

Fossil gregarines in Dominican and Burmese amber: examples of accelerated development?

GEORGE POINAR, JR.

Abstract

An eugregarine protozoan from the body of a sminthuridid springtail (Sminthuridae: Symphypleona: Collembola) in Dominican amber is characterized. It possesses a thin-walled gametocyst that dehisces inside the host's body and formed a long spore duct projecting some distance from the springtail. The previously described *Primigregarina burmanica*, represented by a trophozoite and three gametocysts adjacent to a cockroach in Early Cretaceous Burmese amber, also has a dehiscence spore duct. The hypothesis is presented that the sudden deaths of the springtail and cockroach hosts resulted in accelerated development of the developing gametocysts, which produced spore ducts within or adjacent to their hosts, a condition unknown in extant eugregarine infections.

Key words: Eugregarine fossils, *Primigregarina burmanica*, Dominican amber, Burmese amber, accelerated development.

Zusammenfassung

Aus dem Dominikanischen Bernstein wird ein eugregariner Einzeller aus dem Körper eines Springschwanzes (Sminthuridae: Symphypleona: Collembola) beschrieben. Er besitzt eine dünnwandige Gametozyste, die im Wirtskörper aufgeplatzt ist und ein langer Sporodukt gebildet hat, der ein Stück weit aus dem Springschwanz herausragt. Die früher beschriebenen Exemplare des Sporentierchens *Primigregarina burmanica*, vertreten durch einen Sporozoiten und drei Gametozysten nahe einer Schabe im unterkreidezeitlichen Bernstein von Burma, weisen ebenfalls einen aufgeplatzten Sporodukt auf. Es wird die Hypothese aufgestellt, dass der plötzliche Tod der Wirtstiere (Springschwanz und Schabe) eine beschleunigte Entwicklung der Gametozysten auslöste, die zur Bildung von Sporodukten in oder nahe den Wirtstieren führte. Dieses Verhalten ist bei rezenten Infektionen mit Eugregarinen nicht bekannt.

Contents

1. Introduction	1
2. Materials and methods	2
3. The Dominican amber specimen	2
3.1. Description	2
3.2. Discussion	3
4. The Burmese amber specimen	3
5. Discussion	4
6. References	6

1. Introduction

Eugregarine protozoa develop in the alimentary tract of terrestrial and marine invertebrates. Occasionally they can occur in the esophagus, crop, pyloric caecae and rectum. Infection is initiated by a sporozoite that enters per os and infects a midgut cell. The sporozoite then develops into a trophozoite that usually is composed of a terminal holdfast portion (epimerite) attached to the host cell and a protomerite-deutomerite portion that projects into the lumen of the alimentary tract. At maturity, the trophozoite detaches from the gut cell, loses its epimerite and forms a sporont. Two sporonts combine and form a cyst. The gametes of both sporonts fuse to form zygotes that mature into spores within the cyst. The final gametocyst is then passed

from the body with fecal material and eventually the spores are released in the environment. The gametocysts form one or more spore ducts through which the spores pass to the outside. Each spore contains a number of sporozoites that, when liberated, begin the cycle anew. Although the developing trophozoites may destroy host gut cells, these cells are later regenerated so the overall effect on the host is usually slight. Only very heavy infections may have a debilitating effect on the host by destroying gut cells or hindering food passage (STEINHAUS 1949).

The present work characterizes a gregarine infecting a springtail (Collembola) in Dominican amber and discusses the dehiscence spore duct of the previously described Burmese amber *Primigregarina burmanica* POINAR, in relation to accelerated development.

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2. Materials and methods

The Dominican amber springtail specimen is in a piece of amber 50 mm long by 30 mm wide by 10 mm deep. It was obtained from mines in the Cordillera Septentrional of the Dominican Republic. Dating of Dominican amber is still controversial with the latest purposed age of 20–15 mya based on foraminifera (ITURRALDE-VINENT & MACPHEE 1996) and the earliest as 45–30 mya based on coccoliths (CÉPEK in SCHLEE 1990). In addition, Dominican amber is secondarily deposited in sedimentary rocks, which makes a definite age determination difficult (POINAR & MASTALERZ 2000). A range of ages for Dominican amber is possible since the amber is associated with turbiditic sandstones of the Upper Eocene to Lower Miocene Mamey Group (DRAPER et al. 1994). Dominican amber was produced by the leguminous tree, *Hymenaea protera* POINAR (1991) and a re-construction of the Dominican amber forest based on amber fossils indicated that the environment was similar to that of a present day tropical moist forest (POINAR & POINAR 1999).

The Burmese amber piece with the cockroach is roughly rectangular in shape with a greatest length of 12 mm, greatest width of 5.4 mm and greatest depth of 3 mm. The amber was obtained from a mine first excavated in 2001, in the Hukawng Valley, southwest of Maingkhwan in Kachin State (26° 20' N, 96° 36' E) in Burma (Myanmar). This new amber site, known as the Noiye Bum 2001 Summit Site, was assigned to the Early Cretaceous, Upper Albian, on the basis of paleontological evidence (CRUICKSHANK & KO 2003), placing the age at 97–110 mya. Nuclear magnetic resonance (NMR) spectra and the presence of araucaroid wood fibers in amber samples from the Noiye Bum 2001 Summit site indicate an araucarian (possibly *Agathis*) tree source for the amber (POINAR et al. 2007).

Observations, drawings, and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnifications up to 600 X. Helicon Focus Pro X64 was used to stack photos for better clarity and depth of field.

3. The Dominican amber specimen

Figs. 1–4

The springtail (Fig. 1) is a member of the family Sminthuridae (Symphypleona: Collembola) and is 504 µm in length. The specimen is deposited in the POINAR amber col-

lection maintained at Oregon State University (accession # P-3-6).

3.1. Description

The partially collapsed gametocyst of the eugregarine protozoan from the springtail is thin-walled, 44 µm in

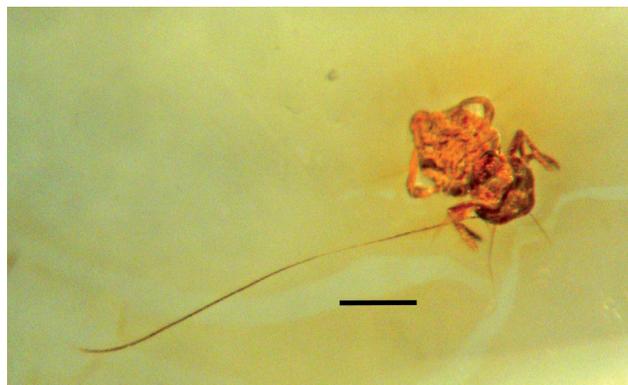


Fig. 1. Sminthuridid with spore duct emanating from the host's body; in Dominican amber. – Bar = 260 µm.



Fig. 2. Sminthuridid showing partially collapsed cyst inside host body (upper large arrow) and spore duct leaving body (lower short arrow); in Dominican amber. – Bar = 46 µm.

length and dehiscing inside the host body (Fig. 2). The thin, transparent wall suggests that the gametocyst may not be fully mature since in most eugregarines, mature gametocysts have thick, dark walls. Its remaining contents closely resemble the contents of the extruded spore duct. The spore duct emanating from gametocyst is 1.46 mm long, nearly three times length of host (Fig. 1). The width of the spore duct varies from 9–14 μm . The content of the spore duct includes multiple layers of spores, many of which are arranged in rows (Fig. 3). The developing spores, which range from 2–3 μm in length, are ovoid, dark and held together by an adhesive mucoid sheath (Fig. 4).

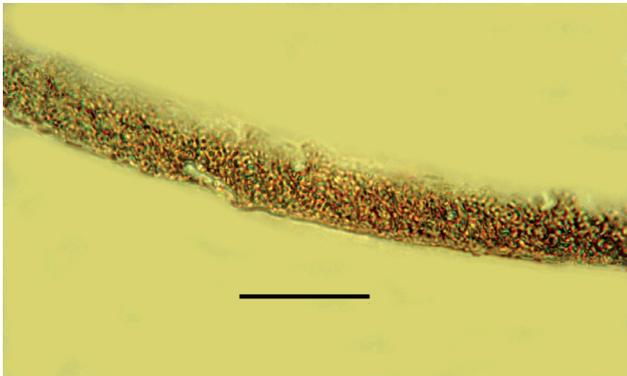


Fig. 3. Portion of spore duct with developing spore stages; in Dominican amber. – Bar = 24 μm .

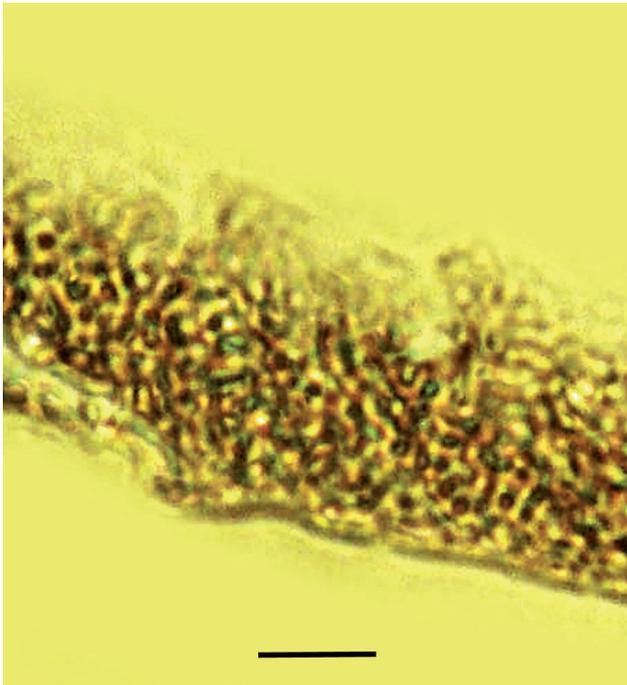


Fig. 4. Detail of portion of spore duct showing developing spores; in Dominican amber. – Bar = 5 μm .

3.2. Discussion

Since collembolans feed on a variety of items in the soil environment, including plant tissues, fecal pellets, fungal hyphae and spores as well as bacteria and nematodes (SNIDER 2007), the question rises whether the cyst was already in the environment and was ingested by the collembolan as a potential food item. However, if the gametocyst was acquired from the environment, it would have been mature with a darkened wall and at dehiscence, the spores would have emerged from the sporoduct in a single row, as is the normal case with eugregarines. The transparent, soft-walled gametocyst and numerous spores in what appears to be different stages of maturity, suggest that the collembolan was the natural host. The position of the host makes it difficult to determine just where the sporoduct originates. It appears to protrude from the intersegmental membrane separating the head and thorax in the neck region, however it is not possible to exclude the mouth as a point of exit.

Extant sminthuridids are hosts to several eugregarines. *Gregarina* sp. was reported from the intestine of *Sminthurus fuscus* L. (Sminthuridae) in Germany (KAMM 1922). *Gregarina podurae* (LÉGER), described from the intestine of *Podura villosa* (Poduridae) and *Orchesella* sp. (Entomobryidae), has spherical gametocysts with a single long spore duct (KAMM 1922), a condition similar to that of the present fossil. Gametocysts of an eugregarine infecting the collembolan, *Tomocerius minutus* TULLBERG (Tomoceridae) in Japan also discharged their spores in long tubes (CHIBA et al. 1966). These extant parasites show a possible behavioral similarity of eugregarines infecting Collembola, namely all with long spore ducts.

In Hispaniola today, representatives of the collembolans, *Sphyrotheca* BÖRNER and *Ptenothrix* BÖRNER are the only two sminthuridid genera reported (PEREZ-GELABERT 2008). Previous records of the Sminthuridae in Dominican amber are limited to two individuals of *Sphyrotheca* (MARI MUTT 1983). The infected fossil specimen reported here is apparently a male. Its length (504 μm) is within the range of the Dominican amber male *Sphyrotheca* sp. (460 μm) (MARI MUTT 1983).

4. The Burmese amber specimen

Apicomplexa LEVINE, 1970
 Eugregarinorida LÉGER, 1900
 Stenophaoricae LEVINE, 1984
 Monoductidae RAY & CHAKRAVARTY, 1933

Primigregarina burmanica POINAR, 2010
 Figs. 5–8

This species was described previously (POINAR 2010a), however, the black and white photographs that accompa-



Fig. 5. Cockroach host of *Primigregarina burmanica*; in Burmese amber. Arrow shows dehiscing gametocyst. – Bar = 900 μm .

nied the description were quite reduced and the new color photographs presented here better express the diagnostic characters. A trophozoite and three gametocysts are adjacent to a cockroach in a piece of Burmese amber (Fig. 5). The trophozoite (Fig. 6) consisted of an epimerite with a short neck expanding into a goblet-shaped disc surrounded by an epicytic collarette. A faint septum separated the transverse protomerite from the closely appressed semi-hemispherical deutomerite. Inside the deutomerite were a number of small oval bodies, very similar to paraglycogen bodies reported in the gamonts of extant eugregarinidids (LANDERS 2002). The structure of the epimerite is a unique and diagnostic character for *P. burmanica*. The three gametocysts are dark brown and spherical. One had not yet dehisced nor formed a bulbous enlargement (= basal disc, spore disc), the second had a bulbous enlargement and the beginnings of a spore duct and the third, the largest of the three (215 μm in diameter), had emitted its spore duct from the basal bulbous enlargement (Fig. 7). Ellipsoidal-ovoid, hyaline spores (oocysts) (3–5 μm in length) were present in the spore duct as well as in the bulbous enlargement at

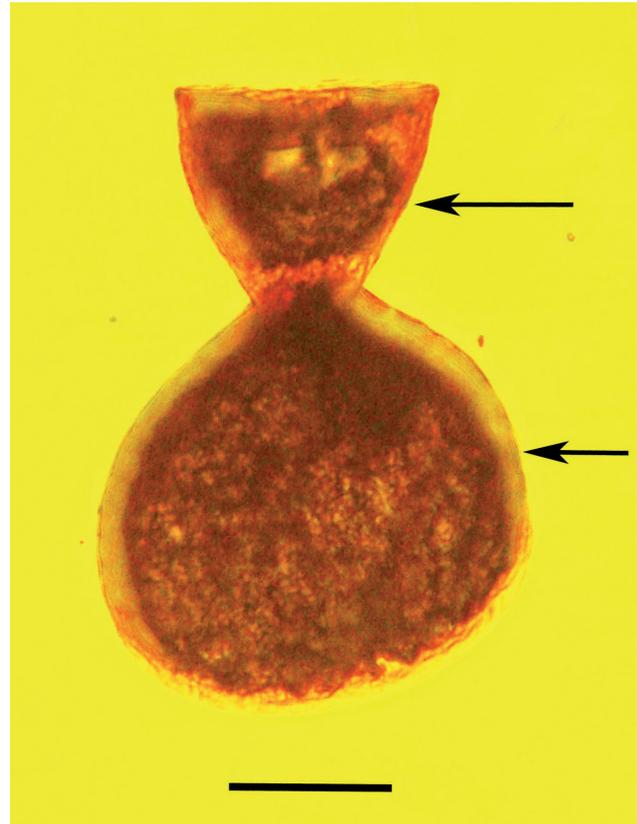


Fig. 6. Trophozoite of *Primigregarina burmanica*; in Burmese amber. Top arrow shows epimerite. Lower arrow shows partially fused protomerite-deutomerite. – Bar = 50 μm .

the base of the spore duct (Fig. 8). The gametocysts are similar to those of the extant cockroach parasite, *Gregarina blattarum* VON SIEBOLD, which also have bulbous enlargements at the base of the spore ducts (SPRAGUE 1941).

5. Discussion

Accelerated (or precocious) development occurs when some stimulus causes an animal or plant to mature faster than normal. This behavior normally is related to the appearance of fruits (with plants) or eggs (with animals) borne ahead of the normal maturation period, usually as a result of increased temperature, moisture, nourishment or a pathological condition. Accelerated development of parasites can also occur in diseased hosts or after death of the host, as is depicted with some invertebrates in amber. An example is the oviposition of female flies in amber (POINAR 2010b). This behavioral action has been interpreted as an attempt to continue the lineage and can be considered accelerated development. Although not directly related to

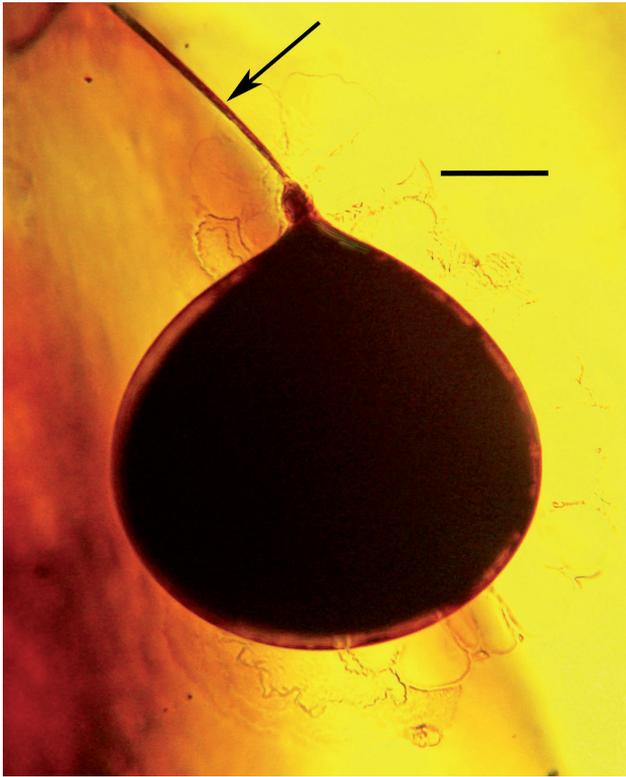


Fig. 7. Detail of gametocyst of *Primigregarina burmanica* with extruded spore duct (arrow); in Burmese amber. – Bar = 55 μm .

accelerated development, evidence that microorganisms can survive in the insect alimentary tract long after the host has succumbed in resin is obtained from “fossil flatus” or gases emitted from the rectum of insects in amber. These microorganisms, mostly bacteria, yeasts and protozoa, live in the alimentary tract where they supply food, essential vitamins and nutrients or are pathogenic. In such cases, most of the extruded gases are carbon dioxide, nitrogen, methane and hydrogen (POINAR 2010c).

Parasitic mermithid nematodes normally leave their insect hosts after reaching a state of maturity that allows them to subsist in the environment long enough to mate and deposit eggs, since no nourishment is obtained in the free-living state. In amber, mermithid nematodes often display accelerated development by leaving their insect hosts prematurely after the latter have become entombed in resin. Only the more mature nematodes, or those that have nearly finished their normal development, leave the host under such conditions, hoping to complete their development in the environment (POINAR 2011). Braconid (Hymenoptera: Braconidae) larvae leaving their ant hosts in Baltic amber (POINAR & MILLER 2002) and dryinid larvae leaving their planthopper hosts (Hemiptera: Fulgoroidea) in Dominican amber (POINAR & POINAR 1999) are yet other examples of accelerated development under stress.

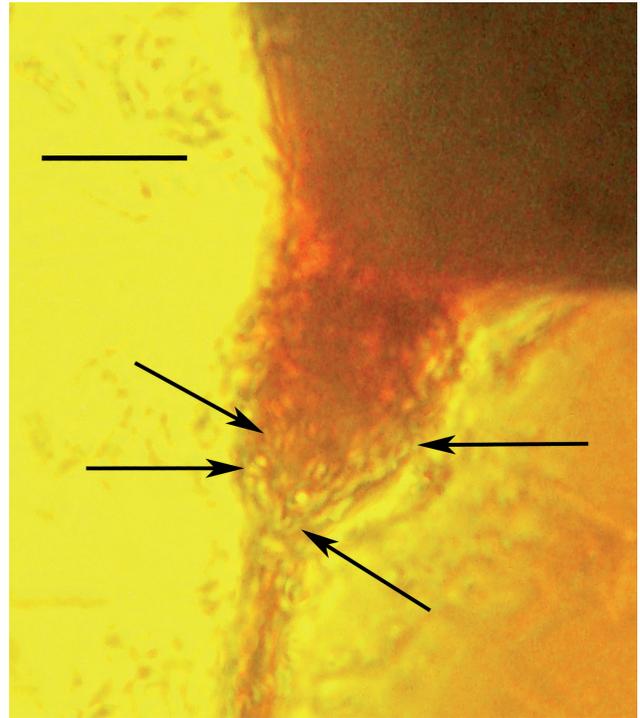


Fig. 8. Spores (arrows) of *Primigregarina burmanica* in bulbous enlargement at base of sporoduct; in Burmese amber. – Bar = 30 μm .

Under normal conditions eugregarines develop as trophozoites, gamonts, zygotes and gametocysts. However if the host becomes the victim of a predator, parasite or another pathogen, one survival feature eugregarines have is accelerated development. If the host dies suddenly, as when it enters a pool of resin, only the final stages of the eugregarine would be able to survive. Gametocysts that had already formed can attempt to release their spore duct and discharge spores to continue the lineage. If host death occurs suddenly as it obviously did in the case of the two amber eugregarines discussed above, the eugregarines, similar to other parasites in resin, can remain alive for a prolonged period, long enough to attempt spore release.

In the case of *P. burmanica*, the gametocysts had already developed when the cockroach was embedded in resin. The protozoan survived in the resin longer than its host. The act of accelerated development in the resin was for the parasites to extrude their spore duct and spores. This explains why spore expulsion occurred in a gametocyst that came directly from the host when normally, the cysts are liberated in the environment before spore expulsion. In the case of the springtail eugregarine, the sudden death of the collembolan resulted in accelerated development of the spore duct and the expulsion of the contents, even though most of the spores were premature.

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Address of the author:

Dr. GEORGE POINAR, JR., Department of Zoology, Oregon State University, Corvallis, OR 97331, U.S.A.
E-mail: poinarg@science.oregonstate.edu

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