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A contribution to the biology of the African canthariphilous anthicids *Formicomus rubricollis* LaFerté, 1848 and *F. gestroi* Pic, 1894

(Coleoptera, Anthicidae)

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Abstract

The geographical distribution, description of the habitat, and data on phenology, diet and breeding conditions are given for *Formicomus rubricollis* LaFerte and *Formicomus gestroi* Pic. Both sexes of these anthicid species are attracted by cantharidin. The role of this terpenanhydrid in the biology of the species is discussed. Furthermore chemical analyses suggest that cantharidin is stored in the reproductive organs of the males and transferred via copulation to the ovaries of the females.

Introduction

The anthicid genus *Formicomus* LaFerté contains more than 390 species. Hitherto little was known about the biology of any of the members of this genus. The reaction of *Formicomus* to the terpenoid cantharidin is remarkable. Eight species of the genus are canthariphilous. These are *F. caeruleus* Thunberg (Van Hille 1986), *F. consul* LaFerté (Young 1984), *F. gestroi* Pic (Hemp 1994), *F. lacustris* Krek. (Van Hille 1970), *F. pedestris* Rossi (Görnitz 1937), *F. rubricollis* LaFerté (Hemp 1994), *F. spatulatus* Van Hille (Hemp 1994) and *F. tropicalis* Krek. (Van Hille 1970).

Both sexes of the African species *F. rubricollis* and *F. gestroi* are attracted by the terpenanhydrid cantharidin. Individuals of these species thus can be easily trapped. Phenological data were collected in a semihumid meadow poor in vegetation during the years 1989/90 from October to April and 1991/92 from November to March at 1430 m o.s.l. in Kidia/Old Moshi (Kilimanjaro, Tanzania). Furthermore the mating behaviour was noted. The role of cantharidin in the biology of the two *Formicomus*-species was of special interest because canthariphilous insects are known to feed on this vesicant, deterrent and antifeedant (Carrel & Eisner 1974, Carrel et al. 1986). An uptake of cantharidin seems to have no nutritional function for canthariphilous insects as they even feed on the synthetic terpenoid (Schütz & Dettner 1992, Holz et al. 1994). A similar system of pharmacophagy is described for Lepidoptera and Orthoptera containing pyrrolizidine alkaloids (Boppré 1986, Boppré & Fischer 1994). The question was, whether *Formicomus*-species are merely attracted by cantharidin to meet mates, or if an analogous system is present as described for *Notoxus monoceros*. In this anthicid species cantharidin accumulates in the reproductive organs of the males and is transferred via copulation to the receptaculum seminis of the female (Schutz & Dettner 1992, Hemp 1994). The terpenanhydrid is transmitted into eggs and thus provides protection for the offspring. In contrast to e.g. canthariphilous *Notoxus, Microhoria* and *Aulacoderus*-species, in which mostly males are attracted by cantharidin partly over great distances (Schütz & Dettner 1992), the investigated *Formicomus*-species are sensitive to cantharidin only in the immediate surrounding. The ratio of the attracted sexes is 1:1.

To investigate the mating and reproduction behaviour and the preferred diet of the African anthicids *Formicomus rubricollis* and *F. gestroi*, freshly killed meloids, beside oedemerids (Carrel et al. 1986, Holz et al. 1994) the only natural source of the terpenoid cantharidin (Capinera et al. 1985, Blodgett 1991), and synthetic cantharidin baits were used to collect these beetles.

Methods and Materials

Insects and breeding conditions

From October 1989 to April 1990 and from November 1991 to March 1992 three traps were put out in a 10 m × 20 m area of a semihumid meadow poor in vegetation (1430 m o.s.l., Kilimanjaro area, Tanzania). The traps were checked three times a day at 7 am, 2 pm and 6 pm. Filter papers soaked with an acetone-cantharidin-solution were placed under gauze to prevent beetles from direct access. The baits were renewed every three to four days. Parallel to the trap controls, temperature and relative humidity were noted.

The beetles were kept in 20 cm × 20 cm plastic boxes under field conditions in Kidia/Old Moshi (Kilimanjaro, 1430 m o.s.l.). The two *Formicomus*-species were separated and bred on material (laterised red soil on volcanic rock, Downie 1972) taken from the habitat of the beetles. Daily moistening with water provided sufficient humidity. Adult beetles as well as the larvae were fed with fish food (Tetra Min) which consists mainly of fish meal, pulverised animals, colouring and vitamins. The uptake of the food was easily seen by the reddish droppings of the beetles and the gut contents of the translucent larvae.

Notes on the behaviour resulted from individuals kept in the laboratory as well as from free living specimens.

Vegetation relevé

The phytosociological data were collected following the method of Braun-Blanquet (1964). In a representative, floristically homogeneous area of a few square metres in xerophilic meadows to some hundred square metres in forests the cover abundance was estimated, separated in strata, according to the following scale:

r = single specimens occurring in the reference area
+ = few species with small cover
1 = species with less than 5 % cover
2 = any number of species with cover 5-25 %
3 = any number of species with cover 25-50 %
4 = any number of species with cover 50-75 %
5 = any number of species with cover of more than 75 %.

Feeding experiments with cantharidin

All baits used to attract anthicids in Old Moshi (Kilimanjaro area, Tanzania) were put under gauze to prevent an direct access to cantharidin. At the end of the field studies about 50 specimens in April 1990 and 30 specimens in March 1992 of both *Formicomus*-species were brought alive to Germany. Because the beetles were captured during the period of January and February of both years, mainly females and hardly any males could be analysed. In the laboratory a part of the beetles had 9 days access to cantharidin. On the ninth day of cantharidin uptake the specimens were frozen (-55 °C).

Chemical analyses of cantharidin contents

For each specimen an elytral fragment, a leg (middle leg with tarsus, tibia, femur and trochanter), one testicle, one accessory gland and one ovary were isolated. Dry weights of the fragments were registered (Sartorius semimicro balance R 200 D). The elytral fragment and the leg were directly used for the determination of the cantharidin titres. In case of the paired structures, testicle, accessory gland and ovary the fresh organs were used for analysis for too little material remained after drying. Only the value of the dried paired structures was used for calculation.

The beetle fragments were placed into injection needles (Pressure Mini-Injector 1 µl, Precision Sampling Corporation) and injected into a Carlo Erba Vega Series 2 GC 6000 gas chromatograph equipped with a CP-Sil 19 CB fused silica capillary column (Chrompack, 12,5 m × 0,32 mm I.D.; 0,25 m phase thickness; temperature program: 80 °C to 280 °C with a heating rate of 15 °C/min; carrier gas: helium, 40 KPa) connected to a Finnigan MAT Ion Trap Detector (ITD). Electron impact ionization (EI, 70 eV) provides mass spectra with a characteristic fragmentation of cantharidin: the base peak with m/z 96 and a fragment with m/z 128.

The cantharidin contents of field-caught and cantharidin-fed specimens were determined by means of calibration curves. The cantharidin contents of the haemolymph resulted from addition and division of the titres of the middle leg with the elytral fragment.

With this method only unbound cantharidin was analysed while bound cantharidin, which is also present in the structures, can not be detected by chemically untreated beetle tissue.

Results

Description and geographical distribution of *Formicomus rubricollis* and *F. gestroi*

Formicomus rubricollis LaFerté (1848) shows a reddish pronotum with a dark head. The elytra are black to green or blue with an apparent metallic lustre. As do most species of the genus *Formicomus*, males possess teeth at the tibia and femur of the fore legs, their average length is 3,3 mm.

This species occurs in Central (Zaire), East (Kenya (Uhmann 1989), Tanzania (Uhmann 1981, Uhmann 1990), Ruanda, Burundi) and South Africa [Mozambique, Sambia, Simbabwe, Botswana, Namibia, South Africa (Uhmann 1984)].

Formicomus gestroi Pic (1894) is a very variable species. Colouring of the adults varies from brown to black, whereas the pronotum mostly is reddish and a vertical nut may be present. The elytra show often a metallic lustre. The males have teeth at the tibia and the femur of the fore legs. The average length is about 3 mm.

The species occurs all over Africa south of the Sahara. It is also found in East Africa (Kenya (Uhmann 1989), Tanzania (Uhmann 1981, Uhmann 1990), Burundi, Ruanda, Uganda), further north in Sudan and southward in Malawi, Angola, Sambia and South Africa. Even in Central (Zaire) and West Africa (Ghana, Gambia) *Formicomus gestroi* is widespread.

Habitat

Formicomus gestroi and *Formicomus rubricollis* are sympatric in the area of Kidia / Old Moshi (Kilimanjaro area, 1430 m o.s.l.). Both species are frequent in open land. Dense populations are present on meadows (see vegetation relevé) and at the rims of bushes with herb vegetation. Aggregations of these beetles can be found on the herb *Lepidium bonariense* L. (Brassicaceae), which reaches heights up to 0,5 m.

A vegetation relevé characterises the habitat of the two *Formicomus*-species where most of the traps were put out.

Relevé of a semihumid meadow poor in vegetation in Kidia / Old Moshi (Kilimanjaro 1430 m o.s.l.).

Cyperus rigidifolius Steudel	3	*Ageratum conyzoides* Linné	+
Hydrocotyle mannii Hook.f.	3	*Apium leptophyllum* (Pers.) Benth.	+
Oxalis latifolia H. B. & K.	3	*Bidens pilosa* Linne	+
Centella asiatica (L.) Urb	2	*Caucalis incognita* Norman	+
Cyperus brevifolius (Rottb.) Hasskn.	2	*Conyza floribunda* H. B. K.	+
Vigna parkeri Bak.	1	*Justicia flava* Vahl	+
Zephyranthes grandiflora Herb.	1	*Justicia striata* (Kl.) Bullock	+
Dichondra repens J. R. & G. Forst	1	*Phyllanthus suffrutescens* Pax	+
Eragrostis schweinfurthii Chiov.	1	*Spilanthes mauritiana* (Pers.) DC.	+
Oplismenus hirtellus (L.) P. Beauv.	1	*Tagetes minuta* Linné	+
Lepidium bonariense Linné	+	*Thunbergia alata* Sims	+

Fig. 1: *Lepidium bonariense* L. parasitized by a mildew fungus.

Diet

Lepidium bonariense is a frequent plant in the region. It often was parasitised by a whitish mildew fungus at the lower leaf surface (Fig. 1). The fungus produced orange sporangia.

Investigations of the gut content of field-caught specimens of both *Formicomus* species revealed that the whole intestines were full of these orange sporangia. In the crop the sporangia could be found nearly in intact packs, whereas further down in the stomach and in the mid gut area garish fatty orange drops appeared. In the end gut the orange colour slowly disappeared and the gut content was soluble in water again.

In the field beetles could be observed tugging with their mandibles at the leaves of *Lepidium bonariense* parasitised by the fungus. Probably the beetles aggregate on these plants to take up the sporangia of the fungus. However, in the laboratory individuals of both *Formicomus* species could also be bred with fish food (Tetra Min), maize porridge, bread, fruit and dead insects. The abdomen of dead *Formicomus* specimens within the plastic boxes were usually hollowed out.

Mating behaviour

On contact males of both species immediately jumped at any individual of the same species. Only after contact the males realise whether the jumped on specimen is a female or not. If the female is ready to mate copulation takes place at once. Otherwise the female tries to remove the male by pushing it with the hind legs. If the female is ready for mating the male hooks its fore legs into depressions at both sides of the prothorax of the mate. Projections of the tibiae of the males fit into these prothoracal notches of the females. The male projects its aedeagus and inserts it into the upward bend opened genital valves of the female. The female holds its antennae tightly pressed to the body whereas the male holds them horizontally away from the body. During copulation the male shakes the female several times. The pair remains mounted at least 24 hours. After several weeks of captivity copulating pairs could be observed again.

Cantharidin-attracted beetles tried intensively to copulate. The specimens jumped on each other, and often four to six beetles got entangled. Even while being collected trapped beetles in small vials, individuals mounted each other and copulated. This behaviour was not observed so often in individuals directly collected from the vegetation.

Reproduction

Three to four weeks after copulation the female produces for several days oval, whitish eggs. The eggs are laid singly or in small heaps into the upper layers of the soil. An isolated female produced a maximum of 25 eggs per day. In choice tests with various plants (also *Lepidium bonariense*, with leaves, flowers, stems and roots) and soil material coming from the habitat of the species, eggs could always be found in hollow spaces in the upper layer of the soil. After 12 days the whitish, little sclerotised larvae, of 1 mm length and 0,2 mm width, hatch.

The larvae, with abdomen first, leave the egg skin by winding and beating movements. In the following 6-8 weeks the now white and distinctly sclerotised larvae grow to a maximum length of 6 mm and a width of 0,6-1 mm (see table 1). Larvae form a longish earthen puparium shortly before pupation. The development from the sluggish larvae forming the puparium to the *pupa exarata* takes eight days.

After the puparium is finished the larvae becomes stiff except for beating movements of the abdomen. Three days later pupation can be noted. After some days the white pupa turns yellowish. On mechanical stimulation the pupa reacts with beating movements. After 10 days the adults emerges. Freshly hatched beetles are immediately canthariphilous.

Table 1. Development of the larvae of *Formicomus gestroi* and *Formicomus ruficollis*. Days: Age of the larvae after hatching; Length: Average length of the larvae, means with standard deviation; Width: Maximum width of thorax, means with standard deviation; n: Number of larvae; –: No values.

Days	Length (mm)	Width (mm)	n
1	1,00 0,01	0,20 0,01	10
5	1,76 0,20	–	17
11	1,90 0,33	–	18
18	2,25 0,64	–	24
21	2,90 0,53	–	19
28	4,00 1,08	0,53 0,24	14
34	4,37 0,84	0,60 0,13	10
41	4,30 0,11	0,70 0,08	6

Most of the larvae were fed with fish food. The uptake of the diet was easily seen from the red colour of the gut content. To find out which food the larvae take up in their natural habitat a choice test was made. In a petri dish (diameter 20 cm) parted into three areas, one part was filled with pure soil from the habitat (laterised red soil on volcanic rock). The second part contained $^{2}/_{3}$ soil mixed with $^{1}/_{3}$ cow dung, the last triangle of the petri dish had soil with plant material. This plant material consisted of small cut leaves of *Grevillea robusta* (Proteaceae), that grew everywhere in the habitat of the beetles. Furthermore pieces of grass leaves, other plants and detritus taken from the area where the beetles occurred were mixed in. The test was started when the larvae hatched from isolated eggs and was stopped when first pupae were found (10.1.-20.2.90). The different soil types were examined for larvae but it was not possible to find all larvae at one session (see table 2, column total number of found larvae).

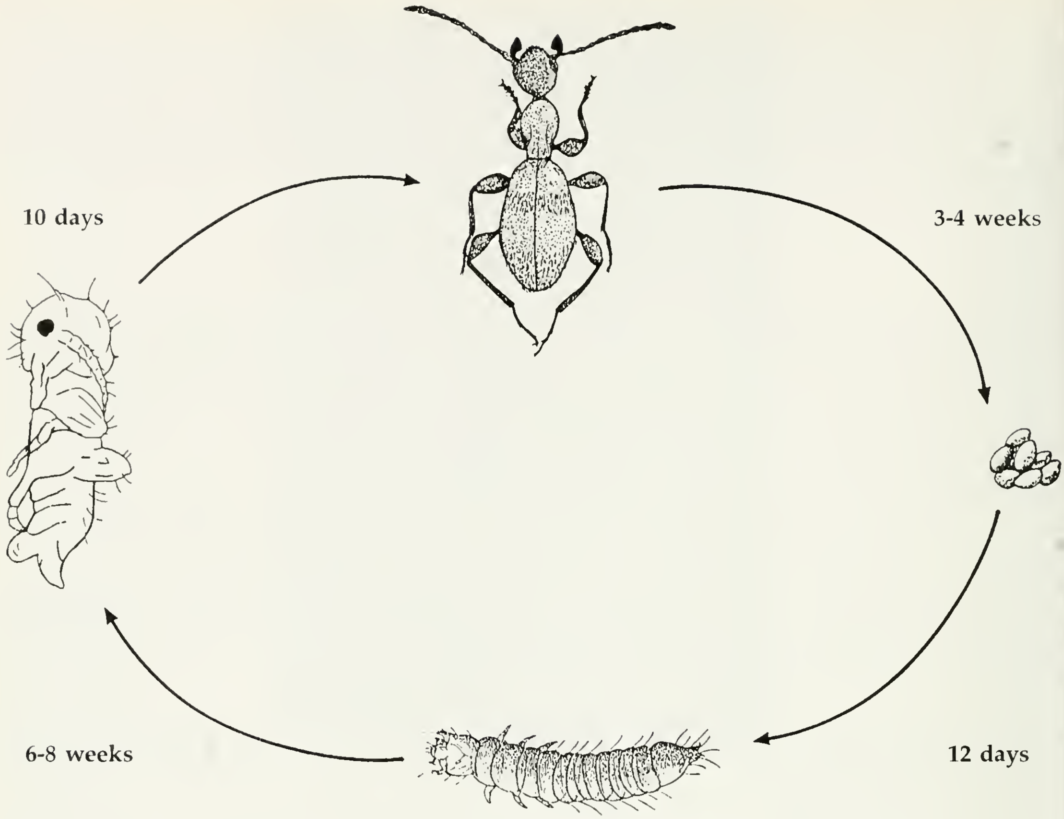


Fig. 2: Life cycle of *Formicomus gestroi*. Lifespan of the adults in the laboratory: max. 5 months; oviposition after copulation: about 3-4 weeks; hatching of the larvae after oviposition: 12 days; development of the larvae to pupa: about 6-8 weeks; pupa: 10 days.

Table 2. Choice test with larvae of *Formicomus*: Number of larvae in the different soil types. Days: Age of the larvae after hatching; n: Total number of found larvae; initial number of larvae: n = 30

Days	Pure soil	Soil with cow dung	Soil with plant material	n
1	1	13	3	17
6	5	9	4	18
14	3	10	11	24
18	3	12	4	19
25	2	8	4	14
32	0	6	4	10
40	1	4	1	6
Total	15	62	31	108

Most of the larvae apparently preferred the soil mixed with cow dung (57,5 %). From 108 larvae found during all controls only 13,8 % of the individuals could be detected in pure soil and 28,7 % in soil mixed with plant material. Many of the larvae in soil mixed with cow dung stuck in dung balls. The guts of these individuals were noticeable more richly filled with material than those of the other soil types.

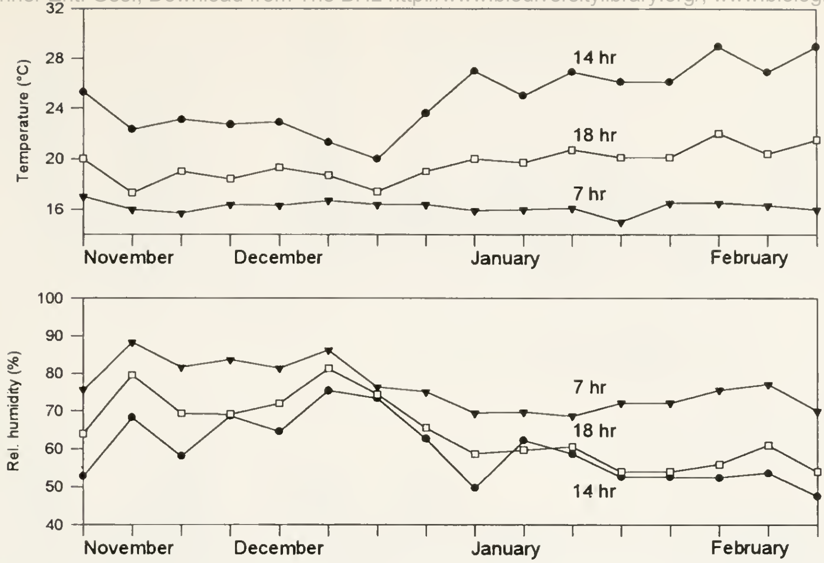


Fig. 3: Temperature and rel. humidity in Kidia/Old Moshi (Kilimanjaro 1430 m o.s.l., Tanzania) at 7 hr, 14 hr and 18 hr in the period of November to February 1991/92.

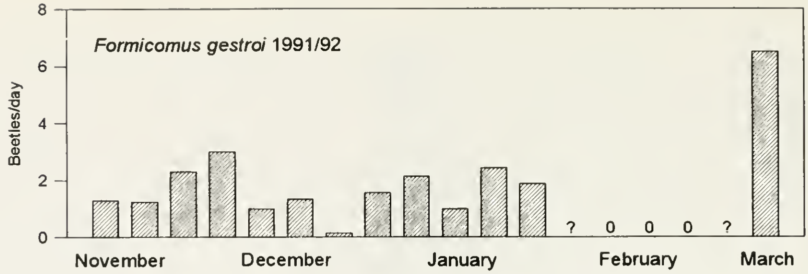
In upper layers of fertilised soil from cultivated areas, that had been passed through a sieve, *Formicomus*-larvae of all sizes were found. Probably the larvae develop quickly specially in loose soil fertilised with organic material. Similar conditions could be noticed also for the species *Formicomus pedestris*. This beetle species was trapped in wheat fields in the Mediterranean region which was fertilised with stall litter. Breeding of this *Formicomus*-species probably was not successful because the larvae were not fed with a suitable diet in the laboratory (Schütz 1989). Fish food given to the African *Formicomus*-larvae in following breeding experiments obviously was the adequate diet. Thus larvae of *F. gestroi* and *rubricollis* grew rapidly, the larvae pupated, and the adults hatched.

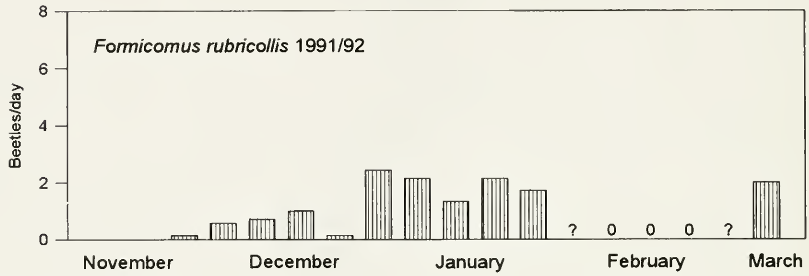
Phenology

During the field studies in Kidia/Old Moshi (Kilimanjaro area, Tanzania) in the months of October 1989 to April 1990 and November 1991 to March 1992 temperature and the relative humidity were noted daily at 7 am, 2 pm and 6 pm (Fig. 3). About the same time the cantharidin traps were checked.

In captivity the beetles laid eggs mid-November, end of December, mid-January and beginning of February. The oviposition in December came together with the small rainy season, those of November and January with the dry seasons. Notably the months of January and February are the hottest and the driest periods of the year (see Fig. 3).

From November to February specimens of *F. gestroi* and *F. rubricollis* were continuously attracted to cantharidin traps (see Fig. 4-7). A maximum was achieved during the period from November to December. Few beetles were trapped end of December/beginning of January 1989/90 as well as 1991/92. Heavy rainfalls during the small rainy season probably caused a decrease of the population density (see Fig. 3). Temperatures fell to 15 °C in the morning hours and the relative humidity increased to over 80 %. Even during the midday hours (2 pm) the temperatures only reached about 20-22 °C with a relative humidity that did not fell below 80 %. Similar weather conditions, that means comparatively low temperatures at a high relative humidity, also predominated during the small rainy season in the evening hours.

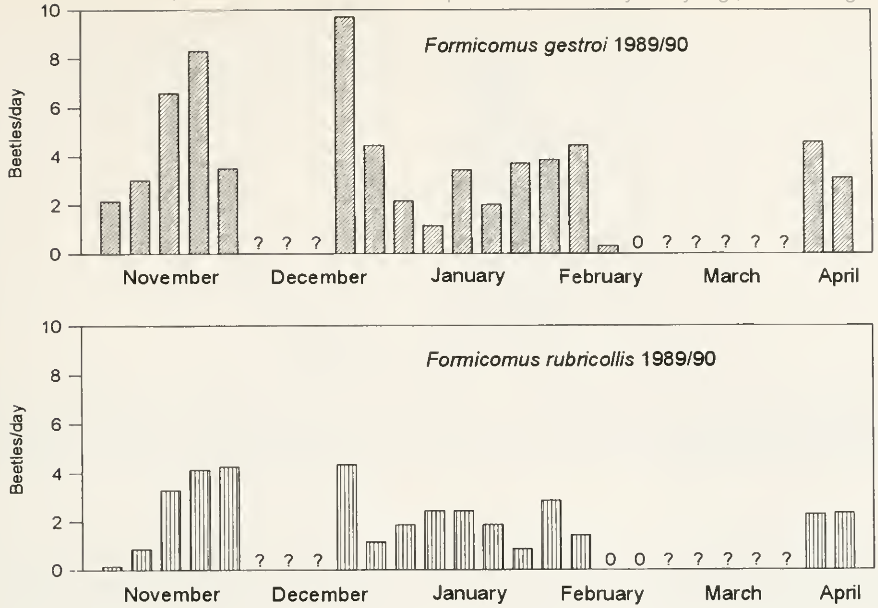




Figs 4-5: Abundance of *Formicomus gestroi* and *rubricollis* 1991/92 from November to March in Kidia (Kilimanjaro-area, Tanzania). ?: No values; 0: No catches in cantharidin traps.

Table 3. Weekly sums and sex ratio of cantharidin attracted *Formicomus gestroi* and *F. rubricollis*. Fg1: Weekly sums *Formicomus gestroi* 1989/90; Fr1: Weekly sums *Formicomus rubricollis* 1989/90; Fg2: Weekly sums *Formicomus gestroi* 1991/92; Fr2: Weekly sums *Formicomus rubricollis* 1991/92; m%, f%: Proportion of males, females of the weekly sum; –: No values.

Week	Fg1	m %	f %	Fr1	m %	f %	Fg2	Fr2
27.10-02.11	15	–	–	1	–	–	–	–
03.11-09.11	21	–	–	6	–	–	–	–
10.11-16.11	46	44,7	55,3	23	43,5	56,5	–	–
17.11-23.11	58	44,8	55,2	29	58,6	41,4	9	0
24.11-27.11	14	50	50	17	41,2	58,8	5	0
28.11-04.12	–	–	–	–	–	–	16	1
05.12-11.12	–	–	–	–	–	–	21	4
12.12-18.12	–	–	–	–	–	–	7	5
19.12-21.12	29	58,6	41,4	13	30,8	69,2	4	3
22.12-28.12	31	38,7	61,3	8	37,5	62,5	1	1
29.12-04.01	15	33,3	66,7	13	23,1	76,9	11	17
05.01-11.01	8	37,5	62,5	17	41,2	58,2	15	15
12.01-18.01	24	50	50	17	41,2	58,8	3	4
19.01-25.01	14	35,7	64,3	13	46,2	53,8	17	15
26.01-01.02	26	42,3	57,7	6	33,3	66,7	13	12
02.02-08.02	27	40,7	59,3	20	20	80	–	–
09.02-15.02	31	32,3	67,7	10	10	90	0	0
16.02-22.02	2	0	100	0	–	–	0	0
23.02-28.02	0	–	–	0	–	–	0	0
06.03-11.03	–	–	–	–	–	–	39	12
30.03-05.04	32	–	–	16	–	–	–	–
06.04-14.04	28	–	–	21	–	–	–	–
Total	421			237			161	89



Figs 6-7: Abundance of *Formicomus gestroi* and *rubricollis* 1989/90 from November to April in Kidia (Kilimanjaro-area, Tanzania). ?: No values; 0: No catches in cantharidin traps.

An increase of trapped beetles was noted again at the beginning of the dry season in January, but the number of caught specimens was not so high anymore during November and December. The mean daily temperatures were in average lower than during the rainy season (15,9 °C during the week from the 5.1. to the 11.1.92 compared to 16,4 °C during the week from the 22.12. to the 28.12.91) and the relative humidity fell for about 10 %. Over midday temperatures raised to 30 °C and the relative humidity sank to 49,7 % (5.1.-11.1.92) and remained relatively low until the evening hours (58,7 % during the week of the 5.1. to the 11-1.92-74,3 % during the week of the 22.12. to the 28.12.91). From the beginning of February few beetles still were attracted. From mid-January the portion of trapped females noticeably increased. For *F. rubricollis* specimens the proportion of females was generally higher, but also in this species from end of January onward the number of females attracted raised from 66,7 % (26.01-1.2.1990) to 90% during the week of 9.2.-15.2.1990. From mid-February to the beginning of March no beetles at all were trapped. From the middle of March and in April with the beginning of the great rainy season in both field study periods (1990 and 1992) beetles appeared again (Fig. 4-7).

Analyses of cantharidin contents

The cantharidin contents of various structures of the both *Formicomus*-species trapped in Kidia/Old Moshi were determined by GC-MS (gas chromatography-mass spectrometry), because first analyses had shown that the structures mostly contained only small amounts of cantharidin. Therefore it was often necessary to identify cantharidin with the help of the diagnostic mass spectra fragments. On the one hand beetles with no access to cantharidin in the laboratory were analysed, on the other individuals that had had the possibility to take up synthetic cantharidin for 9 days in the laboratory.

While preparing males it was found conspicuously, that the paired testes showed enormous differences in size. When a spermatophore was present in the abdomen of the male, one of the testicles was small (about half of the size of the other) and of amorphous shape. The other testicle showed the normal 3-armed shape. Freshly hatched males where a spermatophore was not yet formed in the abdomen always possessed equally shaped 3-armed testes.

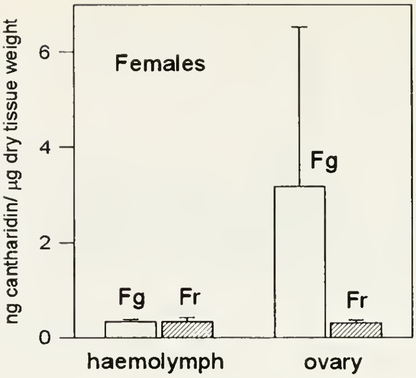


Fig. 8: Cantharidin contents (ng cantharidin/g dry tissue weight, means with standard deviation) in the haemolymph and ovaries of the females of *Formiconus gestroi* (Fg, left columns, n=4) and *F. rubricollis* (Fr, right columns, n=3), with 9 days access to cantharidin.

Table 4. Cantharidin contents (ng cantharidin/g dry tissue weight, means with standard deviation) of various structures of *Formiconus gestroi* and *Formiconus rubricollis*. Fg: *Formiconus gestroi*; Fr: *Formiconus rubricollis*; m, f: Males, females; +: Traces of cantharidin (less than 0,01 ng/g). Number of investigated beetles per group: n=3, except cantharidin fed females of *F. gestroi*: n = 4.

Structure	No access to cantharidin				9 days access to cantharidin		
	Fg m	Fg f	Fr m	Fr f	Fg f	Fr m	Fr f
Haemolymph	+	+	+	+	0,33 0,05	0,51 0,12	0,33 0,07
Ovary	–	+	–	+	3,20 2,90	–	0,30 0,05
Testicle 1 *	+	–	+	–	–	0	–
Testicle 2 *	+	–	+	–	–	8,63 4,90	–
Spermatophore	+	–	+	–	–	7,33 1,55	–
Acc. gland	+	–	+	–	–	0,14 0,2	–

* Testicle 1: about half of the size of testicle 2; Testicle 2: intact, 3-armed testicle

Compared to field-caught females of both *Formiconus*-species, which only contained traces of the terpenanhydrid in the analysed structures, all of the seven females (4 *F. gestroi*, 3 *F. rubricollis*) that had the opportunity to take up cantharidin for 9 days showed distinct cantharidin titres (table 4). In females of *F. gestroi* the titres of cantharidin were higher than those of *F. rubricollis*. Fig. 8 shows that in the ovaries of *F. gestroi* females more cantharidin was stored than in the haemolymph.

Also field-caught males of both *Formiconus*-species only showed traces of cantharidin in the analysed structures (table 4). On the other hand distinct cantharidin titres were present in males of *F. rubricollis* that fed for 9 days on synthetic cantharidin in the laboratory (Fig. 9).

High cantharidin titres were found in the spermatophore and the 3-armed testicle of the males of *F. rubricollis*. In the amorphously shaped smaller testicle no cantharidin at all was present. In the accessory glands only small amounts of free cantharidin could be analysed (Fig. 9).

Tests with cantharidin

Mating ratio of cantharidin-fed specimens

Cantharidin-attracted individuals of *Formiconus gestroi* and *F. rubricollis* jumped at each other immediately on contact. Even in the traps the beetles mated when males and females were trapped together. Individuals

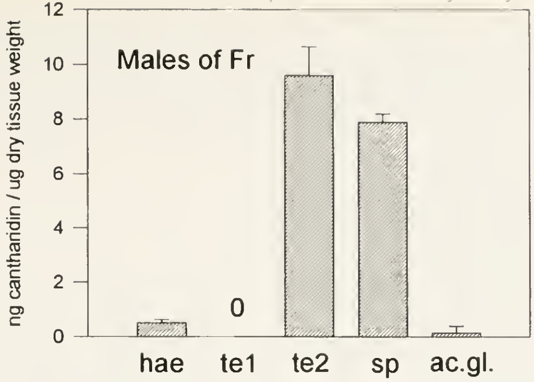


Fig. 9: Cantharidin contents (ng cantharidin/g dry tissue weight, means with standard deviation) in the haemolymph (hae), the amorphous shaped testicle (te1), the 3-armed testicle (te2), the spermatophore (sp) and the accessory glands (ac.gl.) of male *F. rubricollis* (Fr) with 9 days access to cantharidin; n=3.

freshly trapped with cantharidin always showed intensive mating behaviour, as could be seen from the high rate of mounted pairs. But a few days later nearly no mounted pairs could be observed anymore. In table 5 all cases from the 7.11.89 to the 14.4.90 are counted in which males and females were trapped together. Mounted pairs in the traps were noted.

Table 5. Mating ratio of attracted *Formicomus gestroi* and *Formicomus rubricollis*. Pairs: Number of cases, in which males and females were trapped together.

Species	pairs	mated	not mated	% mated
F. gestroi	76	43	33	56,6 %
F. rubricollis	28	18	10	64,3 %

Table 5 shows the high ratio of individuals that mated immediately after contact in the cantharidin traps. Out of 76 cases, in which females and males were trapped together, 56,6 % of all *Formicomus gestroi* pairs mated. The ratio for *F. rubricollis* pairs is even higher: from 28 cases 18 pairs copulated (=64,3 %). Not included in the table are those copulations which took place later in captivity. Cantharidin attracted beetles are thus apparently very eager to copulate.

Ratio of cantharidin attraction in dependency from age

To find out whether an attraction by cantharidin depends on the age of the individuals two boxes were attached together. One airtight plastic box contained synthetic cantharidin whereas into the other beetles of different ages were placed. Recently attracted beetles probably were young and unmated individuals; these specimens in general were smaller (specially the females) than beetles being in captivity for a longer time. Beetles directly caught out of the vegetation in the habitat by an exhaustor, on the other hand, were of all age stages. Finally beetles kept for 4 weeks were probably all quite old and already mated.

Tested individuals that were directly taken from the traps again were strongly attracted by cantharidin (100 %, 93 %, see table 6). The attraction ratio of beetles caught from the vegetation clearly was not as high (86 %, 68 %) as that of cantharidin attracted specimens. The lowest attraction ratio was found with beetles having lived in captivity for about 4 weeks (25 %, 30 %).

Table 6. Ratio of cantharidin attraction of *Formicomus gestroi* and *F. rubricollis*. Number of beetles per time (1/2 hr to 9 hrs) attracted to synthetic cantharidin. hr: Hour(s); %: Attracted beetles in %; -: No values.

Time	½ hr	2 hrs	7 hrs	9 hrs	%
12 *F. gestroi*, freshly attracted	1	2	4	12	100
15 *F. rubricollis*, freshly attracted	1	6	–	14	93
14 *F. gestroi*, caught from vegetation	3	–	12	12	86
44 *F. rubricollis*, caught from vegetation	10	–	–	30	68
12 *F. gestroi*, 4 weeks in captivity	0	0	1	2	25
30 *F. rubricollis*, 4 weeks in captivity	–	7	–	9	3

Bait experiments with meloids

Beside synthetic cantharidin baits meloid beetles were used to attract *F. gestroi* and *F. rubricollis*. The meloid specimens were collected in the savanna (1000 m o.s.l.) at the foot of mount Kilimanjaro near Moshi. A trap contained 5 freshly killed intact meloids (*Mylabris aperta, M. vestita* and *M. amplectens*) and was placed in the habitat of the anthicids in Kidia / Old Moshi. Data about the cantharidin contents of meloids have been published for e.g. *Lytta* and *Epicauta*. Blodgett (1991) analysed four species of the genus *Epicauta* and found total amounts of cantharidin from 33,5 g to 372,9 g per beetle.

During the bait tests with meloid beetles a second trap was put out, which contained a filter paper soaked with 1 ml of a acetone-cantharidin solution (about 200 g cantharidin/ml acetone). The concentration of cantharidin in the trap with the synthectic bait was probably much lower than that with the meloid beetles. On the other hand it must be considered that an evaporation of cantharidin is much easier from a filter paper than through the cuticle of killed beetles.

The bait test was made at the beginning of January 1992 at a time of relative high population densities of the *Formicomus*-species (see Fig. 4-5). The traps were posted about 3 m from each other into a meadow poor in vegetation.

Table 7 shows no distinct difference in the attraction of synthetic and natural cantharidin baits. Anthicids that were caught in the meloid trap were observed feeding on the meloids. At the end of the test the abdomen of the meloids was totally hollowed out.

Table 7. Comparison of the attraction ratio of synthetic and natural cantharidin baits.

5.1.-12.1.90	*F. gestroi*	*F. rubricollis*
meloid beetles	22	17
synth. bait	21	13

Duration of attraction of cantharidin baits

During the cantharidin bait tests in Kidia/Old Moshi (Tanzania, Kilimanjaro) it was obvious that the number of caught individuals decreased with the age of the baits. Therefore a test was made to show whether the age of a bait has an influence on the ratio of attracted anthicids. Furthermore it was of interest whether leaves of *Lepidium bonariense* that were parasitised by a mildew fungus also attract anthicid beetles. An acetone-extract (about 30 g cut leaves in 5 ml hot acetone) of parasitised leaves was made and trickled on filter paper.

Two traps contained cantharidin (filter paper soaked with 1 ml of a acetone-cantharidin solution, concentration 200 g cantharidin / ml acetone). The bait of only one of these traps was renewed every three days. The second trap contained a filter paper that was impregnated with cantharidin 5 days before the test was started. In a third trap the *Lepidium bonariense*-extract was tested. The extract also was renewed every three days. The fourth trap without bait served as a control. The four traps were put out under *Lepidium bonariense* plants at distances of about 40 cm of each trap. The order of the traps was changed every three days. The traps were checked during 12 days at 7 am, 2 pm and 6 pm.

The most effective attraction was observed at the bait that was renewed every three days. Into the trap with the 5-days-old bait that was not renewed during the test only four beetles were attracted during the first 6 days. Later no anthicids were trapped anymore. Only one beetle was found in the trap containing the *Lepidium bonariense*-bait and no beetles at all came to the control. In the trap with the cantharidin bait that was renewed every three days a high number of beetles was noted throughout the duration of the test. All in all 50 anthicids were taken out of this trap.

Discussion

Biology

Males and females of *Formicomus gestroi* and *F. rubricollis* meet by chance in their habitat. Because both sexes of these species are canthariphilous, cantharidin might play a role as an aggregation pheromone for these beetles. Natural cantharidin sources are present in form of meloid beetles e.g. *Mylabris aperta*, *M. vestita* and *M. amplectens*, which are especially very frequent in the savanna, where they even damage crop fruits. These meloids however were never found in Kidia / Tanzania at 1430 m o.s.l. during the field studies. This is also an explanation why the cantharidin titres of field-caught anthicids at a sea level of 1430 m are very low. *Formicomus gestroi* and *F. rubricollis* also occur in lowland areas where meloids could serve as cantharidin source.

The investigated *Formicomus*-species are polyphagous; the beetles fed on material of animal as well as of plant origin. In their natural habitat e.g. (meadows with poor vegetation) the beetles could be observed taking up the sporangia of a mildew fungus parasitising the leaves of *Lepidium bonariense*.

Breeding of both species is successful using fish food as diet. In the field the larvae can be found in the upper layers of soil of cultivated areas fertilised with organic material. The species *Formicomus pedestris* obviously has similar habitat preferences (Schutz 1989). Individuals of this species were trapped with cantharidin in the Mediterranean region (Istria, Yugoslavia) from a wheat field fertilised with stall litter. Here as well this *Formicomus*-species was reproducing itself in loose soil, where organic manure was available for the larvae.

Mating behaviour: Males jumped at every individual of the same species. Copulation took place if the jumped-on individual was a receptive female. The mates kept together in copula about one day. The male was mounted on the female, the foreleg hooked in depressions of the pronotum of the female.

Reproduction: 3-4 weeks after copulation the female laid up to 25 eggs, singly or in small heaps into the upper layers of the soil. The larvae hatched after 12 days and pupated 6-8 weeks later. Shortly before pupation the larvae built a cocoon out of soil. The pupa stayed 10 days in the puparium before the adult emerged.

Phenology: During the field studies individuals of *F. gestroi* and *F. rubricollis* were attracted with cantharidin from November to February. A maximum of caught beetles was observed in November/December. The start of the rainy season caused a population decrease in both species. Probably some of the beetles died in the heavy day-long rainfalls because in January with the beginning of the dry season, the population density was clearly lower. A breakdown of the populations of both *Formicomus*-species was noted in February. Parallel to this the proportion of attracted females rose to 90-100 %. An explanation for this phenomenon might be that females have a longer lifespan than males.

A reason for the breakdown of the populations of both species may be the weather conditions. Only few larvae hatched and developed in January, which is the hottest and driest season of the year. 4-6 weeks later (February) the old beetles already died and fresh ones had not yet hatched. On the other hand, eggs that were laid by females end of January/beginning of February had good chances to develop. During this period precipitation was sufficient again. These larvae developed and built up a new generation in March and April. Also during November to December new beetles of *F. gestroi* and *F. rubricollis* appeared continuously so that individuals were trapped over the whole period.

Behaviour and cantharidin

Cantharidin-attracted beetles are very eager to copulate. A great part of the attracted individuals already mated in the traps (56,6 % of *F. gestroi*, 64,3 % of *F. rubricollis*).

The proportion of cantharidin attracted beetles obviously decreases with increasing age. While freshly baited beetles reacted again very strongly to cantharidin shortly after their captivity (nearly 100 % of the tested individuals), only 25-30 % of cantharidin-attracted beetles that were kept for 4 weeks showed a positive reaction to the terpenanhydrid again.

A possible interpretation for these observation is that cantharidin plays a role as an aggregation pheromone in the biology of the species. If the beetles are ready for copulation they are very sensitive towards cantharidin. Therefore the meeting of mates is increased by a positive reaction to cantharidin.

Synthetic (= unbound cantharidin) and cantharidin from natural sources (= meloid beetles, = bound and unbound cantharidin) showed no difference in the proportion of attracted beetles in the field.

Cantharidin baits lose their impact with time. Only fresh baits show considerable attraction on anthicids. It was also tested whether a fungus whose sporangia were eaten by the *Formicomus*-species attracts the specimens. However, no positive reaction of the beetles was noted to an acetone extract of *Lepidium bonariense* leaves parasitised by a mildew fungus.

Analysis of cantharidin contents of body compartments of *Formicomus gestroi* and *F. rubricollis*

Field-caught individuals of *F. gestroi* and *F. rubricollis*, which had no access to synthetic cantharidin in the laboratory, only contained traces of the terpenanhydrid.

Cantharidin-fed specimens on the other hand stored this natural product in their bodies (Dettner 1997). Most of the unbound cantharidin was found in the 3-armed testicle of males, while the other testicle, which was much smaller and of amorphous shape, contained no cantharidin at all. Different shaped testes occurred only, when at the same time a spermatophore was present in the abdomen of the males. Otherwise the testes were equally shaped. Probably, tissue as well as the whole amount of stored cantharidin is used from one testicle to form the spermatophore, while the other testicle remains intact. Thus the cantharidin titres of the intact testicle and the spermatophore are comparingly high. The accessory glands contained only small amounts of cantharidin.

Obviously the females store cantharidin in their ovaries. In the ovaries of *F. gestroi* females high titres were analysed. Probably – the data material is too few to be certain – these individuals already were mated and the cantharidin was transferred during copulation from the males to the females. Females of *F. rubricollis* on the other side, which certainly were already mated, did not show higher cantharidin titres in the ovaries compared to the haemolymph. These results are not surprising considering field-caught individuals, that all contain only traces of the terpenanhydrid. Before the females had access to cantharidin in the laboratory they were already mated. In captivity the analysed females obviously did not mate a second time with cantharidin-fed males and thus only contained the small amount of cantharidin that is normally found in field-caught beetles. The high cantharidin contents in the ovaries of the above mentioned *F. gestroi* females thus might originate from cantharidin-fed males that copulated again with these females in captivity.

Cantharidin-fed *Formicomus* females obviously do not store the terpenanhydrid in the reproductive organs. Higher cantharidin titres are only found in the ovaries when the female copulated with cantharidin-fed males.

Comparison of cantharidin storage of the investigated *Formicomus*-species with other canthariphilous species

Cantharidin-fed specimens of *Notoxus monoceros* L. (Anthicidae) store the terpenanhydrid in huge amounts (Schütz & Dettner 1992). Different from the *Formicomus*-species high titres are found in the accessory glands of the males that are much higher than in the testes (Hemp 1994). Cantharidin accumulation is also different in females. Whereas the cantharidin transferred during copulation is stored in a receptaculum seminis in females of *Notoxus monoceros* females of the *Formicomus*-species accumulate the terpenanhydrid in the ovaries. Receptacula seminis are absent in *Formicomus* females.

The lowest titres of cantharidin are found in the haemolymph in both *Notoxus monoceros* and the *Formicomus* species.

As in *Notoxus monoceros* (Schütz & Dettner 1992) only males of the *Formicomus*-species invest stored cantharidin into their offspring. Accumulated cantharidin of the testes is transferred to the females via copulation. The females, at least in *Notoxus monoceros* (Hemp 1994), use this cantharidin to protect their eggs.

An oral uptake of cantharidin by the females is only used for self-protection in *Notoxus monoceros* females as well as in females of the *Formicomus*-species.

Analogous to the canthariphilous anthicid species, males of the pyrochroid *Schizotus pectinicornis* transfer cantharidin to the females during copulation (Holz et al. 1994). In anthicids as well as in pyrochroids accumulated cantharidin is used to protect the offspring. Carrel & Eisner (1974) found out that cantharidin is a highly effective feeding deterrent to certain predaceous insects. In eggs of the anthicid species *Notoxus monoceros* and *Formicomus pedestris* (Hemp 1994) and in the eggs and larvae of the pyrochroid *Schizotus pectinicornis* partly high amounts of cantharidin could be found (Holz et al. 1994).

Studies on the biology of canthariphilous species as well as close investigation of other morphological features may help to solve problems concerning the phylogeny of heteromeran beetle families containing canthariphilous species. Important features are the elytral notches present on the tips of the elytra of the males of some anthicid genera (Schutz & Dettner 1992), pedilids (Abdullah 1964c) and the primitive meloid genus *Protomeloe* (Abdullah 1964a,b). Also mesothoracic glands, present in all anthicids (Schütz 1989) and meloids (Basavanna & Thontadarya 1961, Berrioz-Ortiz 1985) are excellent characteristic features for phylogenetic questions. The mesothoracic gland e.g. in the genus *Formicomus* is the most highly evolved gland within the Anthicidae (Hemp & Dettner 1997) and shows that together with the different behaviour to cantharidin this genus belongs to a different tribe as e.g. *Notoxus* or the genera possessing elytral notches (*Microhoria, Tenuicomus, Clavicomus, Aulacoderus*) within the anthicinae (Hemp & Uhmann, in prep.).

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