحيوان و النيماتولوجيا الزراعية، كلية الزراعة، جامعة القاهرة، الجيزة، ١٢٦١٣ شارع الجامعة، جمهورية مصر العربية

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قد تم من خلال هذه الدراسة العملية متابعة و تسجيل جميع الجوانب البيولوجية الأساسية للبزاقة التابعة (العائلة: فيرونيسيليدي) "ليفيكولس استهلماي ايجيبيتي " علي و روبنسون ... و هي تحت نوع جديد تم تسجيله و اكتشافه في البيئة الزراعية المصرية في حديقة بالزمالك بمحافظة القاهرة جمهورية مصر العربية . من أهم المفاهيم البيولوجية الرئيسية و التي تم تسجيلها هي فترة الحضانة، نسبة الفقس، مدة وضع البيض، مدة الجبل بالإضافة الي دورة الحياة. و قد تم تسجيل هذه الملاحظات تحت ظروف مختبرية خلال موسم نشاط البزاقات في المعمل بداية من شهر مارس حتي أوائل شهر ديسمبر. لم تحظي هذه البزاقة في الأبحاث السابقة بالدراسة الكافية لتفهم أساسيات دورة حياتها و سلوكها بالشكل الكامل خاصة بالنسبة للرخويات الأرضية كتحت أنواع جديدة و موجودة في البيئة المصرية. تم تسجيل الإخصاب الذاتي لهذا النوع من البزاقات من خلال هذه الدراسة، حيث أن الإخصاب الذاتي هو نظام التكاثر الطبيعي لعملية وضع البيض مع ملاحظة وضع عدد كبير من كتل البيض و الذي يجعل من هذه البزاقة أفة جديدة و خطيرة لأي بيئة زراعية جديدة في المستقبل.