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Effective method of incubating stick insect eggs using a breathable material above a water surface

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Abstract
Incubation is an integral part of a successful breeding of stick insects. There are many incubation methods, most of them work by placing the eggs on a moist substrate such as vermiculite, sand, or peat. However, if placed on a moist surface and in containers without adequate ventilation, the eggs of several species are susceptible to mold. The occurrence of moldy phasmid eggs kept in very humid conditions has also been described by Potvin (1991). The method presented is very effective, combining moisture and ventilation in a good ratio, thus preventing the formation of mold. It is easy to maintain and is an efficient way of incubating most of the stick insect species bred.

Keywords: Stick insect, hatching, humidity, incubation, eggs

Introduction
Stick insects (Phasmatodea) are an herbivorous order of insects found mainly in tropical regions all around the world (Bradler & Buckley 2018) [2]. They have gained popularity mainly because of their unique appearance and, in the case of some species, their ease of breeding. Many stick insect species have a high egg laying rate, which is a reason for relatively high mortality of eggs and young nymphs in the wild (Baker 2015) [1]. The mortality can be prevented in breeding by providing suitable conditions for egg incubation. Some phasmids do not need much additional care in captivity for successful hatching (Extatosoma tiaratum). However, there are species that have higher humidity requirements, and the incubator needs to be adapted accordingly. The eggs of some Phyllium species have adapted to humid environment by developing structures, together with an adhesive secretion, which react to contact with water and allow the eggs to adhere to various surfaces, increasing the chances of the embryo developing without difficulty (Büscher *et al*. 2023) [3]. During incubation of Ramulus siamensis eggs, Potvin (1995) [7] found that hatchability decreased in drier conditions. Therefore for the eggs of many phasmids, sufficiently high humidity or direct contact with water is generally benefical.
The aim is to present an incubation method which provides sufficient humidity for the correct development of the embryo of most phasmid species, while at the same time providing good ventilation to prevent mold from forming.

Materials and methodology
The incubator is based on the following principle: The stick insect eggs are held just above the surface of the water, at a height of about one centimetre. This helps the development of the eggs, as they are constantly exposed to the moisture from underneath. Leclercq´s experiment (1946) [6] showed that higher humidity shortened hatching period, however, the reduction in time is only a matter of a few days.
The walls are made of transparent plastic, it allows the eggs to be checked and the hatched nymphs to be seen. The upper part of the incubator is made of mesh, which prevents high humidity from building up and keeps the incubator ventilated. Moisture or high relative humidity is sufficient to allow fungal spores to germinate and grow (Kowalski 2000) [5], air circulation during incubation is therefore important. If the eggs are placed in a way that they don't touch, the risk of mold growth is reduced. It is advisable to spray the eggs with water on a daily basis or every second day; this is to prevent the embryo from drying out if the air humidity in the room in which the incubator is placed is too low.

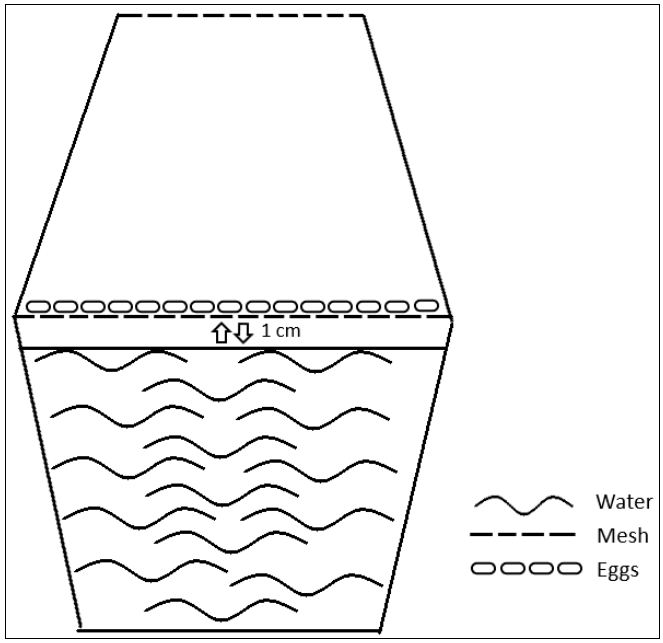

Fig 1: layout of the incubator

Eggs of 20 stick insect species were placed in this type of incubator to see if the species would hatch from the eggs without getting stuck. If the nymph fails to hatch, this indicates that it may need to be kept at a higher humidity.

Fig 2: The incubator (closed)

Fig 3: The incubator (open)

Results and Discussion

For 18 out of 20 species, the method proved suitable, with a 100% success rate of nymphs hatching without being stuck in the egg. Mold was not observed during incubation, caused by the air circulation created by using mesh in the upper part. In spite of the fact that it is a type of incubation method where the high humidity does not accumulate too much, which support the eggs to develop successfully, the results are very good. However, 2 species failed to incubate satisfactorily (*Oreophoetes topoense*, *Periphetes graniferum*), apparently due to insufficient humidity. Oreophoetes topoense thrives in more humid conditions (Conle *et al*. 2009), as does *P. graniferum*, although this species appears to be more tolerant of lower levels of humidity. While in the first species almost all embryos dried up completely, the moisture was sufficient for *P. graniferum* to develop, but not for the nymphs, which had problems hatching and 60 % of them got stuck in the egg.

Table 1: Incubated species, PSG number, origin, incubation period (in months) and percentage of nymphs that hatched without being stuck in the egg

Species	PSG n.	Origin	I.P.	Trouble-free hatching
Achrioptera manga	327	Madagascar	4-5	100 %
Anarchodes annulipes	290	Malaysia	5	100 %
Anisomorpha buprestoides	12	USA	3-4	100 %

Diapherodes gigantea	260	Grenada	4-5	100 %
Diesbachia tamyris	175	Indonesia	5	100 %
Epidares nolimetangere	99	Malaysia	3-4	100 %
Eurycantha horrida	409	Papua New Guinea	6	100 %
Extatosoma tiaratum	9	Australia	4-7	100 %
Lonchodiodes samarensis	230	Philippines	3-4	100 %
Lonchodiodes sp. "Ilocos"	392	Philippines	4-5	100 %
Oreophoetes topoense	356	Ecuador	3-4	5 %
Oxyartes lamellatus	182	Vietnam	3-5	100 %
Periphetes graniferum	357	Philippines	4	40 %
Peruphasma schultei	270	Peru	3-4	100 %
Phobaeticus magnus	358	Thailand	6	100 %
Phyllium philippinicum	278	Philippines	4	100 %
Pseudophasma fulvum	390	Colombia	4-6	100 %
Pseudophasma scabriusculum	406	Peru	5	100 %
Pseudophasma subapterum	299	Venezuela	3-4	100 %
Sungaya inexpectata	195	Philippines	4-6	100 %

Conclusion

Incubation of stick insect eggs using mesh (or other breathable material) above the water surface is a simple and effective method which, in the case of most species, will allow problem-free development of the embryo and hatching of the nymphs. An important fact is that no mold was observed during the incubation. This is a very common problem not encountered with this method. On the other hand, it turned out that this type of incubation is not suitable for species that require a higher humidity. Either the eggs do not hatch because the embryo dries out, or the hatched nymphs have difficulty getting out of the egg. Overall, however, the method has been successful and can be recommended for stick insect species that do not have a need for high humidity.

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