

The defensive secretions of *Pachyloidellus goliath* (Opiliones, Laniatores, Gonyleptidae)

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Abstract. The defensive secretions of the Argentinian harvestman *Pachyloidellus goliath* and their spreading mechanism are studied here. It is established that they consist of two fluids: a colourless one, originating from the mouth, and a yellow one, produced by the scent glands. The former is of an aqueous nature, and reaches the gland opening and the lateral areae of the scutum by means of well-defined, tegumentary grooves; the yellow fluid is then injected into it, and diffuses along the grooves, so that the evaporation surface of the defensive substances becomes increased. A preliminary chemical analysis indicates that the gland fluid is composed by a mixture of phenols and quinones. What is known up to now about the dispersal mechanisms of the secretions within the order, as well as the chemical nature of the defensive compounds in Gonyleptoidea, are summarized.

Key words. *Pachyloidellus*, Gonyleptidae, Opiliones, Argentina, scent glands, defensive secretions.

Introduction

The presence of a pair of prosomal exocrine glands, whose secretions are considered to be mainly defensive (Holmberg 1986), is characteristic of the order Opiliones. While the presence of these glands is verified uniformly in the whole order, there are variations concerning the chemical nature of the produced substances, as well as the mechanisms used to direct the effects of these secretions toward the aggressor.

The first aspect was studied in about twenty species, of the suborders Palpatores and Laniatores (Eisner et al. 1978; Duffield et al. 1981; Roach et al. 1980; Wiemer et al. 1978). The secretions of Palpatores consist of short-chained acyclic compounds, including ketones, alcohols and an aldehyde; in one species, naphthoquinones were identified (Ekpa et al. 1985). The majority of the studied Laniatores secrete alkylated benzoquinones and phenols, although these substances might be only characteristic of the superfamily Gonyleptoidea (table 1), for in the sole Triaenonychidae examined (superfamily Travunioidea) bornyl esters and a dimethylphenylethylamine were found (Ekpa et al. 1984). Data on the chemistry of the secretions in other laniatorid groups are not available, nor in the Cyphophthalmi.

Quite larger is the diversity of the delivery mechanisms of the repellent products (table 2), which range from a simple evaporation through the gland opening (Juberthie 1961 b), to a deliberate directing at the aggressor by means of the forelegs (Juberthie 1961 a; Eisner et al. 1971). In several species of the family Cosmetidae, the previous appearance of an aqueous fluid of enteric origin was described; this fluid reaches the gland opening and then receives the discharge of the gland products (Eisner et al. 1971, 1977). A similar procedure was reported in one Stygnommatidae (Duffield et al. 1981) and a Phalangiiidae (Clawson 1988), although without a definitive demonstration.

In the present article we describe the defensive secretions of *Pachyloidellus goliath* Acosta (Gonyleptidae, Pachylinae), an endemic species from the higher altitudinal belt of the Sierras Grandes, province of Córdoba, Argentina (Acosta 1993). The large size of this arachnid, as well as its abundance and easy collection indicated it as a very suitable material for study. It is simple to elicit the emission of secretions in this species. Just by seizing an example — by hand or with forceps —, the appearance of a clear, odorless fluid, that usually forms a pair of droplets between the scutum border and the basis of legs II and III, is almost immediately detected. This liquid becomes gradually opalescent and yellowish, at the time that it begins to move along the lateral areae of the scutum. At this moment a strong, sour odour is perceived, very conspicuous and characteristic, similar to that produced by the insect called “chinche molle” (*Agathemera crassa* Blanchard, Phasmodea). This resemblance possibly gave the common name to these opilions. As cited by Ringuélet (1959), referring to an unpublished version of Frers, in some places of the Sierras de Córdoba the name “chinchina” is applied to *Pachyloidellus butleri* (Thorell), whose secretions, regarding their odour, are like those of *P. goliath*; the latter is known by the inhabitants of Pampa de Achala as “chichina” (F. Pereyra, R. Pizzi, pers. comm.); maybe as a deformation of the former name.

The observation of a clear liquid that turns to yellowish suggested us the possibility that in *P. goliath* two fluids of different composition and origin indeed exist, as described in Cosmetidae (Eisner et al. 1971, 1977). A careful analysis confirmed this mechanism (a colourless liquid coming from the mouth, a yellow one secreted by the glands), whose details are hereby described. We provide as well observations on the gland morphology and the chemistry of the involved substances.

Materials and Methods

The material of *Pachyloidellus goliath* — 258 males and 227 females — was collected with U. V. light (Acosta 1983) and by turning over stones at the locality of El Cóndor, Pampa de Achala, Córdoba, in October and December 1988 and January 1989. In the laboratory, the specimens were maintained in 30 x 30 x 30 cm terraria, and fed with pieces of fresh killed *Musca domestica* L. In the beginning of the research, we had a great mortality of specimens, due principally to the high temperatures of the city of Córdoba; a rearing chamber, so as to recreate the colder climatic conditions of Pampa de Achala, was unfortunately not available to us. To prevent that effect, the terraria were kept outdoors during the night, and part of the day in the refrigerator (approximately 12 °C), with good results.

The dissection of the glands and the observation of the displacement of the secretions were carried out under stereomicroscope.

To obtain the secretions in a small quantity, the manipulation of the specimens normally suffices, but to get them in larger amounts — for example, for the chemical analysis — it was necessary to gently squeeze the animal's body with forceps. A second “milking” procedure was to apply a faint electrical stream (6 Volts) on the membranous coxa-trochanter joint of the two first pairs of walking legs.

For the chemical analysis, the yellow secretion was collected by absorption with pieces of filter paper (3 x 5 mm), touching the emission zone, and then extracted with methylene chloride previously dried with calcium chloride. This solution was allowed to concentrate by mild evaporation at room temperature, and the stock stored at -4 °C for analysis. The following methods were employed in it: Thin-layer chromatography (TLC, Merk DC Alufolien Kieselgel 60F 254), mass spectrometry (Finnigan Mass Spectrometer), gas chromatography (GLC, Varian 2400) and infrared spectrophotometry (Nicolet I. R. Spectrophotometer). The

colourless secretion was taken from the mouth region with a Pasteur micropipet, just before it mixed with the yellow fluid, and then analyzed by I. R. spectrophotometry.

Results

Morphology of the glands

Like in other harvestmen, the scent glands of *P. goliath* are comparatively large with relation to the animal's size (2,1–3,1 mm long, for scutum lengths between 8,5 and 11,6 mm), and they occupy a great part of the prosoma. Their aspect parallels a "bagpipe", i. e., a simple sac, with a narrowed, short outlet. When filled with secretion, the colour of the glands is yellowish — by transparency of their contents —, and they become whitish or greyish if empty.

Each gland opens through an orifice, situated on the anterolateral angle of the carapace, on a slight tegumentary mound near the second pair of legs (figs 2, 3). This opening is a kind of oblique spine directed backwards and a little sideways, and appears obliterated by a whitish substance, which has irregular, crevice-like spaces, through which the gland fluid comes out.

Origin and spreading of the secretions

Colourless fluid: As already mentioned, the first to be observed when a specimen is disturbed is the colourless liquid, that moves by capillary action from the mouth to the dorsal scutum, along tegumentary grooves. A simple experience, based on Eisner et al. (1971), allowed us to confirm the enteric origin of this fluid. Ten examples were fed during a week with water containing a non-absorbable dye (Ponceau 4 R); after this time we observed that the clear mouth exudate had turned to a light pink colour.

The mouth is surrounded by four membranous lobes (coxapophyses), which are extensions of the pedipalps and legs I coxae (figs 1–3). The liquid canalizes between the coxapophyses of the pedipalp and leg I of each side, following then an ample channel between the same coxae — very movable —, up to the scutum border. A second way passes between the coxapophyses I and the labium, that guides the liquid to a cleft delimited by the coxae I and II — scarcely movable —, to reach the dorsal surface between the respective legs. The joints of the coxae II, III and IV are unmovable, and are fastened together by a dentated edge (fig. 1). In some specimens killed by chilling it was possible to see how the clear liquid — that evidently began to be secreted — cristallized in the mentioned grooves, giving us additional proof for the direct observations. The two ways described convey the fluid to a channel delimited between the carapace border and the coxae I, II and III, and which ends above the latter. The dorsal faces of the coxae I and II bear apophyses bordering that space, that possibly act to avoid its collapse; we didn't observe these apophyses to intervene in the liquid displacements. From this lateral channel, the clear fluid ascends through a notch that interrupts the rebordered margin of the scutum (above leg II), and that bifurcates into two grooves (fig. 3). One of them, short and narrow, leads to the gland opening. The other, narrow on the beginning but wide on the rest, extends along the scutum's margin, up to its posterolateral corner; this groove is limited by a row of low granules and the scutum border itself (fig. 2).

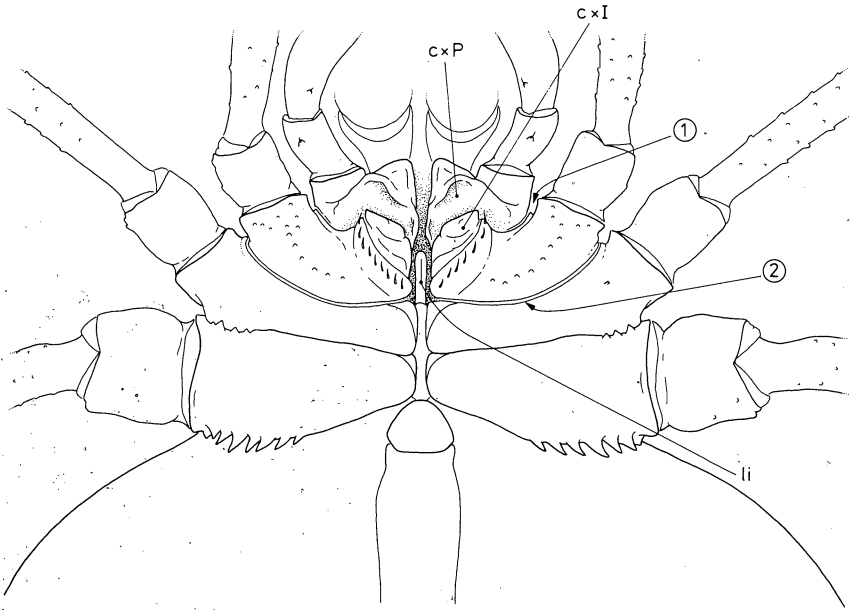


Fig. 1: Morphology of the mouth region and associated structures of *Pachyloidellus goliath*, ventral view. cxP: coxapophyses of the pedipalps, cxI: coxapophyses of the first pair of legs, 1: channel between the pedipalps and first pair of legs coxae, 2: channel between the coxae of legs I and II, li: labium.

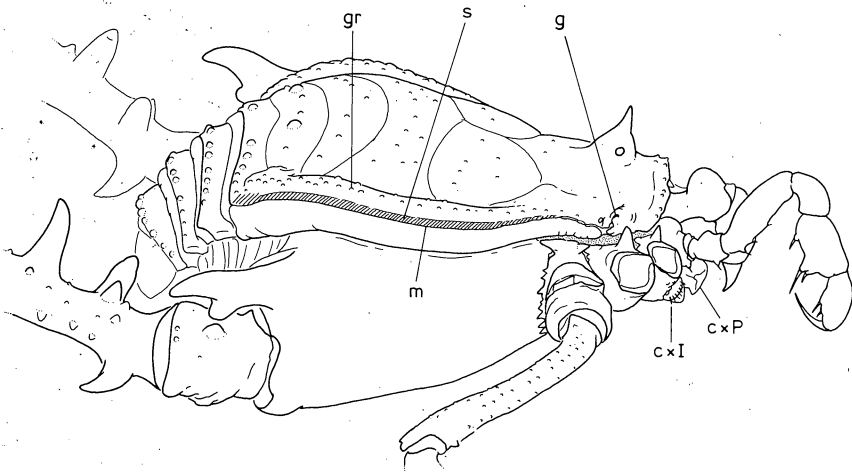


Fig. 2: Male of *Pachyloidellus goliath*, lateral view. gr: granules, s: lateral groove of the scutum (the hatching indicates the fluid displacement), m: scutum margin, g: gland opening; other references like fig. 1.

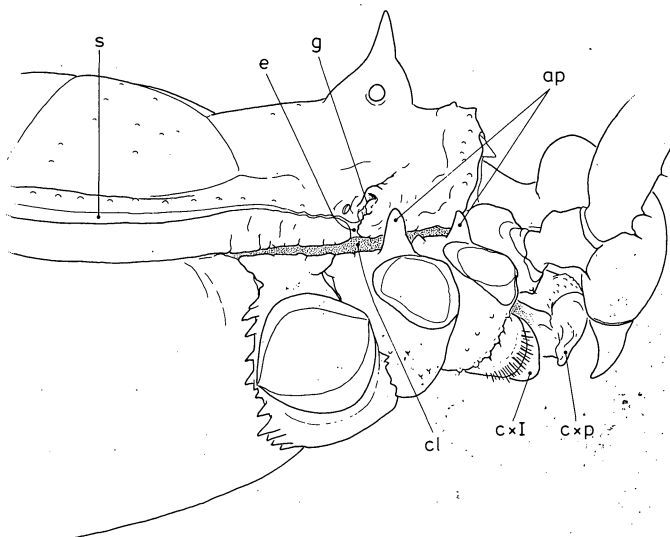


Fig. 3: Detail of structures around the gland opening. e: notch on the rebordered carapace edge, cl: lateral channel above the coxae I, II and III, ap: dorsal apophyses of coxae I and II; remaining references like figs 1 and 2.

Yellow fluid: Once the clear liquid reaches the gland opening the second fluid appears; its coloration varies from light yellow to orange. This substance is intermittently discharged into the mouth exudate, reaching by diffusion all the places previously occupied by it (the mouth region included); both liquids evaporate together a little later. By interrupting the normal displacement of the clear fluid toward the scutum, by absorption with filter paper on the channels between and above the coxae, we observed that the yellow liquid accumulated at the gland threshold, probably too dense to run by itself along the scutum laterals. This suggests that the main function of the colourless liquid is to dilute and disperse the glandular secretion, the true repugnatory substance.

Comments: The specimens of *P. goliath* seem to be easily disturbed, judging from their quickness in emitting their secretions. It is sometimes possible to perceive the characteristic odour merely by lifting up the terrarium cover or by turning over the stones under which they shelter in their natural habitat. The secretions are normally released in very little amounts, and are detected more by their odour than visually. A careful observation reveals that the lateral grooves of the scutum are moist, coated with a thin and scarcely conspicuous pellicle of liquid.

It is clear that our conditions of observation represent extreme circumstances for the animal, that provoke the fluids oozing in large amounts, overflowing in many cases the described tegumentary channels. Of 80 individuals to which electric stimulation was applied, three expelled the yellow liquid as a jet, a way of emission that is common in some Triaenonychidae (Lawrence 1938; Maury 1987) but absolutely exceptional in our species.

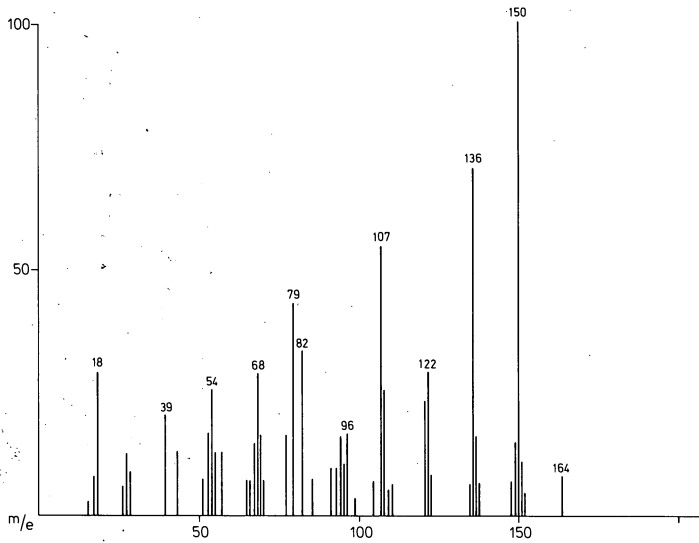
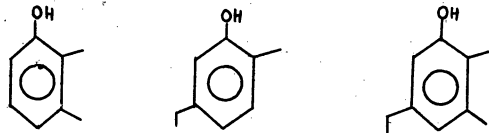


Fig. 4: Mass spectrometry of the glandular secretion (Finnigan Mass Spectrometer, EV = 70, T° = 50 °C, EMV = 1300 V).

Chemical analysis

Yellow fluid: As a first approach we carried out a TLC, using chloroform-benzene-methanol (15:5:2) as solvent, observing the presence of three compounds, one of them prevalent. We repeated the essay by varying the solvent (chloroform-ethanol-methanol, 15:5:5), without differences in the spot resolution.

Assuming the existence of only three components, we made a mass spectrometry (fig. 4). The results allowed us to detect three phenols: 2,3-dimethyl, 2-methyl-5-ethyl and 2,3-dimethyl-5-ethylphenol. Schematically:



Rupture patterns

122	136	150
107	121	136
91	107	135
	91	91
	77	

These rupture patterns are equivalent to those obtained by Duffield et al. (1981) in *Stygnomma spinifera* for the same compounds. Coincidentally, the quinones found in different Gonyleptoidea (table 1) possess a similar molecular weight, and the same

kind of substituents, what would yield exactly the same rupture patterns to those obtained from the phenols.

With the aim to determine with certitude the number of involved compounds, we made a gas chromatography, obtaining a chromatogram that indicates the presence of six compounds (fig. 5). To elucidate if they are phenols or quinones, we made use

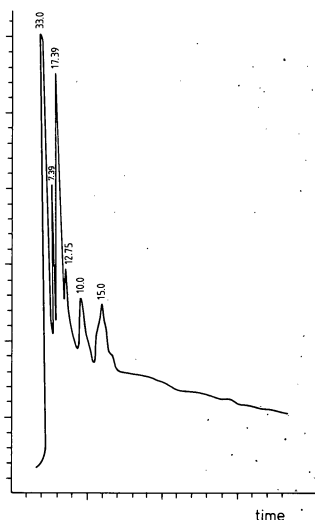


Fig. 5: Gas chromatography (N/H) of the glandular secretion (Varian 2400).

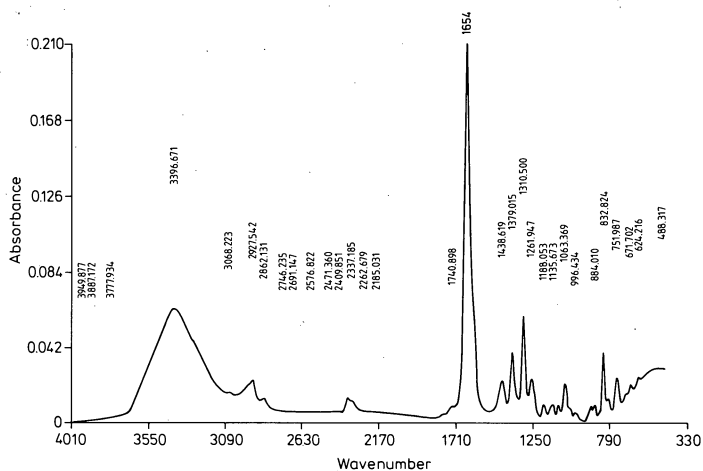


Fig. 6: Infrared spectrophotometry of the glandular secretion (Nicolet I. R. Spectrophotometer). Absorption bands: alcohols from 3200 to 3400, quinones from 750 to 1750.

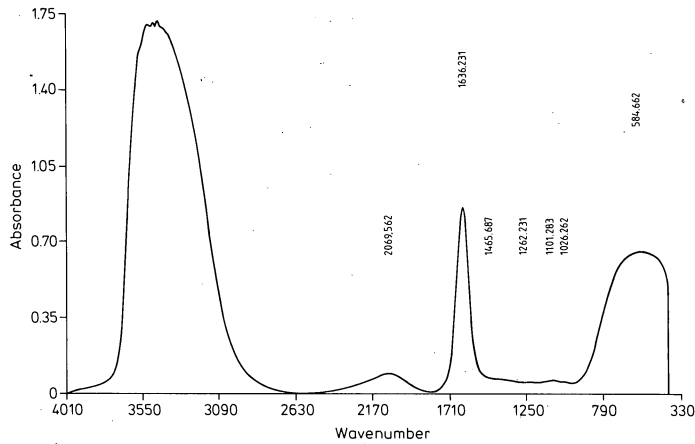


Fig. 7: Infrared spectrophotometry of the mouth exudate.

of an I. R. spectrophotometry (fig. 6), where the characteristic peaks of ketones (quinones) were observed, but also one peak corresponding to the alcohols (phenols) in a relatively low proportion of the total sample.

With these results, the yellow secretion is likely to be a mixture of quinones and phenols. These are, in accordance to the obtained data: 2,3-dimethyl-1,4-benzoquinone, 2,3,5-trimethyl-1,4-benzoquinone, 2,3-dimethyl-5-ethyl-1,4-benzoquinone, 2,3-dimethylphenol, 2-methyl-5-ethylphenol, 2,3-dimethyl-5-ethylphenol. We were not able to compare these analyses with authentic samples of the same substances; for this reason, they should be regarded as subject to confirmation.

Colourless fluid: The mouth exudate showed an infrared spectrum (fig. 7) practically coincident with that of water (verified with a spectrum of the Sigma Chemical Compounds Library), the trace elements being undetectable.

Discussion

According to Holmberg (1986), the suborder Laniatores is the only one whose defensive secretions are composed by two fluids. Actually, this was only proved in one species of Gonyleptidae, four of Cosmetidae, and — tentatively — one of Stygnomatidae (table 2). It is nevertheless possible that in the first family the phenomenon occurs more widespread. Recently, Clawson (1988) cited for the first time the mixture of oral and glandular fluids in one Palpator, *Leiobunum vittatum* (Say) (Phalangidae), but he gave no further details of his observations.

The emission of the enteric liquid in *P. goliath* is clearly related to the delivery mechanism of the repellent substance used by this species. Within the order Opiliones an ample variety of such mechanisms occurs (table 2). In most cases special devices and/or behavioral patterns are observed, that increase the repellent effects. This is achieved basically in two ways: (1) the opilion distributes the substance on its body, increasing the evaporating surface like a "chemical shield"

Table 1: Summary of the published data on the chemistry of the defensive secretions in the superfamily Gonyleptoidea.

Family	Species	Chemical composition								References
		Quinones				Phenols				
		1	2	3	4	5	6	7	8	
Gonyleptidae	<i>Acanthopachylus aculeatus</i>	+	+	+	—	—	—	—	—	Fieser & Ardao 1956
	<i>Pachyloidellus goliath</i>	+	+	—	+	+	+	—	—	This article
	<i>Nesopachylus monoceros</i>	+	+	—	—	—	—	—	—	Roach et al. 1980
	<i>Zygopachylus albomarginis</i>	+	—	—	—	—	(*)	—	+	Roach et al. 1980 Eisner et al. 1977
Stygnommatidae	<i>Stygnomma spinifera</i>	—	—	—	—	+	+	+	—	Duffield et al. 1981
Cosmetidae	<i>Vonones sayi</i>	+	+	—	—	—	—	—	—	Eisner et al. 1971
	<i>Paecilaemella eutypta</i>	+	+	+	—	—	—	—	—	Eisner et al. 1977
	<i>Paecilaemella quadripunctata</i>	+	+	—	—	—	—	—	—	Eisner et al. 1977
	<i>Cynorta astora</i>	—	—	—	—	+	+	—	—	Eisner et al. 1977
	<i>Cynorta nannacornuta</i>	+	+	—	—	—	—	—	—	Roach et al. 1980
	<i>Eucynortula albipunctata</i>	—	—	—	—	+	+	—	—	Roach et al. 1980

Notes: (1) = 2,3-dimethyl-1,4-benzoquinone, (2) = 2,3,5-trimethyl-1,4-benzoquinone, (3) = 2,5-dimethyl-1,4-benzoquinone, (4) = 2,3-dimethyl-5-ethyl-1,4-benzoquinone, (5) = 2,3-dimethylphenol, (6) = 2-methyl-5-ethylphenol, (7) = 2,3-dimethyl-5-ethylphenol, (8) = 2,3,4-trimethylphenol, (*) = Eisner et al. (1977) "tentatively" report a methylethylphenol, not identified.

that surrounds the animal, and (2) the harvestman attempts to put the substance in contact with the aggressor, by darting it as a jet or by directing it with the legs. *P. goliath* belongs to the first category.

A spreading mechanism similar to that observed in our species was described for representatives of Biantidae, Phalangodidae and Stygnommatidae (table 2); in all cases there are well-defined grooves along the scutum's lateral areas, where the fluids flow. In *Zygopachylus albomarginis* Chamberlin (Gonyleptidae, Cranainae) the secretions run near the borders of the scutum as well, but not in grooves, and following a row of tubercles instead (Cokendolpher 1987); the described morphology of grooves and notch is however similar in this species to that of *P. goliath* (L. E. A., pers. obs.). In the case of *Pachyloides thorellii* Holmberg — the only Gonyleptidae Pachylinae mentioned up to now in the literature — it was supposed that the liquid

Table 2: Mechanisms of delivery of the defensive secretions in the order Opiliones. (P): Palpatores, (L): Laniatores, (C): Cyphophthalmi. The author who described each mechanism is indicated after the specific name.

1. — Evaporation from the gland opening or its surrounding, without liquid displacement.

1.1. Exhalation of odour from the ozopore, with small or no emission of liquid.

Phalangiidae (P): *Rhampsinitus levis* / Juberthie 1961b

1.2. Emission of a secretion globule at the gland opening.

Phalangiidae (P): *Phalangium opilio* / Juberthie 1961b

Phalangodidae (L): *Querilhacia querilhaci* / Juberthie 1961b

Travuniidae (L): *Peltonychia clavigera* / Juberthie 1961b

Trianenychidae (L): *Adaeulum robustum* / Lawrence 1938

2. — Mechanisms that increase the evaporating surface.

2.1. Emission as fine spray, that moistens the animal's dorsum.

Leiobunidae (P): *Leiobunum formosum* / Blum & Edgar 1971

Leiobunum speciosum / Blum & Edgar 1971

2.2. Displacement of the liquid along the lateral area of the scutum.

2.2.1. The liquid runs by capillarity in tegumentary grooves.

Biantidae (L): *Metabiantes leighi* / Lawrence 1938

Phalangodidae (L): *Scotolemon lespesi* / Juberthie 1961b

Scotolemon doriae / Juberthie 1961b

Scotolemon lucasi / Juberthie 1961b

Stygnomatidae (L): *Stygnomma spinifera* (2) / Duffield et al. 1981

Gonyleptidae (L): *Pachyloidellus goliath* (1) / This article.

2.2.2. The liquid spreads along a row of tubercles.

Gonyleptidae (L): *Zygotachylus albomarginis* / Cokendolpher 1987

3. — Mechanisms that direct the liquid toward the aggressor.

3.1. Emission in form of a fine jet upwards and backwards.

Trianenychidae (L): *Larifugella natalensis* (3) / Lawrence 1938

Larifuga capensis (3) / Lawrence 1938

Trianenychoides cekalovici / Maury 1987

Trianenychoides breviops / Maury 1987

3.2. Emission of a secretion globule on the gland opening, that is directed to the aggressor with the forelegs.

Sironidae (C): *Siro rubens* / Juberthie 1961a

Parasiro coiffaiti / Juberthie 1961a

Cosmetidae (L): *Vonones sayi* (1) / Eisner et al. 1971

Paecilaemella eutypta (1) / Eisner et al. 1971

Paecilaemella quadripunctata (1) / Eisner et al. 1977

Cynorta astora (1) / Eisner et al. 1977

(1) The emission of a colourless, enteric fluid, and its mixing with the glandular secretions was proved here.

(2) The mechanism described in (1) is considered "probable".

(3) This delivery fashion is described as a "frequent" mechanism; sometimes the emission is limited to a fluid droplet on the ozopore (Lawrence 1938).

could run along a channel above the coxa IV (Juberthie 1961b); this seems to us not very probable, because the morphology of the structures related to the fluid displacement is basically identical to that found in *P. goliath* (Juberthie 1961b; pers. obs.) and possibly they function in the same way too. It is to be noted that an equal morphology is to be observed in other Argentinian and Chilean Gonyleptidae, all belonging to the subfamily Pachylinae (L. E. A., pers. obs.): *Pachyloidellus butleri* (Thorell), *Pachyloidellus fulvigranulatus* (Mello-Leitão), *Acanthopachylus aculeatus* (Kirby), *Discocyrtus dilatatus* Sörensen, *Neopucroliella pertyi* (Thorell), *Parabalta sicaria* Roewer, *Parabalta cristobalia* Roewer, *Daguerreia maculata* Canals, and *Pygophalangodus gemignanii* Mello-Leitão. The main differences — presumably of generic level — are to be seen in the shape of the dorsal apophyses of coxae I and II. We think, therefore, that the emission of enteric fluid, its mixing with the glandular secretion, and its displacement in grooves along the scutum laterals could be the characteristic mechanism of the group, at least of the subfamily (in two examined Gonyleptinae, *Sadocus polyacanthus* (Gervais) and *Geraecomorbius sylvarum* Holmberg, we did not find well-defined grooves on the lateral areae). A definitive judgement depends certainly on new observations, especially on living material.

Besides its defensive function, other possible roles of the gland secretions were suggested (aggregation pheromone; alarm pheromone, to warn conspecifics in case of threat; repellent action against parasites); the evidence for these hypotheses is however not always convincing (Holmberg 1986). Clawson (1988) has observed that females of *Leiobunum vittatum* (Say) and *L. flavum* Banks seem to mark with secretion the sites where they layed eggs, maybe to prevent other females from ovipositing in the same place. *P. goliath* is a gregarious species, but we do not know whether the secretions play any role in the mutual attraction. The alleged function of alarm pheromone does not seem very likely for this species. In fact, if disturbed in their shelters (for example, when the protecting rock is removed), the characteristic odour is perceived without a visible effect of "generalized flight", as we may expect if this hypothesis were true; after some minutes, the specimens begin to walk slowly away, without any doubt disturbed by the sunlight. Many individuals of *P. goliath* carry larvae of acari, sometimes very numerous, affixed by their mouth parts onto the prosoma, the abdomen and the legs. By applying a drop of glandular fluid to one of these larvae we verified its death almost immediately. But, in any case, this action seems to us incidental, for the fluids run only along the borders of the scutum, and that place is avoided by the acari in their distribution on the opilion.

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Resumen

Se estudian las secreciones defensivas y su mecanismo de distribución en un opilión argentino, *Pachyloidellus goliath*. Se determina la presencia de dos líquidos: uno incoloro, proveniente de la boca, y uno amarillo, secretado por las glándulas repugnatorias. El primero, de naturaleza acuosa, alcanza la abertura glandular y las áreas laterales del escudo siguiendo canales tegumentarios definidos; en él es vertido el líquido amarillo, que se dispersa por difusión en dichos canales, aumentándose así la superficie de evaporación de la sustancia defensiva. El análisis químico preliminar muestra que el líquido glandular se compone de una mezcla de fenoles y quinonas. Se resume lo publicado hasta el momento sobre los mecanismos de distribución de los fluidos defensivos en el orden, y sobre la química de los productos glandulares en la superfamilia Gonyleptoidea.

Zusammenfassung

In dieser Arbeit werden das Verteidigungssekret des argentinischen Weberknechts *Pachyloidellus goliath* und sein Verteilungsmechanismus studiert. Es wurde festgestellt, daß das Sekret aus zwei Komponenten besteht: aus einer farblosen Flüssigkeit, die vom Mund abgegeben wird, und einer gelben, die von den Stinkdrüsen produziert wird. Die erste ist wässrig. Sie wandert vom Mund zur Stinkdrüsenöffnung und weiter entlang einer Rinne nahe dem Seitenrand des Abdominalscutums (Lateralareae). Anschließend wird die gelbe Flüssigkeit in die farblose eingespritzt und löst sich entlang der Rillen in der farblosen, so daß sich die Verdunstungsfläche des Substanzgemisches vergrößert. Eine vorläufige chemische Analyse zeigt, daß das Sekret der Stinkdrüse eine Mischung von Phenolen und Chinonen ist. Die bisherigen Kenntnisse über die Verteilungsmechanismen der Abwehrsekrete bei Weberknechten und die chemische Komposition dieser Stoffe bei den Gonyleptoidea werden zusammengefaßt.

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