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# BIOLUMINESCIENCE AMONG THE DIFFERENT SPECIES OF ORDER COLEOPTERA

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## ABSTRACT

Researchers looked at seventeen different species of Brazilian railroad-worms belonging to eight different genera (Phrixothrix, Stenophrixothrix, Mastinocerus, Mastinomorphus, Taximastinocerus, Brasilocenis, Euryopa, and Pseudophengodes). These specimens were collected in the southeast and west central regions of Brazil, close to the Parque Nacional das Emas. Mastinomorphus sp. was discovered in open marshy regions of west central cerrados, in contrast to the other species that were researched, which normally live in pluvial tropical forests. The life cycle of Mastinomorphus species is described in full here. The bioluminescence of the head may vary from yellow-green to red (Am.LX = 562-638 nm) in larvae and larviform females of the species that was researched, whereas the bioluminescence of the lateral lanterns can range from green to orange (Amax = 535-592 nm). The colour of the emission may vary from green to yellow in mature males (Amax = 549- 580 nm), with the exception of the Euryopa spp. species, which produce orange light (Amax = 592-598 nm). In contrast to the North American Phengodini, none of the adult males of the species under study glowed when exposed to ultraviolet light. Luminescence was found in all stages of life of the species. The bioluminescence emitted by the larval and female head lanterns may help to illuminate the surrounding environment, but the lateral lanterns emitted by larvae, adult males, and females seem to play a significant role in the animals' ability to defend themselves.

***Keywords:*** *bioluminescence, coleopteran.*

## INTRODUCTION

The family Phengodidae is comprised of around 170 known species, the majority of which are located in the Neotropical Region (W. Wittmer, personal communication). Although just 49 species native to Brazil have been described up to this point (W. Wittmer, personal communication), there are most likely still many more waiting to be found. The group was examined by Wittmer (1976), who catalogued a total of 27 American taxa belonging to the tribes Phengodini, Mastinocerini, and Pennicilloporini (Phengodinae).

The phenomenon of bioluminescence has been documented in the genera Phengodes, Phrixothrix, Stenophrixothrix, Mastinocerus, Zarhipis, Cenophengus, Cydiscus, Falsophrixothrix, Dioptoma, and Diplocadon, according to Herring (1987). Phrixothrix vivianii Wittmer (Witmer 1993), Euryopa clarindae Wittmer, and Euryopa laurae Wittmer are the names given to the three new species of phengodids that were found by our group in west-central Brazil near the Parque Nacional das Emas. In addition to this, we published the spectrum distribution of ten different phengodid species from Brazil as well as an approximation of the physical and chemical characteristics of their luciferin-luciferase system.

In this paper, we report on the biological information that we obtained from both in-field and in-laboratory observations as well as bioluminescence spectra for other phengodid species. These species include members of the genera Phrixothrix, Stenophrixothrix, Mastinocerus, Mastinomorphus, Taximastinocerus, Euryopa, and Pseudophengodes. In this article, the life cycle of Mastinomorphus sp.j. is detailed as well. Previously, the larva and larviform female of this species were thought to belong to Mastinocerus nigricollis (Viviani and Bechara 1993).

**Bioluminescence Origin**

The phase in the cycle known as bioluminescence, which occurs when a species produces and emits light as a consequence of a chemical reaction, is referred to as the light-producing stage. The word "life" may be translated from the Greek word "organism," while the Latin word "lumen" meaning "light." Throughout the course of the cycle, the composite energy is transformed to the energy of light.

**The phenomenon of bioluminescence**

This event takes place when a pigment known as lucifer, an enzyme known as luciferase, and a source of oxygen are all present in the same environment. The reaction results in the production of a molecule known as oxyluciferin, which is responsible for the emission of light. In the process of bioluminescence, luciferase is an essential component because it catalyses the luminescence reaction.

**Coleoptera (beetles)**

The beetle genus has the greatest diversity of luminous species and the greatest number of species overall. In addition, it contains members of the families Lampyridae and Phenodidae, which are known as click bugs and railway worms, respectively (Elateridae). Additionally, luminescence was discovered in the larvae of Xantholinus (Staphylinidae).

Fireflies use the length, spread, and recurrence of their belly lamps as sexual signals, and the resulting flames are greenish-yellow in colour. Insects that are often known as "bugs" give out a continuous green light, and the abdominal light organ in their abdomens give forth a constant green-orange light. Female glow beetles are cylindrical in shape, and railroad worms are responsible for transmitting the most colour among light beetles, including the most explosive larvae. Greenish-orange light is produced by the LEDs that run horizontally down the body.

The length, span, and intensity of the lights that are released from the abdomen of fireflies are employed for sexual functions. Fireflies create flames that are yellow and green in colour. The organs on the abdominal belly of a crawling reptile produce a constant green-orange light while the reptile is flying, but the organs on the abdominal belly of bugs emit a continuous green light. Railroad worms are responsible for the transmission of the glow beetle colours that are considered to be the most significant, which includes the most explosive larvae and females.

It is common knowledge that fireflies use their light to communicate with potential mates. Within some animal species, bioluminescence is responsible for drawing together members of the same social group, which in turn increases the probability of sexual reproduction. a female Chlorophytum that lacks wings and is stationary in certain animals.

which As a result of the long wavelength of the ambient light, the morphology of the signal was not significantly affected. These data imply that long-wavelength lighting has less of an influence on firefly courting than broad-spectrum white lighting does. Because of this, it's likely that employing long-wavelength lighting rather than broad-spectrum white lighting might aid in the conservation of fireflies.

In a separate piece of research (Adams and Miller 2013), the researchers found fireflies, a kind of beetle that employs D-luciferin as a substrate and is one of the most well-known instances of luminescence. The beetles' luminescence is caused by enzymes known as fatty acyl-CoA synthetase, which are often present in insects. These enzymes are responsible for giving insects their characteristic glow. According to the most current sequencing of firefly genomes and transcription, ACS replication and subsequent diversification were the drivers of the evolution of bioluminescence. This information was gleaned through studying firefly genomes. There are a variety of species that are capable of producing bioluminescence, and it has advanced significantly in the insects that play a significant part in mating, predation, and defence.

## OBJECTIVES

1. To study of bioluminescence
2. To study coleoptera.

## Materials and Methods

**Reagents.** The following chemicals were purchased from Sigma: adenosine triphosphate (ATP), D-luciferin, ethylenediaminetetracetic acid (EDTA), dithiothreitol (DTT), tm(hydroxymethylamino) methane (Tris), magnesium sulphate (MgSO4), and Triton X-100 (St. Louis, MO). Merck was the vendor for the acquisition of sodium phosphate (Darmstadt, Germany). Every solution was made using water that has been bidistilled and deionized with MilliQ.

**Insects.** During the years 1990-1995, specimens of phengodids were collected and field observations were done at the following five locations: (1) Fazenda Santana-Sousas, located in the municipality of Campinas, state of Sao Paulo: Phrixothrix hirtus (Olivier, 1909), Mastinocerus sp.2, and Mastinocerus sp.4. At an elevation of 700 metres, this region is limited on three sides by open fields, transition zones, and the Atibaia River. The territory is occupied in great part by a remnant mesophil tropical forest that is 100 hectares in size. (2a) Fazenda Santa Cruz located in the municipality of Costa Rica within the state of Mato Grosso do Sul: Phrixothrix vivianii Wittmer, Phrixothrix heydeni Olivier, Stenophrixothrix brasiliensis (Pic), Mastinocerus nigricollis (Pic), Taximastinocerus hickeri (Pic), Mastinomorphus sp.1 This location is included in the morphoclimatic zone of the central west Brazilian cerrados, which is comprised of four primary formations: cerrado, gallery forest, marshy regions, and grassland; (2b) Parque Nacional das Emas, state of Goi£s: E. clarindae, E. laurae, and Mastinomorphus sp.j. This area include the section of cerrado that is located on the Rio Formoso's eastern bank, as well as its gallery forest. It is situated sixty kilometres to the north of the location referred to in 2a. (3) In the municipality of Sales6polis, in the state of Sao Paulo, in the Estacao Biologica de Boracia: Stenophrixothrix pollens (Berg), Brasilocerus impressicollis (Wittmer), Phrixothrix hirtus, and Pseudophengodes brasiliensis Wittmer. At an elevation of 850 metres and situated 110 kilometres to the northeast of Sao Paulo, this region is a perfect example of a typical Atlantic forest reserve. (4) Sitio Cambar, which is located in the municipality of Capao Bonito, in the state of Sao Paulo: Brasilocerus sp. (impressicollis?). A virgin Atlantic forest, secondary forest, eucalyptus belts, and open pastures are all found on this property.

Light traps with white (300 W), blue (8 W), and UV (8 W) bulbs were effective in attracting adult males (Zaragoza Caballero 1989) shortly after sunset (in December at 1900 hours), and continued to be effective throughout the night. Both the females and the larvae were pulled out from the surface of the soil as well as the dirt in their natural habitats. In the laboratory, larvae and females were grown in circular plastic jars with heights of 11 centimetres and diameters of 7 centimetres, as well as in aquariums with dimensions of 20 centimetres by 13 centimetres by 13 centimetres. They were given chicken liver extract in addition to alive and dead isoptera and diplopods as their diet. Adults were also preserved by placing them in circular jars with damp absorbent paper to keep them alive. Every bug was nurtured at a temperature of 21 degrees Celsius and subjected to seasonal photoperiods.

**Bioluminescence Spectra.** The SPEX model Fluorolog spectrofluorometer from Edison, New Jersey was used to record the bioluminescence spectra, and the only photomultiplier that was active was the one attached to the sample. An internal algorithm was used to adjust the spectra so that they would account for the spectrum photosensitivity of the apparatus (MCorrect). Previously, Viviani and Bechara (1993) examined the bioluminescence spectra with a PerkinElmer model LS-5 spectrofluorometer (Oak Brook, IL). They then used the fluorescent standards quinine sulphate and rubrene to adjust the results for the spectrum photosensitivity of the equipment. The correction of the in vivo spectra obtained for nigricollis and vivianii adult males and impressicollis larvae and also for the in vitro luciferase standard reactions gave essentially the same result with both sets of equipment. This was due to the fact that the in vivo spectra were obtained from adult males of nigricollis and vivianii and impressicollis. Immobilized adult females and live last instars were placed in glass capillaries, and the anterior or lateral lantern portion of the glass was covered with black paper so that independent spectra could be obtained. These capillaries were then placed in a spectrophotometric cuvette and stored in the sample section of the equipment. After about five minutes of being controlled, the spectra were examined. At this point, the insect's movement had practically ended, and it was generating a steady low-level light. We have found that the optimum time to collect consistent larval bioluminescence spectra is when the insect is close to ecdysis. This is because the insect is less active during this time, which helps to prevent the distortions that are induced by abrupt movement. As was done in the previous study, adult male phengodids were given an injection of 5 /xl of commercial epinephrine at a concentration of 1 mg/ml to cause them to glow (Viviani and Bechara 1993). A clear tape was used to restrain adult men on glass laminae before they were positioned in front of the emission window. In every instance, the lantern whose spectrum was to be scanned was positioned such that it was directly in front of the centre of the emission window. This was done to prevent any distortion from occurring as a result of the various detection and self-absorption geometries. Every spectrum is an average calculated from at least three separate scans performed on each individual specimen of a specific species.

## RESULTS

**Life History Observations.** Habitats. In the central cerrados of the state of Sao Paulo, we have recorded 10 species, while in the southeastern Atlantic Forest area of the state, we have recorded 7 species (Table 1). According to the specimens of adult males and larvae that have been collected, southeastern phengodids are most often found in tropical forests. Because adult males may be attracted to light traps from both the gallery forest and surrounding open fields, including virgin cerrados and pastures, and because our data on larvae are still incomplete, it is not clear which habitats are the actual habitats of west central species. However, it is not clear which are the actual habitats of west central species. The only species of Mastinomorphus that we looked at that lived in open marshland habitats was Mastinomorphus sp.j.

**Oviposition and Eggs**. 22 females of the species Mastinomorphus sp.j. were seen engaging in opposition. During the months of December and January, each female placed a dense cluster of eggs during a single oviposition in cracks in the earth or in chambers they created out of dirt. After laying the eggs, the females surrounded the eggs until they hatched. During this time, the females were hostile and attacked any item that tried to interfere with their territory when they were startled by a fingertip or a pencil. The females all perished within a week after the hatching of their eggs. Similar behaviour was shown by a female Phrixothrix sp., however unlike the male, it did not create soil chambers and instead perished before the larvae could eclose. The number of eggs deposited by Mastinomorphus sp. females ranged from four to fifty, with a mean and standard deviation of fourteen and four, respectively (n = 22). The eggs have a coloration that is somewhere between orange and brown, are spherical and firm, and have an average diameter of one millimetre. The incubation duration that was observed in the laboratory at a temperature of 21 degrees Celsius and under seasonal photoperiods ranged from 33 days to 51 days on average (42 days on average, n = 22).

**Larvae.** The normal home for millipede larvae is the dark soil under rotting logs and leaves in tropical woods, which is also where phengodid larvae may be found. P. hirtus larvae were discovered in soil that had a pungent odour of decaying vegetable materials and was abundant in fungus. Under a rotting wood, in the soft soil, along with millipedes, passalid, and elaterid larvae were discovered, as well as a larval female of the same species. In addition, it was typical for the larvae of B. impressicollis to be found on moss-rich soil in rain forests. At the foot of termite mounds that were occupied by larvae of Pyrearinus termitilluminans (Elateridae), it was common to see P. vivianii larvae feeding on little black millipedes (Bechara 1988). On the dark soil of marshy places with low grass, larvae of the species Mastinomorphus sp.i were discovered. In conclusion, larvae of two undetermined species, most likely Stenophrixothrix, were discovered to be arboreal in the Atlantic woodland. These larvae were observed to be active at heights of up to 1.7 metres on the leaves and branches of plants.

During the rainy season, both phengodid larvae and millipedes were active. Millipedes were also active. The larvae of P. hirtus and B. impressicollis were discovered from October through April, whilst the larvae of P. vivianii and P. heydeni were gathered from October through February. Both of these species are native to the west central cerrado. The larvae of the southeastern species spent the winter dormant and buried under the surface of the ground. The highest depth that was sampled was 30 cm, at which point P. hirtus and Mastinocerus sp.2 larvae could still be discovered.

Nocturnal in their activity, larvae of all species were most active on nights with high humidity. However, it is unknown whether or if larvae are active in the field at other times of the day except morning and afternoon. Daytime observations in the laboratory have shown the presence of Phrixothrix spp. larvae feeding on millipedes on many occasions. Specimens of P. hirtus were discovered at temperatures ranging from 13 to 25 degrees Celsius. In the month of December, P. vivianii larvae were seen to begin their activity at 2100 hours; however, on nights that were warm (24°C) and dry, they did not begin to show until much later, at 2300 hours. Larvae wandered on the surface of the earth as part of their typical pattern of behaviour. Occasionally, they would bury themselves and then later return to the surface.

Phengodid larvae are predators that specialise on eating millipedes, although they will also consume other types of food. Phrixothrix and Brasilocerus spp. were both fed several kinds of millipedes in the laboratory, including a wood millipede called Julus sp. It was discovered that P. hirtus larvae were preying on these millipedes out in the wild. Species of Brazilian millipedes were consumed by a P. laticollis larva that was generously provided by J. Sivinsky of the University of Florida. It was discovered that the larvae of B. impressicollis feed on live wood termites when they are kept in a laboratory setting. They injected a black liquid into these Isoptera and then sucked it out, which suggests extracorporeal predigestion similar to what happens in the larvae of lampyrid and elaterid species (Colepicolo et al. 1987). The larvae of Mastinomorphus sp.i were given chicken liver extract to eat in the laboratory.

Even when kept in humidified vials and given sugar water, the lifespan of adult male phengodids in the laboratory is quite brief, often lasting no more than one week. This is the case even when they are fed sugar water. The mature males of Mastinomorphus sp.x and P. heydeni that emerged in the laboratory had a lifetime of seven days, according to the observations that were made. Both in the lab and in the field, attempts to determine whether or not Mastinomorphus sp. females are capable of mating with males that have been captured in the wild were unsuccessful. However, we do not know if the females were open to the idea of reproduction or not. Adult females are notoriously difficult to study because they are often confused with larvae. When adult females of the Mastinomorphus sp. I species oviposited, we were able to identify them. They are much bigger than the males of the respective species. More information on the behaviour of females may be found in the section devoted to oviposition.

**Bioluminescence.** Eggs After day 15, a photon-counting equipment was used to detect a very faint and almost undetectable light emanating from the embryos. There is no information available on the spectrum distribution of this luminescence. Viviani and Bechara (1993) revealed that the bioluminescence intensity of fertilised phengodids eggs was temperature dependent. They also determined that the apparent activation energy was 58 kilojoules per mole. After 25 days of incubation, Z. integripennis eggs, according to a study by Tiemann (1967), emit a dim light.

## CONCLUSIONS

One of the most important compounds involved in the bioluminescence scintillation process is called luciferase. It does this by promoting the oxygenation of the combination that is often referred to as luciferin, which results in the production of an energy-rich peroxide medium. There are various orders of insects that exhibit bioluminescence, and these insects use it for a variety of purposes, including defence, mating, and hunting. Researchers have looked at the phenomenon of bioluminescence from a variety of angles throughout the years.

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