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# Bioluminescent Coleoptera of Biological Station of Boracéia (Salesópolis, SP, Brazil): diversity, bioluminescence and habitat distribution

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Abstract: Brazil hosts the richest biodiversity of bioluminescent beetles in the world. Several species are found in the Atlantic rain forest, one of the richest and most threatened tropical forests in the world. We have catalogued the biodiversity of bioluminescent species mainly of Elateroidea superfamily occurring in one of the last largest and most preserved remnants of Atlantic rain forest, located at the Biological Station of Boracéia of São Paulo University (Salesopolis, SP, Brazil). This site accounted with the largest diversity ever found for a single place in São Paulo State, with 39 species: Lampyridae (30), Phengodidae (5), Elateridae (3) and Staphylinidae (1). This fauna has unique species that were not found in any other places of Atlantic forest in São Paulo state, especially fireflies from the Lampyrinae tribes (Cratomorphini, Lamprocerini, Lucidotini), the subfamilies Amydetinae and Photurinae, and the phengodids *Pseudophengodes* and *Brasilocerus* sp.2. Most species are found in dense Ombrophyl forest or at their border, and a few ones are found dwelling in the few open fields around the forest. There is a predominance of glowing patterns in the green region among forest inhabiting species when compared with open field fireflies.

Keywords: Lampyridae, Phengodidae, Elateridae, Staphylinidae, bioluminescence, fireflies, bioindicators.

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Resumo: O Brasil tem a maior biodiversidade de coleópteros bioluminescentes do mundo. Muitas destas espécies são encontradas na Mata Atlantica, um dos mais ricos e ameaçados ecossitemas. Catalogamos a biodiversidade de espécies bioluminescentes da superfamília Elateroidea em um dos maiores e mais preservados remanescentes de Mata Atlântica da Serra do Mar, localizado na Estação Biológica de Boraceia (E.B.B.) em Salesopolis, SP, reserva administrada pelo Museu de Zoologia da Universidade de São Paulo. Este local contou com a maior biodiversidade de espécies bioluminescentes já encontrada num único local dentro do estado, com 39 espécies: Lampyridae (30), Phengodidae (5), Elateridae (3) e Staphylinidae (1). Esta fauna tem espécies únicas não encontradas em nenhum outro lugar investigado de Mata Atlântica no estado de São Paulo, especialmente das tribos de Lampyrinae (Cratomorphini, Lamprocerini, Lucidotini), as subfamílias Amydetinae e Photurinae, e os fengodídeos *Pseudophengodes* e *Brasilocerus* sp.2. A maioria das espécies ocorre em floresta ombrófila densa, e poucas ocorrem em campos abertos. Existe uma predominância de espécies com padrões de brilho contínuo na região do verde no interior da mata quando comparadas a espécies de vagalumes de campo aberto.

Palavras-chave: Lampyridae, Phengodidae, Elateridae, Staphylinidae, Bioluminescência, vagalumes, Bioindicadores.

#### Introduction

Brazil has the largest diversity of luminescent beetles in the world, with *ca* 500 described species, corresponding to *ca* 23% of described species in the world (Costa 2000), distributed in the three main families of beetles: Lampyridae, Phengodidae and Elateridae. Furthermore, bioluminescence was also registered for a staphylinid species (Costa et al. 1986). These species occur in the Atlantic Rainforest, Cerrado (Savanna), Pantanal (Marshes) and Amazon Rainforest. Among these ecosystems, the Atlantic rain forest is one of the richest and also most threatened ones, currently with only *ca*. 8% of the original cover. Therefore, it is essential to make species lists to aid conservation programs, through the selection of proper bioindicators, besides providing subsidies for bioprospection programs and studies on bioluminescence (Viviani 2007).

Studies using Coleoptera as bioindicators focused mainly on the families Scarabeidae, Carabidae (many dealing with Cicindelinae subfamily) and Staphylinidae which include soil species (Bohac 1999, Rainio & Niemelã 2003, Marinoni & Ganho 2006, Durães et al. 2005). These taxa have been used as environmental indicators, when a species indicates the degradation or recovery of the environment; ecological, when a species or group of species indicates the quality of a habitat, or of biodiversity when a species or group are used as indicators of biodiversity in a general sense. On the other hand, bioluminescent beetles are not used for such purposes yet, despite their unique capacity to produce light which may render them convenient bioindicators at night. Fireflies and other bioluminescent beetles, because depend on their bioluminescence for reproduction (Lloyd 1983, Lewis 2010) and other biological functions (De Cock 2009), may constitute sensitive indicators for investigating the impact of artificial illumination in the environment at night (Lloyd 2006, Viviani et al. 2010).

Bioluminescent beetles display different bioluminescent signals for different biological purposes in different life stages, usually: defense in the immature stages (De Cock 2009) and sexual attraction and/or defense in the adult stage (Lloyd 1983, Viviani & Bechara 1997). Lampyrid fireflies produce complex patterns, usually flashes, for sexual attraction (Lloyd 1983, Branham & Wenzel 2000, Lewis 2010). Several authors studying North-American and Euroasiatic species (Lloyd 1983, Ohba 1983, Branham & Wenzel 2000, Lewis 2010), found four bioluminescence communication systems: (system 1) sedentary females release pheromones to attract non-signalling flying males; (system 2; former system 1) sedentary females produce continuous glows sometimes coupled with pheromones to attract non-signalling flying males; (system 3; former system 2) males emit a primary signal to which sedentary females answer and (system 4) found in synchronous fireflies such as Pteroptyx in which several males congregate in leks and emit synchronous flashes to attract flying females.

Despite their biodiversity, taxonomic and systematic studies on bioluminescent beetles in Brazil are still scarce. The bioluminescent elaterids of the tribe Pyrophorini were reviewed by Costa (Costa 1975, Casari-Chen & Costa 1986). However, the families Lampyridae and Phengodidae lack recent revisions and their identification remain troublesome. Some studies on the biology and ecology were performed for some species of Elateridae (Costa 1975, Costa et al. 1999) and Phengodidae (Viviani & Bechara 1997, Costa et al. 1999). In the famíly Lampyridae, bionomic studies were done for *Aspisoma* spp. (Costa et al. 1988, Viviani 1989a, b). Viviani (2001) described the biology of several lampyrid species of São Paulo state and Rosa (2007) described immature stages and the biological data of *Photuris fulvipes* Blanchard 1837. More recently, a survey of the species occurring near and in the urban areas of Campinas, Rio Claro and

Sorocaba in São Paulo state, was done (Viviani et al. 2010). However, there are not detailed surveys in large remnants of Atlantic rain forest, mainly close to the sea shore.

The Serra do Mar (Sea shore) preserves the last largest remnants of primary Atlantic rain forest. Among the areas we investigated, the Biological Station of Boracéia of São Paulo University (Salesópolis), which is located in one of the last and largest contiguous areas of Atlantic rain forest in Eastern São Paulo state, showed a surprising diversity of bioluminescent beetles. Here we report a survey of bioluminescent species done in this site during the past 20 years.

#### Materials and Methods

Habitats description. According to Heyer et al. (1990), the Biological Station of Boracéia is located in the Serra do Mar, 80 km East of São Paulo city, at about 23° 38' S latitude, 45° 52' W longitude, 900 m above the sea level, about 12 km from the Atlantic coast (Figure 1,2). The area is mostly covered by continuous dense mesophyl Atlantic rain forest (Figure 1), with high pluviometric indexes (3000-3550 mm/year), among the highest in Brazil, mainly during the period from October through April. It is constituted by mist or cloud forest, with continuous canopy averaging 5-10 m, highest in the valleys bottoms (up to 30 m), constituted by one tree and one bush layers with few emergents, mostly palms (including Euterpe edulis), eventual bamboo clumps, tree ferns and climbing wines. The forest is also rich on bromeliads, orchids and epyphitic ferns. Epyphitic mosses grow on branches, roots, twigs, old leaves, rocks and logs. The undergrowth is relatively open in most of the forest. The ground is covered by complexes of horizontal moss covered roots.

There is some kind of differentiation found in the soil. Along the main road to Rio Claro dam, clay yellowish soil is predominant whereas in the road to Guaratuba's river, darker and redder basaltic soil is predominant. The following specific habitats are also found: **Humid forest (Marshy):** The forest is cut by many small streams and rivers with eventual swampy areas with dense undergrowth constituted by herbaceous grasses including heliconias; **Open fields:** A few sites consist of man made clearings around the station buildings, and around tall electricity wires, being constituted mainly by grasses; **Marshies:** Some open marshy areas are also found, mainly along Claro's River and its small lakes, and man-made clearings to allow electric wires conduction. These areas consist mainly of grasses and typical marshy plants, including *Typha latipholia*.

Collecting sites. Figure 2 shows the map of Biological Station of Boracéia and the main collecting sites. The species were collected along the two main dirty roads that cross the station (Figure 1), the main road to Claro's river dam and a bifurcation to Guaratuba's river. The following sites were investigated: (1) road banks and along the main dirty road in the segment from Sede (station's buildings) (23° 39′14.42"S; 45 ° 53′22.03"O; 847 m) until Poço Preto (23° 38' 51.86" S; 45° 52' 17.81" O; 869 m); (2) trail that goes from the main road to Claro's river fall (23° 39' 00.79" S; 45° 52' 57.86" O; 861 m); (3) old stone extraction site along the main road (23° 38' 16.51" S; 45° 50' 24.78" O; 893 m), close to the dam; (4) small lake along the main road (23° 38' 47.09" S; 45° 51' 38.15" O; 862 m); (5) trail behind the station's buildings (also called Sede) (23° 39' 11.66" S; 45° 53' 24.36" O; 847 m); (6) road to Guaratuba's River (23° 39' 14.92" S; 45° 54' 53.08" O; 834 m) which goes until water collecting facility of Guaratuba's river (called Estrada do Guaratuba); (7) a cleared marshy area around a small tributary of Claro's River along the road to Guaratuba's river (23° 39' 25.49" S; 45° 54′ 53.53" O; 823 m), and in smaller trails, inside the forest, open fields and marshies along the roads 1 and 6.

Collecting techniques. The collecting and observations were annually made from 1990 to 1997, in the period from October to April and, eventually, in July. The collecting started again in 2002, and was done annually until 2010.

Adult lampyrids, elaterids and the phengodid *Pseudophengodes brasiliensis* Wittmer 1976 were located by their own light at night and collected in flight with insect collecting net, or on the grass. Luminescent click-beetles and *Cratomorphus distinctus* Olivier 1895 fireflies were attracted to green chemiluminescent light sticks. Adult male phengodids were collected, in rare occasions, on soil and on the grass. Lampyrid larvae were collected on the grass and soil at night, by the location of their glows (Viviani 2001). Phengodid larvae were also collected at night in the soil, in banks, and eventually on the grass by location of their luminescence (Viviani & Bechara 1997). Click beetle larvae were collected on decayed logs or over the soil in rare occasions at night.

Identification. Specimens of fireflies and their larvae were identified by comparison with personal collection, which was previously identified by comparison with the collections of Museu de Zoologia da Universidade de São Paulo (MZUSP, São Paulo), Muséum d'Histoire Naturelle de Paris (MNHN, Paris) and British Natural History Museum (BMNH,London). Some species were recently identified by Dr. Simone P. Rosa during her visit at the MNH . Elaterid species were previously identified by C. Costa (MZUSP) and Simone P. Rosa. Several lampyrid and phengodid species could not be identified, and were catalogued by the name of the genus followed by a specific number, according to Viviani & Bechara (1997) and Viviani (2001). In the field, several adult fireflies could be also identified by their specific bioluminescent signal, according to Table 2. The specimens were deposited in the collection of Universidade Federal

de Sao Carlos (UFSCAR, Sorocaba) under curatorship of V. Viviani, and in the MZUSP.

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Bioluminescent signals recordings. Bioluminescent signals of various species were recorded with video camera Sony Hi8 CCD-TRV or with Sony Cybershot DSC-H50 photographic camera. The bioluminescent signals were classified in the following classes: (1) glows which are emissions that last several seconds; (2) pulses, in which the light intensity increases and decreases without completely disappearing between the peaks; (3) single flashes which are fast emissions lasting fractions of seconds; (4) bimodal flashes which are two consecutive flashes without interval; (5) multimodal flashes which are more than two consecutive flashes without interval between them, and (6) slow-crescendo which is a flash with gradual increase of intensity and short sustaining at the peak of intensity. The bioluminescent color were previously recorded spectrofluorometrically (Viviani & Bechara 1995, 1997) or more recently with a Hitachi F4500 spectrofluorometer with the excitation shutter closed. The type of bioluminescence communication system of fireflies studied here was classified according to Branham & Wenzel (2000) and Lewis (2010) in the following systems: (system 1) sedentary females release pheromones to attract non-signalling flying males; (system 2; former system 1) sedentary females produce continuous glows sometimes coupled with pheromones to attract signalling flying males; (system 3; former system 2) males emit a primary signal to which sedentary females answer.



Figure 1. Aerial view of the Biological Station of Boracéia and surrounding areas: (upper panel) map and (Lower panel) aerial view by Google-Earth.

The properties of the bioluminescence signal of various species and the communication system is also shown in Table 2.

## **Results and Discussion**

**Taxonomic survey.** Thirty-nine species of bioluminescent Coleoptera were catalogued: thirty Lampyridae; three Elateridae, five Phengodidae e one Staphylinidae (Table 1).

Lampvridae (Figure 3, 4). Figures 3 and 4 show some representative species of Biological Station of Boracéia. Among Lampyridae, the following number of species was found in different subfamilies and tribes: (Photurinae) 11; (Lampyrinae) 18; Cratomorphini: 5; Photinini: 5; Lamprocerini: 2: Lucidotini: 2;Lampyrini: 1; (Amydetinae): 1. There was a predominance of the subfamily Photurinae in relation to other localities investigated in São Paulo state (Viviani et al. 2010) (Table 1). According to observations of bioluminescence activity of lampyrids in the field, the most frequent species were Photinus fuscus Germar 1824 and Pyrogaster sp.2 inside the forest, but Magnoculus sp.3, Cratomorphus distinctus Olivier 1895 and several species of Photurinae were also quite frequent, although less abundant, in the period from October to December. Among the 30 species, 5 pertaining to the tribes Lamprocerini (Lamprocera flavofasciata Kirby 1808, Lucio castelnau Kirsh 1865, Lucio sp., Cladodes stellatus Gorham 1880, Cladodes sp.) and Lucidotini (Lucidota sp.) have diurnal habits. Larvae of some species were associated to the respective adults after rearing in the laboratory (Costa et al. 1988; Viviani 2001). Among them there were: Lucio castelnau Kirsh 1865, Lamprocera flavofasciata Kirby 1895, Pyrogaster moestus Germar 1824, Cratomorphus distinctus, C. concolor Perty 1830, Cratomorphus sp.2, Aspisoma physonotum Gorham 1884. However, there are several larvae that could not be associated to the respective adults yet, but which could be identified up to the genus level. It is noteworthy the morphological variety of these larvae (Figure 5), with species displaying diverse pigmentation, some of them totally camouflaged with their environment, such as larvae that live on decayed leaves on the soil (Figure 5).

Phengodidae (Figure 6). A single species of the tribe Phengodini, *Pseudophengodes brasiliensis* Wittmer 1976, and four species of Mastinocerini were found. Among the latter, two species are known by adults (*Brasilocerus* sp.2, *Phrixotrix hirtus* Olivier 1909), whereas other two only by larvae (*Stenophrixotrix* sp., *Mastinocerus* sp.). One of them is a very small arboreal larva very common inside the forest (*Stenophrixotrix* sp.), quite similar to *Brasilocerus*, but with much lower dimensions. The most common species were *Brasilocerus* sp.2, in the larval stage, and *Pseudophengodes brasiliensis* Wittmer in the adult stage.

**Elateridae** (**Figure 7**). Three luminescent species were found: *Pyrophorus divergens* Eschscholtz 1829, *Pyrearinus micatus* Costa 1978 and *Haspodrilus pyrotis* Germar 1841.

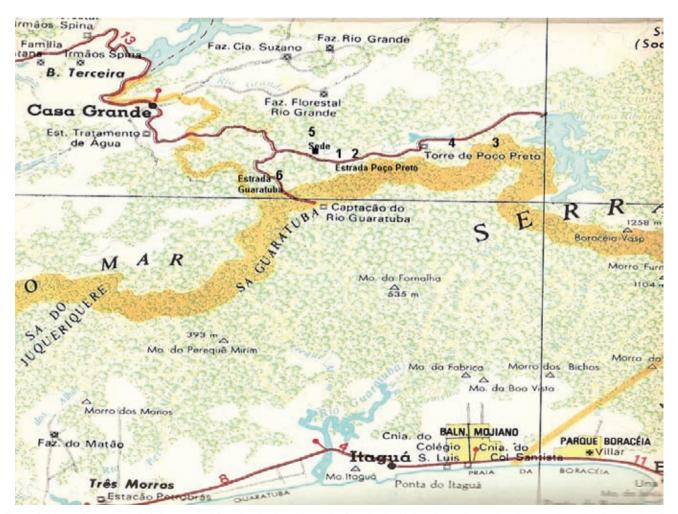


Figure 2. Views of the Atlantic Rainforest at the Biological Station of Boracéia (Salesópolis, SP, Brazil): (a) Guaratuba's River road crossing dense ombrophyl forest, and (b) open marshy field cutting the forest.

**Habitat distribution**. Several species could be associated to their respective habitats, according to observations made along several years. Table 1 lists the species found in their respective habitats, and Figure 8 shows the frequency of species of different families

in different habitats. Most of the species (22) was found in dense ombrophyl forest, followed by secondary growths and at the borders of forest (8), marshies (3) and open fields (2). Photurinae species were usually common in humid forest. *Photuris* sp.7 e *Cratomorphus* 

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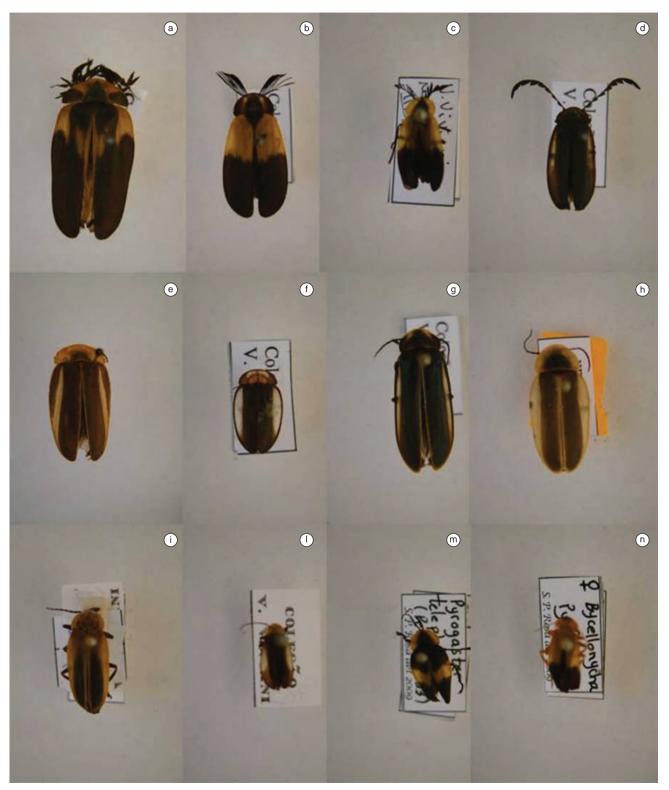


Figure 3. Some typical lampyrids of Biological Station of Boracéia: (a) Lamprocera flavofasciata Kirby 1895; (b) Cladodes sp.; (c) Cladodes stellatus Gorham 1880; (d) Lucidota sp.; (e) Cratomorphus distictus Olivier 1895; (f) C. besckei Olivier, 1895 (g) Macrolampis sp.2; (h) Photinus sp.; (i) Pyrogaster moestus Germar 1824; (l) Photuris sp9 (m) Pyrogaster telephorinus Perty 1808 and (n) Bicellonychia sp.9.

concolor Perty 1830 were found in open fields. Aspisoma physonotum Gorham 1884, Pyrogaster moestus Germar 1824 were collected at the edges of the forest and in secondary growths, and Macrolampis sp.2, Photinus sp. e Bicellonychia lividipennis Motshulsky 1854 were found in open marshies. Elaterids and phengodids were usually found in dense forest and secondary growths, but they were not found in open fields and marshies, similarly to the previously reported species found in other localities of São Paulo state (Viviani et al. 2010).

Among the observed and collected species, Lamprocera flavofasciata Kirby, 1808, Lucio castelnau Kirsh 1865, Lucio sp., Cratomorphus beckerii Olivier 1895, Photinus fuscus Germar 1824, Macrolampis sp.2, Pyrogaster lunifer, Pyrogaster telephorinus Perty 1833, Pyrogaster sp.2, Photuris lugubris Gorham 1881, P. ellyptica Olivier 1886, Photuris sp.9, Bicellonychia sp.9, Pseudophengodes brasiliensis Wittmer 1976 and Brasilocerus sp.2 were unique of the Atlantic rain forest of Biol. Station of Boracéia, and were not found in any other investigated place yet.

Only 13 species were found in other localities of São Paulo state, generally in semi-seasonal rain forest, transition areas and open fields.

The lampyrid species Cratomorphus distinctus Olivier 1895, Photinus sp.1 and Photinus sp., the phengodids Phrixotrix hirtus Olivier 1909 and Mastinocerus sp. and the elaterids Pyrophorus divergens Eschscholtz 1829 and Pyrearinus micatus Costa 1978 are also found in semi-seasonal rain forest inside São Paulo state (Viviani et al. 2010). Aspisoma physonotum Gorham 1884 and Pyrogaster moestus Germar 1824 are also found in transition between open fields and forest, especially at the edges of the semi-seasonal forest and secondary growths in the municipalities of Campinas, Sorocaba and Rio Claro (Viviani et al. 2010). Photuris sp.7 was found in open fields close to the forest, from the seashore up to the semi- seasonal forest in Sorocaba. Cratomorphus concolor Perty 1830 is commonly found in open fields inside the state (Viviani et al. 2010). Among the marshy species, only Cratomorphus sp.2 and Bicellonychia lividipennis Motshulsky 1854 that are commonly found inner São Paulo state, were also found in the Biological Station, but were not so common, probably because the lack of extensive open marshy fields, which are commonly found in central and eastern the state.



Figure 4. The glow pattern of two lampyrids: (a-c) Cratomorphus distinctus Olivier 1895; (d-f) Magnoculus sp.3.

Table 1. Species of bioluminescent Coleoptera collected at the Biological station of Boraceia, and their respective habitats.

Estrada Poco Pero (1.2), Estr Guaranba (6)   X   X   X	Tavon	I ocal (cita numbar)	Donco Omb	Humid	Rordore/cocondory	Fields	Morehy	Concon
Estrada Poco Peto (1.2), Estr.Guaratuba (6)	144011		For.	for.	growths	T ICHTS	Man Suy	1000000
Estrada Poco Preto (1.2), Estr.Guaratuba (6)	LAMPYRIDAE (30 spp.)							
Estrada Poco Pero (1.2), Estr Guaratuba (6)   X	LAMPYRINAE							
Estrada Poco Peto (1.2), Estr. Guaratuba (6)	Cratomorphini (5)							
Estrada Guaratuba (6)	Cratomorphus distinctus Olivier, 1895	Estrada Poco Preto (1,2), Estr.Guaratuba (6)	×		×	×		November-January
Estrada Guaratuba (6)	C. concolor Perty,1830	Estrada Guaratuba (6)				×		November-January
Sede  Trail to Rio Claro's fall (2)  Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Research house (Sede)  Research house (Sede)  X  Trail Research house (Sede)  X  Trail Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (1,6)  X  Trail Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (4,6)  X  Trail Research house (Sede)  Estrada Poco Peto/Estr Guaratuba (4,6)  Estrada Poco Peto/Estr Guaratuba (4,6)  Estrada Poco Peto, Estr Guaratuba (4,6)  Trail Research house (Sede)  Estrada Poco Peto, Estr Guaratuba (4,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail La Rocco Peto, Estr Guaratuba (4,6)  Trail La Rocco Peto, Estr Guaratuba (4,6)  Trail La Rocco Peto, Estr Guaratuba (4,6)  Trail La Rocco Peto Estr Guaratuba (4,6)  Trail La Rocco Peto Estr Guaratuba (4,6)  Trail La Rocco Peto (1,4)  Estrada Poco Peto (	C. beskey Olivier, 1895	Estrada Guaratuba (6)	×					November-January
Trail to Rio Claro's fall (2)	Cratomorphus sp.2	Sede					×	November-January
Trail to Rio Claro's fall (2)	Aspisoma physonotum Gorham, 1884				×	×		October-April
Trail to Rio Claro's fall (2)	Lamprocerini (5)							
Research house (Sede)	Cladodes stellatus Gorham, 1880	Trail to Rio Claro's fall (2)	×					October-December
1837   Estr. Guaratuba (6), Estr. Poco Preto (1)   x     Estrada Poco Preto (1)   x     Estrada Doco Preto (1)   x     Estrada Cuaratuba (6)   Estr. Poco Preto (1)   x     Estrada Poco Preto (1)   x     Estrada Poco Preto/Estr. Guaratuba (1,6)   x     Research house (Sede)   x   x     Research house (Sede)   x   x     Estrada Poco Preto/Estr. Guaratuba (1,6)   x     Trail to Rio Claro's fall (2)   x     Stone extraction, Estr. Guaratuba (3,6)   x     Trail Research house (Sede)   x     Trail Resear	Cladodes sp.	Research house (Sede)	×		×			October-January
Estrada Poco Preto (1)	Lamprocera flavofasciata Blanchard 1837	Estr. Guaratuba (6), Estr. Poco Preto (1)	×					October-January
Estrada Guaratuba (6), Estr. Poco Preto (1)  Estrada Guaratuba (6)  Estrada Guaratuba (6)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (1,6)  Trail Research house (Sede)  X  X  X  X  X  X  X  X  X  X  X  X  X	Lucio castelnau Kirsh1865	Estrada Poco Preto (1)	×					
Estrada Guaratuba (6)  Estrada Poco Preto (1)  Estrada Poco Preto (15)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Sy. 1854  Trail Research house (Sede)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail Research house (Sede)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail Research house (Sede)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail Io Rio Claro's fall (2)	Lucio sp.	Estr. Guaratuba (6), Estr. Poco Preto (1)	X					November
Estrada Guaratuba (6)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)	Photimini (5)							
Estrada Poco Preto (1)  Satisfacia Poco Preto/Estr. Guaratuba (1,6)  Research house (Sede)  X  X  X  X  X  X  X  X  X  X  X  X  X	Photinus sp.	Estrada Guaratuba (6)					×	November-January
Estrada Poco Preto/Estr. Guaratuba (1,6)  Acude/Estrada Guaratuba (4,6)  Research house (Sede)	Photinus sp.1	Estrada Poco Preto (1)	×					November-January
Acude/Estrada Guaratuba (4,6)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Estr. Guaratuba (4,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)	Photinus fuscus Germar 1824	Estrada Poco Preto/Estr. Guaratuba (1,6)	×					October-February
Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)  Trail to Rio Claro's fall (2)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)	Photinus sp.2		×				×	October-January
Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Estr. Guaratuba (4,6)  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)	Macrolampis sp.2	Acude/Estrada Guaratuba (4,6)					×	October-December
Research house (Sede)  Research house (Sede)  Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Estr. Guaratuba (4,6)  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)	Lucidotini (2)							
Research house (Sede)  Estrada Poco Preto/Estr. Guaratuba (1,6)  Estrada Poco Preto/Estr. Guaratuba (4,6)  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  X  Stone extraction, Estr. Guaratuba (3,6)  Trail Research house (Sede)  Trail Research house (Sede)  Trail Research house (Sede)  Trail to Rio Claro's fall (2)	Lucidota discoidalis Laporte, 1833	Research house (Sede)	×					October-January
Estrada Poco Preto/Estr. Guaratuba (1,6) x  Estrada Poco Preto/Estr. Guaratuba (4,6) x  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6) x x  Trail to Rio Claro's fall (2) x  Stone extraction, Estr. Guaratuba (3,6) x  Stone extraction, Estr. Guaratuba (4,6) x  Trail Research house (Sede) x  Estrada Poco Preto/Estr. Guaratuba (1,6) x  Trail Research house (Sede) x  Trail Research house (Sede) x  Trail Research house (Sede) x  Trail to Rio Claro's fall (2) x  Trail to Rio Claro's fall (2)	Lucidota sp. (1)	Research house (Sede)	×		×	×		November-December
Estrada Poco Preto/Estr. Guaratuba (1,6) x  Estrada Poco Preto/Estr. Guaratuba (4,6) x  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6) x x  Trail to Rio Claro's fall (2) x  Stone extraction, Estr. Guaratuba (3,6) x  Stone extraction, Estr. Guaratuba (4,6) x  Trail Research house (Sede) x  Estrada Poco Preto, Estr. Guaratuba (1,6) x  Trail Research house (Sede) x  Trail Research house (Sede) x  Trail Research house (Sede) x  Trail to Rio Claro's fall (2)	Lampyrini (1)							
Estrada Poco Preto/Estr. Guaratuba (4,6) x  Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr. Guaratuba (1,6) x  Trail to Rio Claro's fall (2) x  Stone extraction, Estr. Guaratuba (3,6) x  Stone extraction, Estr. Guaratuba (4,6) x  Trail Research house (Sede) x  Estrada Poco Preto, Estr. Guaratuba (1,6) x  Trail Research house (Sede) x	Incertae sedis AMYDETINAE (1)	Estrada Poco Preto/Estr. Guaratuba (1,6)	×					October-December
Poco Preto/Lake (1,4)  Estrada Poco Preto, Estr.Guaratuba (1,6)  Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  y, 1854  Lake/Estrada Guaratuba (4,6)  Trail Research house (Sede)  Estrada Poco Preto, Estr.Guaratuba (1,6)  Trail to Rio Claro's fall (2)	Magnoculus sp.3 PHOTURINAE (11)	Estrada Poco Preto/Estr. Guaratuba (4,6)	×					October-December
Estrada Poco Preto, Estr. Guaratuba (1,6) x x x Trail to Rio Claro's fall (2) x x Stone extraction, Estr. Guaratuba (3,6) x x x x x x x x x x x x x x x x x x x	Photuris sp.7	Poco Preto/Lake (1,4)				×		Ocotber-January
Trail to Rio Claro's fall (2)  Stone extraction, Estr. Guaratuba (3,6)  y, 1854  Lake/Estrada Guaratuba (4,6)  Trail Research house (Sede)  Estrada Poco Preto, Estr.Guaratuba (1,6)  Trail to Rio Claro's fall (2)	Photuris lugubris Gorham 1881	Estrada Poco Preto, Estr.Guaratuba (1,6)	×	×				October-January
Stone extraction, Estr. Guaratuba (3,6) x  y, 1854 Lake/Estrada Guaratuba (4,6) x  Trail Research house (Sede) x  Estrada Poco Preto, Estr. Guaratuba (1,6) x  Trail to Rio Claro's fall (2)	Photuris ellyptica Olivier, 1886	Trail to Rio Claro's fall (2)	×					Ocotber-January
y, 1854 Lake/Estrada Guaratuba (4,6) x  Trail Research house (Sede) x  Estrada Poco Preto, Estr.Guaratuba (1,6) x  Trail to Rio Claro's fall (2)	Photuris sp.9	Stone extraction, Estr. Guaratuba (3,6)	×					October-December
Trail Research house (Sede)  Estrada Poco Preto, Estr.Guaratuba (1,6)  Trail to Rio Claro's fall (2)	Bicellonycha lividipennis Motshulsky, 1854	Lake/Estrada Guaratuba (4,6)					X	October-January
Trail Research house (Sede) x Estrada Poco Preto, Estr.Guaratuba (1,6) x Trail to Rio Claro's fall (2)	Bicellonycha sp.9							January
Estrada Poco Preto, Estr.Guaratuba (1,6) x Trail to Rio Claro's fall (2)	Bicellonycha sp.	Trail Research house (Sede)	×					
Trail to Rio Claro's fall (2)	Pyrogaster lunifer Escholtz	Estrada Poco Preto, Estr.Guaratuba (1,6)		×				October-December
	Pyrogaster telephorinus Perty, 1833	Trail to Rio Claro's fall (2)						November-January

Taxon	Local (site number)	Dense Omb	Humid	Borders/secondary Fields Marshy	Fields	Marshy	Season
		For.	for.	growths			
Pyrogaster moestus Germar 1843	Trail Res. house, Estr. Guaratuba (5,6)	X		X			October-December
Pyrogaster sp.2	Estrada Poco Preto/Estr. Guaratuba (1,6)	×					October-December
PHENGODIDAE (5)							
Mastinocerini							
Phrixotrix hirtus Olivier,1909	Estr. Poco Preto, Estrada Guaratuba (1,6)	×		×			October-April
Brasilocerus sp.	Estrada Poco Preto/Estr. Guaratuba (1,5,6)	×					October-April
Mastinocerus sp	Estrada Guaratuba (6)	×		×	×		November
Stenophrixotrix sp.	Estrada Poco Preto/Estr. Guaratuba (1,6)						October-April
Phengodini							
Pseudophengodes brasiliensis Wittmer 1976 Estrada Poco Preto, Estr.Guaratuba (1,6)	Estrada Poco Preto, Estr.Guaratuba (1,6)	×	×				Ocotber-December
ELATERIDAE (3)							
AGRYPININAE							
Pyrophorini							
Pyrophorus divergens Escholtz 1829	Estrada Guaratuba/Stone Extraction (3,6)	×		X			November-January
Pyrearinus micatus Costa, 1978	Stone Extraction, Estr. Guaratuba (3,6)	X					November-January
Hapsodrilus pyrotis Germar, 1841	Estrada Guaratuba/Stone Extraction (3,6)	×					November-January
STAPHYLINIDAE (1)		×					
Xantholinus sp.	Research house (Sede)	X	Х				October-January

Table 1. Continued...

Activity and Sazonality. Most bioluminescent species investigated here were active at twilight and night. Only some lampyrid species from the tribes Lucidotini (Lucidota sp.) and Lamprocerini (Lucio sp., Lamprocera sp.) were diurnal. These species are in general differentiated from the nocturnal ones by the presence of very developed antennae and rudimentary light organs. Usually they display only spot-like light organs in the last abdominal segments (Lucidota sp.), or in some cases just the vestigial light organs carried from the larval stage (Lucio castelnau Kirsh 1865, Lamprocera flavofasciata Kirby 1895). Among adults of typically bioluminescent species, the activity starts sometimes after sunset and lasts 30 min-2 h after dark. The majority of species start the activity when it is almost dark, with environmental light intensities below 0.3 LUX. Only *Macrolampis* sp.2 is twilight active firefly, starting its activity around 30 LUX and almost stopping the activity below 5 LUX. Observations performed at the end of November showed that adults of Pyrogaster moestus Germar 1824 at the edges of the forest start their activity when there is still considerable environmental light background around 8:00 PM. Photinus fuscus Germar also starts almost at the same time, but in the much darker environment inside the forest whereas Magnoculus sp.3 and Cratomorphus distinctus Olivier 1895 start their activity in the dark between 8:30 PM-10:00 PM. Table 2 shows the time activity of the observed species.

The great majority of adults of lampyrid species are active from October through December. Between January and April, only adults of some lampyrid species are active, whereas from May to August, corresponding to the winter season, the great majority of species were not found at least in the adult stage. Only *Photinus* sp.2 was found active in a warmer night in July 1996. Adult elaterids are active only from November through January. Among phengodids, adult males of

Pseudophengodes brasiliensis Wittmer are active between October and December, whereas an adult Brasilocerus sp.2 was found in April. However, in the larval stage both lampyrid and phengodid species were found active between October and April, with most activity and larger specimens being found in the hot and humid months from January through April.

Bioluminescence: luminescent patterns and photoecology. We found that most firefly species found in the Biol. Station of Boracéia fit under communication system 3 (former system 2), similarly to other species investigated in São Paulo state (Viviani 2001, Viviani et al. 2010), since males have more developed lanterns than the females and emit stronger bioluminescent signals. Only a single species, which was not identified yet, apparently from the tribe Lampyrini, is classified in system 2. The females are larviform and were found at the basis of the grass inside the forest and along the road to Claro's dam during the humid night of October-December, emitting an intense greenish pulse signal. Males, on the other hand were rare, usually found in flight emitting very weak glow through a small lantern. Continuous glows in both sexes, probably representing a transition from system 2 to 3, are found in Magnoculus sp.3 (formerly referred by Viviani (2001) as Amydetes sp.3), and Cratomorphus distinctus Olivier 1895. Their signals can be easily mistaken by the glows of flying luminescent Pyrearinus micatus click beetles and Pseudophengodes brasiliensis. Males of Magnoculus sp.3, have very developed antennae suited for pheromone detection, and also emit intense continuous glows during flight, resembling the signaling pattern of Phausis reticulata, observed in North Carolina (USA). Males and females of C. distinctus Olivier 1890 have very similar lanterns emitting glows or pulses. However, adult males typically



Figure 5. Some typical lampyrid larvae of Biological station of Boracéia: (a) Cratomorphus distinctus Olivier 1895 preying on Bradybaena semilaris Férussac 1821 snail; (b) Lamprocera flavofasciata Kirby 1895; (c) Lucio castelnau Kirsh 1865; (d) camouflage in a lampyrid larva.

fly high below the forest canopy emitting continuous greenish glows whereas females stay lower on the grasses and ivies emitting pulses.

Lamprocera flavofasciata Kirby 1895, Lucio spp., Lucidota spp. are diurnal and use pheromones and communication system 1, with less developed lanterns that produce continuous glows. Similarly, phengodid beetles use a communication system 1 with males with developed antennae suited to detect pheromones and larviform females emitting intense bioluminescence throughout the body.

Adult males of Mastinocerini have lateral lanterns dorsally located which are activated for few seconds upon mechanical stimuli, and neotenic females which emit green-yellow light by lateral lanterns and yellow to red light by cephalic lanterns (Viviani & Bechara, 1997). Bioluminescence function in these species is related to defense in the case of lateral lanterns, and illumination in the case of cephalic lanterns, displaying secondary roles in sexual attraction, which is mediated mainly by pheromones (Sivinsky 1981, Viviani & Bechara 1997).

Among phengodids, *Pseudophengodes brasiliensis* Wittmer (Figures 6a, b) is especially interesting, because it is the only reported species of the tribe Phengodini with adult males displaying luminescence (Viviani & Bechara 1997). Furthermore, adult males display lampyrid-like lanterns which emit continuous yellowgreen glow when flying inside the forest. This pattern is atypical, considering that Mastinocerini males activate their lanterns upon stimulus, suggesting defensive function. The bioluminescent signal

of adult males of *Pseudophengodes*, is very similar to the continuous glows of flying *Magnoculus* sp.3 (Figures 4d, f), and of luminescent click beetles abdominal lanterns, suggesting its use in sexual attraction and also illumination of the environment.

It is noteworthy that there is a slight predominance of continuous glows among forest dueling species (Figure 4), in relation to open fields species found in other localities (Viviani 2001, Viviani et al. 2010), suggesting an adaptation of this kind of pattern in this environment. It was suggested that continuous glows could be advantageous in discontinuous environments such as forests, where intermittent flashes can loose detection efficiency depending on the angle of the receiver (Viviani 2001). Furthermore, inside the forest, continuous glows could be less risky than in open environments where continuous glows can be easy target to aerial predators. Indeed, we often observed attacks by bats and owls to glowing fireflies such as *Magnoculus* sp.3 (Figure 4f) or slow-crescendo flashes such as *Pyrogaster moestus* Germar 1824 when they fly across the road running through the forest.

We also observed the predominance of green colors over yellow inside the forest (Figure 9). This pattern could be explained by the presence of darker environment inside the forest, where the necessity to contrast the bioluminescence signal against the greenish background of the grass is less critical than in open fields where the reflectance of the foliage at twilight can mask the green emissions. (Lall et al. 2009). In this context, *Macrolampis* sp.2 firefly, which lives



Figure 6. Some typical phengodids of Biological Station of Boracéia: (a,b) adult male of *Pseudophengodes brasiliensis* Wittmer 1976 (dorsal, ventral); (c) *Brasilocerus* sp.2 larva and (d) *Phrixotrix hirtus* Olivier 1909 larva.

Table 2. Characteristics of bioluminescent signals of beetles of Biological Station of Boracéia.

TAXON	BL color adult	λmax (nm)	BL pattern	System	Activity time	Environmental Light Intensity (LUX)
LAMPYRIDAE						
LAMPYRINAE						
Cratomorphini						
Cratomorphus distinctus Olivier 1895	green	554	glow	2	20:20-20:40	
C. concolor Perty, 1830	green	554	glow	2		
C. beskey Olivier, 1895	yellow-green		multi	3		
Cratomorphus sp.2	green		glow	2		
Aspisoma physonotum Gorham, 1884 <b>Lamprocerini</b>	yellow-green	561	pulse	3		0.3
Cladodes stellatus Gorham, 1880	green		glow	1	Diurnal	
Cladodes sp.	green		glow	1	Diurnal	
Lamprocera sp.			glow	2	20:30-21:00	
Lucio castelnau Kirsh 1865			glow	1	Diurnal	
Lucio sp.	green		glow	1	Diurnal	
Photinini					Diurnal	
Photinus sp.	yellow-green		single	3		0.9
Photinus sp.1	yellow	563	single	3		0.3
Photinus fuscus Germar 1824	yellow	569	single	3	20:05	
Photinus sp.2	yellow		single	3		
Macrolampis sp.2	yellow	572	slow	3	19:05-19:30	35-30
			crescendo			
Lucidotini						
Lucidota discoidalis Laporte, 1833	green		glow	1	Diurnal	
Lucidota sp.	green		glow	1	Diurnal	
Lampyrini		550				
Incertae sedis AMYDETINAE	green		glow/pulse	2		
Magnoculus sp.3 PHOTURINAE	green	551	glow	2	20:30-22:00	
Photuris sp.3	green	563	single	3		
Photuris sp.7	green	552	bimodal	3	20:30	
Photuris lugubris Gorham 1881	yellow		single	3	20:10	
B.lividipennis Motshulsky 1854	yellow-green	561	single	3		
Bicellonychia sp.9	,			3		
Bicellonycha sp.				3		
Photuris ellyptica Olivier, 1886	yellow-green			3		
Photuris sp	green		single	3		
Pyrogaster lunifer Eschscholtz	green	553	single	3		
Pyrogaster telephorinus Perty, 1833	yellow-green		228.0	3		
Pyrogaster moestus Germar 1843	green	557	slow crescendo	3	19:55	0.3
Pyrogaster sp.2	yellow-green	559	single	3		
PHENGODIDAE	Julion Broom	337	Single	3		
Mastinocerini						
Phrixotrix hirtus Olivier, 1909	yellow-green		glow	1		
Brasilocerus sp.2	green		glow	1		
Stenophrixotrix sp.	yellow		<i>5</i>	1		
Stenophrixotrix sp. Phengodini	J ··			1		
Pseudophengodes brasiliensis Wittmer 1976	green	542	glow	2	20:30-22:00	
ELATERIDAE AGRYPININAE						

<sup>(</sup>P) Protoracic lanterns, (A) Abdominal lantern.

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Table 2. Characteristics

TAXON	BL color adult	λmax (nm)	BL pattern	System	Activity time	Environmental Light Intensity (LUX)
Pyrophorini			glow			
Pyrophorus divergens Escholtz, 1833	(P)green/ (A)yellow		glow			
Pyrearinus micatus Costa, 1978	(P)green/ (A)green		glow			
Hapsodrilus pyrotis Germar,1841	(P)green/ (A)yellow		glow			
STAPHYLINIDAE						
Xantholinus sp.	green		glow			

(P) Protoracic lanterns, (A) Abdominal lantern.

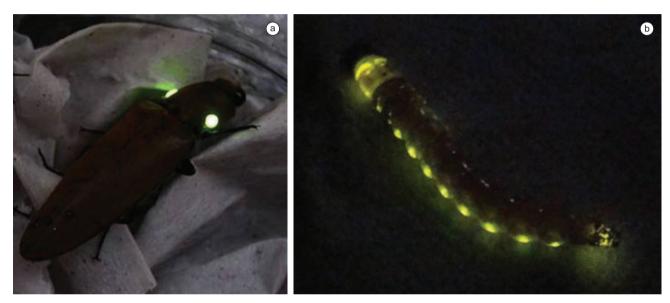
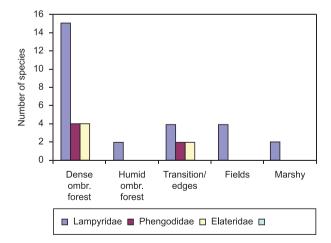
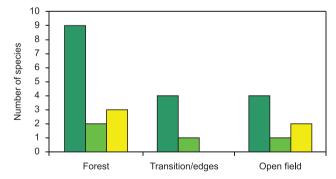


Figure 7. Pyrophorus divergens click beetle: (a) adult and (b) its larva.



**Figure 8.** Frequency of bioluminescent Coleoptera species in different habitats of the Biological Station of Boracéia.

in open marshies, was the only twilight species found, displaying a yellow bioluminescence. Noteworthy, visual, spectrofluorometric and video camera recordings confirm that the bioluminescence color may slightly change from yellow-orange (574 nm) to yellow-green (564



**Figure 9.** Frequency of bioluminescent colors (green, yellow-green and yellow) found in Coleoptera species in different habitats of the Biological Station of Boracéia.

nm). The biological function of such subtle *in vivo* color modulation, if there is one, remains to be investigated (Viviani et al. 2007).

Elaterids also use bioluminescence as a communication system, but it is much less studied; instead of typical intermittent flashes as in fireflies, click beetles use two different sets of lanterns that usually emit different colors (Bechara 1988). The thoracic lanterns usually emit greenish light upon mechanical stimulation or when posed on

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the grass, suggesting defensive function, and a ventral abdominal lantern that emits green to yellow light depending on the species when the animal is flying. The abdominal lantern is usually used by flying males whereas the thoracic lanterns, besides being used for defensive purposes, may also attract flying males of the same species or different ones, strongly indicating its sexual attraction function (Lall et al. 2009).

Regarding larvae, bioluminescent signals have usually defensive functions (De Cock 2009), such as false-eye-spot displays that can startle eventual predators, such in larvae of *Cratomorphus* spp., or aposematic, such in the case of phengodid larvae and several firefly larvae. Lateral lanterns along the body, such as those that occur in railroadworms and click beetles, which live in tunnels inside of decayed logs or inside the soil, may startle negative phototropic organisms living in such microhabitats. Finally, head lanterns in railroadworms have been associated with illumination during the searching for preys (Sivinsky 1981, Viviani & Bechara 1997).

# **Conclusions**

The Biological Station of Boracéia displays the highest diversity of bioluminescent beetles ever reported for a single place in São Paulo state with 39 species, mainly of fireflies (30 species), and unique bioluminescent species that were not found in any other investigated place yet. Most species were found into dense Ombrophyl forest or at its border, and a few ones are found in open fields surrounding the forest. Unique species were found adapted inside the forest, whereas species in common with other places of São Paulo state were found both inside the forest or open fields. These species display typical bioluminescent signals, with predominance of glowing and green emission patterns inside the forest, which may constitute specific photoecological adaptations to the closed environment of the Atlantic rain forest. This unique biodiversity provides valuable material for evolutional, phylogeography and photoecological studies.

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