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Spray mechanism of crepidogastrine bombardier beetles (Carabidae; Crepidogastrini)*

Thomas Eisner[1], Daniel J. Aneshansley[2], Jayne Yack[3], Athula B. Attygalle[4] and Maria Eisner[1]

[1]Department of Neurobiology and Behavior, W347 Mudd Hall, Cornell University, Ithaca, NY 14853, USA
[2]Department of Agricultural and Biological Engineering, Riley-Robb Hall, Cornell University, Ithaca, NY 14853, USA
[3]Department of Biology, College of Natural Sciences, Carleton University, Ottawa, Ontario, Canada K1S 5B6
[4]Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853, USA

Summary. The defensive glandular apparatus of primitive bombardier beetles of the tribe Crepidogastrini (Carabidae) is described for the first time. As exemplified by two African species (*Crepidogaster ambreana* and *C. atrata*), the apparatus conforms to the basic bombardier plan, in that the glands are bicompartmented and the secretion is quinonoid (it contains 1,4-benzoquinones and hydrocarbons), hot, and discharged audibly. In a number of morphological respects the crepidogastrine apparatus resembles that of the classical bombardiers of the tribe Brachinini (rather than that of bombardiers of the paussoid lineage), reinforcing the view, already held on taxonomic grounds, that the Crepidogastrini and Brachinini are closely related. That the Crepidogastrini may be primitive relative to Brachinini is underscored by the finding that, unlike brachinines, crepidogastrines do not pulse their secretory emissions. Moreover, they discharge their secretion as a mist, rather than forcibly in the form of jets.

Key words. Bombardier beetle – *Crepidogaster* – evolution – chemical defense – benzoquinones – hydrocarbons – Coleoptera – Carabidae

Introduction

Bombardier beetles comprise two lineages within the family Carabidae (Fig. 1). One lineage, the brachinoid lineage, includes the familiar bombardiers (genera *Brachinus* and *Stenaptinus*, among others) in which the bombarding mechanism was first worked out (Schildknecht 1957; Schildknecht & Holubek 1961; Schildknecht *et al.* 1968, 1970; Aneshansley *et al.* 1969). The other lineage, the paussoid lineage, includes beetles of several tribes that also bombard (Eisner & Aneshansley 1982; Aneshansley *et al.* 1983; Eisner *et al.* 1992, 2000), but which on account of more restricted distribution and secretive habits are generally less known. The relationship of the brachinoids and paussoids is subject to some disagreement. While some investigators take the view that the ability to bombard evolved only once in the Carabidae, in other words, that bombardier beetles are of monophyletic origin and that the paussoids and brachinoids are sister groups (Eisner *et al.* 1977, 2000; Crowson 1981; Aneshansley *et al.* 1983; Erwin & Sims 1984; Bousquet 1986; Deuve 1988), others argue that the bombarding mechanism evolved independently in paussoids and brachinoids (Moore & Wallbank 1968; Forsyth 1972; Moore *et al.* 1987; Ball & McCleve 1990). A recent investigation of *Metrius contractus*, a member of the most primitive paussoid tribe, the Metriini, failed to shed light on the controversy (Eisner *et al.* 2000). In all major respects the bombarding mechanism of *M. contractus* is typically paussoid and devoid of features indicative of a shared recent ancestry with brachinines.

The one group of bombardiers that had not been investigated was the Crepidogastrini, a tribe generally considered to be the most primitive of extant brachinoids (Erwin & Sims 1984), and a counterpart therefore, in evolutionary status, to the Metriini within the paussoids. Crepidogastrines were consequently of some interest in that they could shed light on the evolution of the brachinoid bombarding mechanism, as well as possibly on the affinities of brachinoids and paussoids.

We were fortunate recently in obtaining 4 live crepidogastrines from Africa, and here report on their bombarding mechanism. We looked both into characteristics of their spray and into the anatomy of their glands, focusing on features that had been studied in other bombardiers. Thus, we looked into whether the crepidogastrine spray was quinonoid and hot, whether it was delivered with accompanying sound and in pulses, and whether it was accurately aimed. Anatomically we looked both into the gross structure of the glands and into the fine structural adaptations of the glandular tissue and its associated ducts.

In what follows we make use of two similar terms – brachinoid and brachinine – that should not be

Correspondence to: Thomas Eisner, e-mail: te14@cornell.edu
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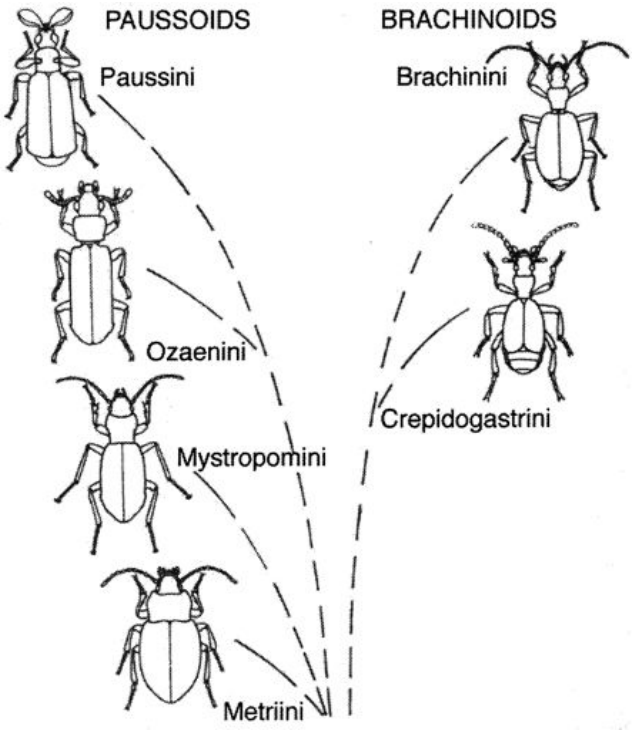

Fig. 1 Phyletic relationship of bombardier beetles (Family Carabidae, subfamily Paussinae). Tribal designation according to Erwin & Sims (1984)

confused. By brachinoid we mean pertinent to the entire brachinoid branch, in other words to the entire complex including both the Brachinini and Crepidogastrini. By brachinine we mean pertinent to the tribe Brachinini alone. We also use the term paussoid, to indicate pertinence to the entire paussoid branch.

Materials and methods

The beetles

One of the beetles, *Crepidogaster (Tyronia) ambreana* (Fig. 2A), stemmed from Madagascar (Antsiranana Province, Mnt. d'Ambre National Park, vicinity of Roussettes Research Station, 1000 m altitude) where it was taken at night on a decayed log in a grassy ditch on January 17, 2001.

The other three beetles, all *Crepidogaster atrata*, were from South Africa (Natal Province, Ntendeka Wilderness, Ngome Camp), where they were taken under stones in grassy habitat between January 31 and February 1, 2001.

The beetles were mailed shortly after collection to our Cornell laboratories, where they were maintained in small containers, on sand, at high humidity, on a diet of freshly cut up mealworms (larvae of *Tenebrio molitor*) and water (soaked cotton wads). The beetles ranged in size from 4.3 mm (*C. atrata)* to 6 mm (*C. ambreana*).

Other bombardiers

For comparative purposes we occasionally refer to unpublished work done on other bombardiers. When such work is referenced to paussoids, it includes observations made on *Metrius contractus*, *Mystropomus regularis*, and several species of Ozaenini. If it is referenced to brachinines, it includes (unless otherwise indicated) unpublished observations on *Brachinus* spp., and *Stenaptinus insignis*.

Also for comparative purposes we include acoustical and morphological data pertinent to brachinines (*Brachinus* spp.) and morphological data pertinent to a paussoid (*Metrius contractus*).

Gland morphology

Dissections were carried out under saline solution. For demonstration of the cuticular conveyance channels of the secretory tissue, clumps of this tissue were freshly excised from the beetles and mounted, unfixed and unstained, under coverslips in glycerin. Preparations were viewed under various lighting conditions (dark field, phase contrast, Nomarski interference contrast).

Preparations made to consist of cuticle alone were divested of their soft cellular components by overnight treatment with warm 10% aqueous potassium hydroxide (KOH).

For scanning electronmicroscopy, preparations were KOH-treated, then critical-point dried and gold-coated.

Tethering of beetles

Beetles were fitted with an aluminum hook, affixed to their elytra with a droplet of dental wax. To prevent them from discharging when thus prepared, they were immobilized by brief placement on refrigerated sand (-4°C). The hook provided a handle by which the beetle could be held, or linked to an adjustable rod, without being caused to discharge.

Elicitation of discharges

Tethered beetles were induced to discharge by pinching one or more of their legs lightly with fine forceps. The samples of secretion that were analyzed chemically were obtained by holding pieces of filter paper against a tethered beetle's rear while its legs were pinched so as to cause it to discharge.

Discharges on indicator paper

We had demonstrated years ago that filter paper impregnated with an acidified solution of potassium iodide and starch discolors instantly in the presence of quinonoid mixtures and can therefore be used for registering the spray patterns of bombardier beetles (Eisner 1958). We used such paper with the two beetles in which we examined the directionality of emissions.

Analytical procedures

Two consecutive discharges from a single beetle (*C. ambreana*), each collected on a piece of filter paper, were analyzed. The papers were extracted separately with 20 µl dichloromethane and a 0.5 µl aliquot of each extract was analyzed by GC-MS using an HP 5890 gas chromatograph linked to an HP 5970 mass selective detector (MSD) fitted with a 30 m × 0.22 mm fused-silica column coated with HP-1. The oven temperature was kept at 40°C for 2 min and raised 8°/min to 270°C. Gas-phase infrared spectra were obtained using an HP 5890 gas chromatograph linked to an HP 5965A IRD instrument. Analyses were performed using a 25 m × 0.32 mm fused-silica column coated with DB-5. The oven temperature was kept at 60°C for 4 min and raised 10°/min to 260°C.

Sound of discharges

The sound made by discharges was recorded onto a Sony DAT PCM-M1 at a sampling rate of 44.1 kHz, using a Sony ECM-MS957 microphone placed about 1 cm above a tethered *C. atrata*. Temporal and spectral characteristics of acoustic signals were analyzed using the Canary Bioacoustics Research Program (Charif *et al.* 1995).

Temperature of discharge

This parameter was measured with a copper-constantan thermocouple. The thermocouple's leads extended by way of ambient-equili-

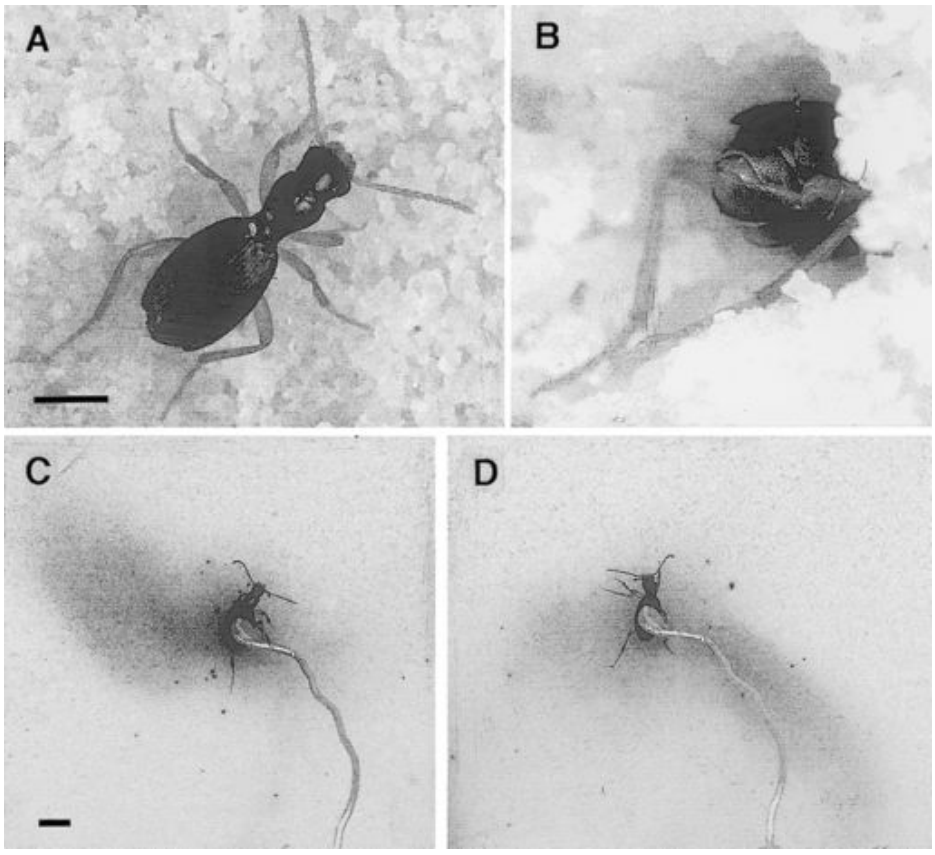

Fig. 2 **A**. *Crepidogaster ambreana*. **B**. Same, partly buried in sand, in typical resting posture. **C**, **D**. Tethered *C. atrata* that has discharged on indicator paper in response to pinching of left midleg (**C**) and right hindleg (**D**). Bars (**A**, **C**) 2 mm

brated (21°C) connectors to an amplifier (Model AD524AD, Analog Devices Inc.) with a gain of 1000. The amplified voltage output of the thermocouple was monitored with an oscilloscope (Model THS-710 STD Tekscope, Tektronix Inc.). The beetle's spray registered as a single sweep on the oscilloscope, which was displayed and analyzed by computer (Waveform Manager Pro V2.5, Metratek).

The thermocouple was calibrated by transferring it abruptly from room temperature (21°C) to an ice bath. The elicited voltage (-0.832 volts) was in accord with expectation for an amplified copper-constantan thermocouple. The time constant of the sensor, calculated from its response characteristic, was 6.2 ms.

To induce the beetle to spray on the thermocouple it was tethered and stimulated by leg pinching, while at the same time the thermocouple was positioned close to the beetle's rear in the anticipated trajectory of the ejection (Fig. 9A).

Results

The beetles

All four beetles tended to remain inactive and in hiding during daytime, under the pieces of moistened paper toweling that we offered as shelter. When disturbed they ran about quickly and erratically. *Crepidogaster ambreana* was of the habit of resting with its front end partly buried in the sand, so that only its "armed" rear projected free (Fig. 2B). All lacked hind wings and could therefore be expected to be flightless. Their elytra,

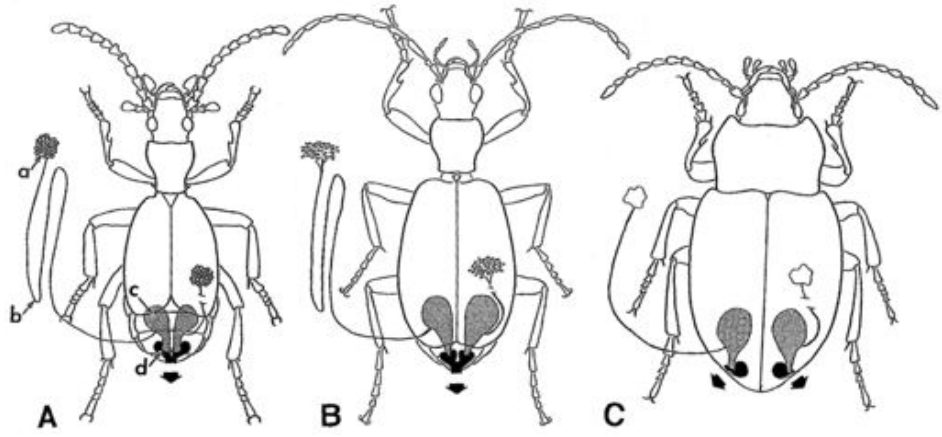

Fig. 3 Comparison of major bombardier beetle types: a crepidogastrine (**A**) (based on *Crepidogaster* sp.), a brachinine (**B**) (based on *Brachinus* sp.) and a paussoid (**C**) (based on *Metrius contractus*). The parts of the glandular apparatus are: (a) glandular tissue, (b) efferent duct, (c) reservoir, (d) reaction chamber

as is true for brachinines also, did not extend the full length of the abdomen but stopped short of covering the abdominal tip.

The glands

These conformed to the fundamental bombardier plan in that they consisted (Fig. 3A) of a mass of glandular tissue (a), linked by way of an efferent duct (b) to the gland reservoir or storage chamber (c), which in turn connects to the reaction chamber (d) that opens to the outside. No major differences were noted in the glands of *C. ambreana* and *C. atrata*.

In common with brachinoids generally, but not paussoids, the crepidogastrine glands open close together on the abdominal tip (Fig. 3A, B). In the paussoids (Fig. 3C) they open to the sides of the abdomen just anterior to the abdominal tip (for example, Eisner & Aneshansley 1982; Eisner *et al.* 1992, 2000).

In general appearance the reservoir/reaction chamber complex of crepidogastrines (Figs. 4, 5) resembles that of brachinines. In both, the reaction chambers are deeply indented where the reservoir links up with them (compare Fig. 5 with Fig. 10B, C). The difference is that whereas in brachinines the reaction chambers are bulbous and symmetrically shaped around that connection, in crepidogastrines the reaction chambers are elongate and asymmetrically formed around the linkage. In paussoids, it should be mentioned, the reaction chambers are somewhat varied in shape (Eisner *et al.* 1989, 1992, 2000; Eisner, unpublished), but they are consistently different from that of crepidogastrines/brachinines. For one thing, in paussoids the reaction chambers are never deeply indented (Aneshansley *et al.* 1983 and Eisner, unpublished).

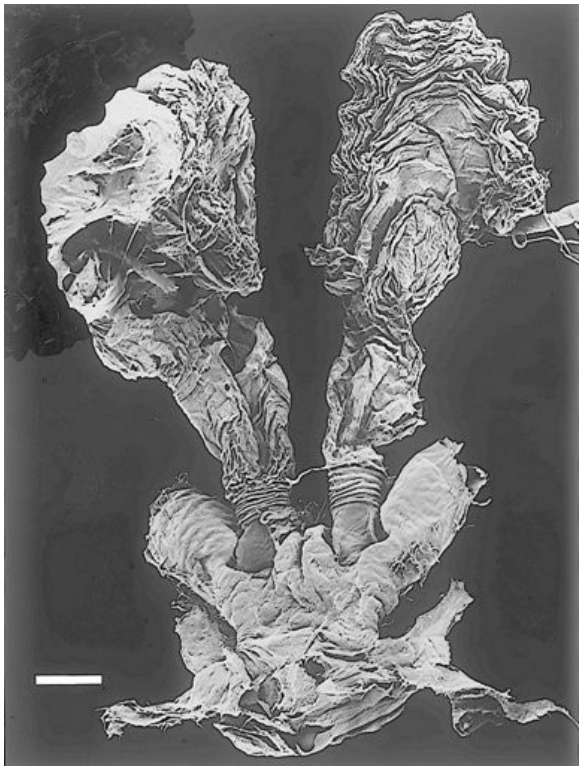

Fig. 4 Scanning electronmicrograph of glandular apparatus of *Crepidogaster atrata* (dorsal view). Bar 0.1 mm

The connection between reservoir and reaction chamber is known to be valvular in brachinines (Dean *et al.* 1990) and it is evidently valvular in crepidogastrines as well. The reservoir narrows in girth where it connects to the reaction chamber, forming a tube that is ordinarily inflected (Fig. 5D, black arrow), indicating that it might be fashioned as a passive valve, held closed by elasticity, without help of muscles. That valvular region bears close similarity in brachinines and crepidogastrines (compare Figs. 5D and 10C). We do not know whether that valve is operated by a special opener muscle, or whether it is forced open simply by fluid pressure when the reservoir is squeezed. Either way, there can be no doubt of the compressibility of the reservoir, which we noted in crepidogastrines to be surrounded by a thick musculature, such as is present in bombardiers generally (Eisner, unpublished observations on brachinines and paussoids). We have no explanation for another feature the crepidogastrines and brachinines share, namely the localized accordion-like folding of the reservoir wall near the junction with the reaction chamber (Figs. 5D, white arrow, and 10C), except to suggest that it might convey flexibility upon that narrowed portion of the reservoir and help keep the pathway clear when fluid is squeezed into the reaction chamber.

Previous work (Eisner, unpublished) had told us that the efferent duct, leading from glandular tissue to reservoir (Fig. 3A, b), differs in structure in brachinines and paussoids. In both groups the ducts consist of a tubular core, encased in what can be thought of as a cylinder of cushioning insulation. But whereas in the brachinines that cushioning cylinder has a ringed appearance, as if consisting of stacked washers (Fig. 10G–I), in paussoids it is fashioned as though it were made of densely packed bubbles (Fig. 11D–F). We found the ducts in both species of *Crepidogaster* to be brachinine-like (Fig. 6E, F).

Secretory conveyance system

Transversing the glandular tissue in *Crepidogaster* and serving to convey secretion from the gland cells to the efferent duct, is a branching system of fine cuticular channels, that is clearly silhouetted in dark field illumination (Fig. 6A). The channels originate in the gland cells, in special microtubular elaborations that are also made of cuticle and serve presumably for the uptake of secretion. In *Crepidogaster*, the microtubules are grouped into convergent clusters, to form tiny individual "florets" (Fig. 6B arrow, C) that impart a blooming appearance upon the branching channel system. The florets are individually beset with an aggregate of granules (Fig. 6C) which, unlike the cuticular microtubules themselves, do not withstand KOH treatment (Fig. 6D).

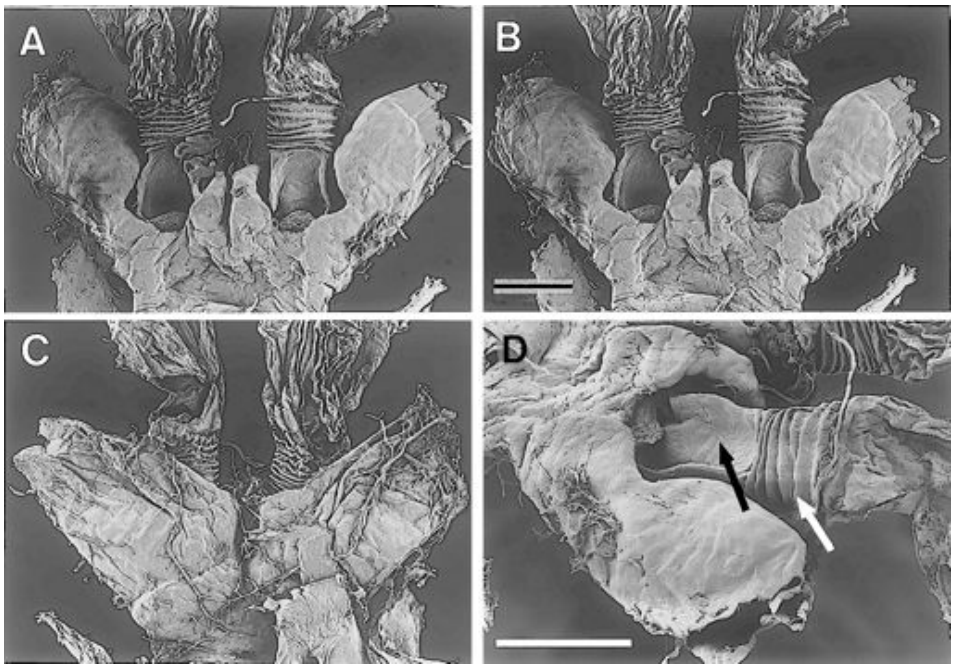

Fig. 5 *Crepidogaster atrata*. **A**, **B**. Reaction chambers in dorsal view (stereo pair). **C**. Same, in ventral view. **D**. Oblique view of junction of reservoir and reaction chamber (black arrow: inflected portion of valve; white arrow: folded prevalvular region of reservoir). Bars (**B**, **D**) 0.1 mm

In brachinines, the microtubules are not clustered into florets, but connect directly to the conveyance channels, sticking out from these like bristles on the shaft of a test-tube cleaning brush (Fig. 10D–F). We found such a filiform arrangement of the microtubules to prevail in both *Brachinus* spp. and *Stenpatinus insignis*, and presume it to be the arrangement typical for brachinines. The conveyance channels in brachinines, like the florets in crepidogastrines, are encrusted with granules (Fig. 10E), which like the granules in crepidogastrines do not survive KOH treatment (Fig. 10F).

We have also had occasion to examine the cuticular drainage system of the glandular tissue of paussoids (Eisner, unpublished), including that of *Metrius contractus* shown here. In all of these paussoids the microtubules are clustered into florets (Fig. 11A–C), basically as they are in *Crepidogaster*, but with fewer granules per floret than in *Crepidogaster*.

As to the cellular arrangement itself in the glandular tissue we found it to be somewhat different in crepidogastrines and brachinines. While in brachinines generally the cells are arranged in lobes (Fig. 10D), they are arranged in globular fashion in *Crepidogaster* (Fig. 6A). Neither arrangement matches the condition prevailing in paussoids, where the cells seem to be tightly compacted (Fig. 11A and unpublished observations on *Mystropomus regularis* and several Ozaenini).

Directionality of the discharges

Three ejections were elicited from tethered beetles on indicator paper, one from the *C. ambreana*, the other two from a *C. atrata*. The latter beetle was stimulated once on the left side (pinching of left midleg), and once on the right side (pinching of the right hindleg), and in each case the discharge was directed predominantly toward the side stimulated (Fig. 2C, D). The patterns left on the indicator paper were different, however, from those typically elicited by brachinine ejections. Brachinines eject their secretion as jets, and their spray patterns are characteristically marked by spots and streaks (Fig. 10A). With *C. atrata* the paper was evenly discolored by the discharges, as if the secretion had been emitted in vapor form or as a fine mist. There were some spots in the discolored zone indicative of droplet impact, but these were sporadically distributed as if generated by sputter rather than impacting jets. The single discharge that could be elicited from the *C. ambreana* (the beetle had been stimulated to the point of secretory exhaustion two days earlier in the course of other procedures) was also ejected directionally (toward a stimulated hindleg) and left an impact pattern on paper indicative of diffuse secretory emission.

Close-up examination of tethered crepidogastrines that were stimulated by seizure of individual legs in forceps, left little doubt that these beetles aim their ejections as brachinines do, by rotation of their abdominal tip. No matter which leg was seized, the beetles directed their "gun emplacement" at it. The beetles thus stimulated were secretion-depleted and capable of aiming only, having been caused to exhaust their glandular reserves in the course of some of the other tests.

Chemistry of the secretion

Seventeen secretory components, clearly resolved chromatographically (Fig. 7), were characterized or partially characterized by GC-MS and (in some cases) GC-IR techniques (Table 1). Amount of secretion was insufficient to establish double bond positions for the singly

and doubly unsaturated hydrocarbons. There were no qualitative, and only minor quantitative differences in the composition of the two spray samples analyzed. The principal components in both samples were 1,4-benzoquinone, 2-methyl-1,4-benzoquinone, pentadecane, and a heptadecadiene.

Sound of discharges

To our own ears, and doubtless because of the small size of the beetles, the glandular discharges were barely audible. We recorded the sound of five consecutive discharges from a *C. atrata*, elicited by sequential pinching of its legs. The sounds were broadband at frequencies under 20 kHz (Fig. 8A), which was the sensitivity range of our recording system. There was no indication that the sound was pulsed, as is typical for brachinines, in which the pulsation is a reflection of the pulsed nature of the spray output (Dean *et al.* 1990). For comparative purposes we include herein an acoustical record of a brachinine spray ejection (recorded with the same equipment as used with *C. atrata*) to illustrate how sharply the pulsation is resolved (Fig. 8B).

Temperature of discharges

The single discharge directed by a *C. atrata* against the thermocouple (Fig. 9A) elicited a voltage change of $+0.87$ volts in 6.6 ms, a value indicative of a spray temperature of 43°C (Fig. 9B). Given the time constant of the thermocouple (6.2 ms), the actual temperature of the spray could be expected to have been higher. From the known time-response characteristics of the thermocouple, it was possible to calculate the temperature that would eventually have been registered by the sensor had it had the opportunity to equilibrate with the heat source. That temperature is 64°C, which we take to have been close to the actual temperature of the spray.

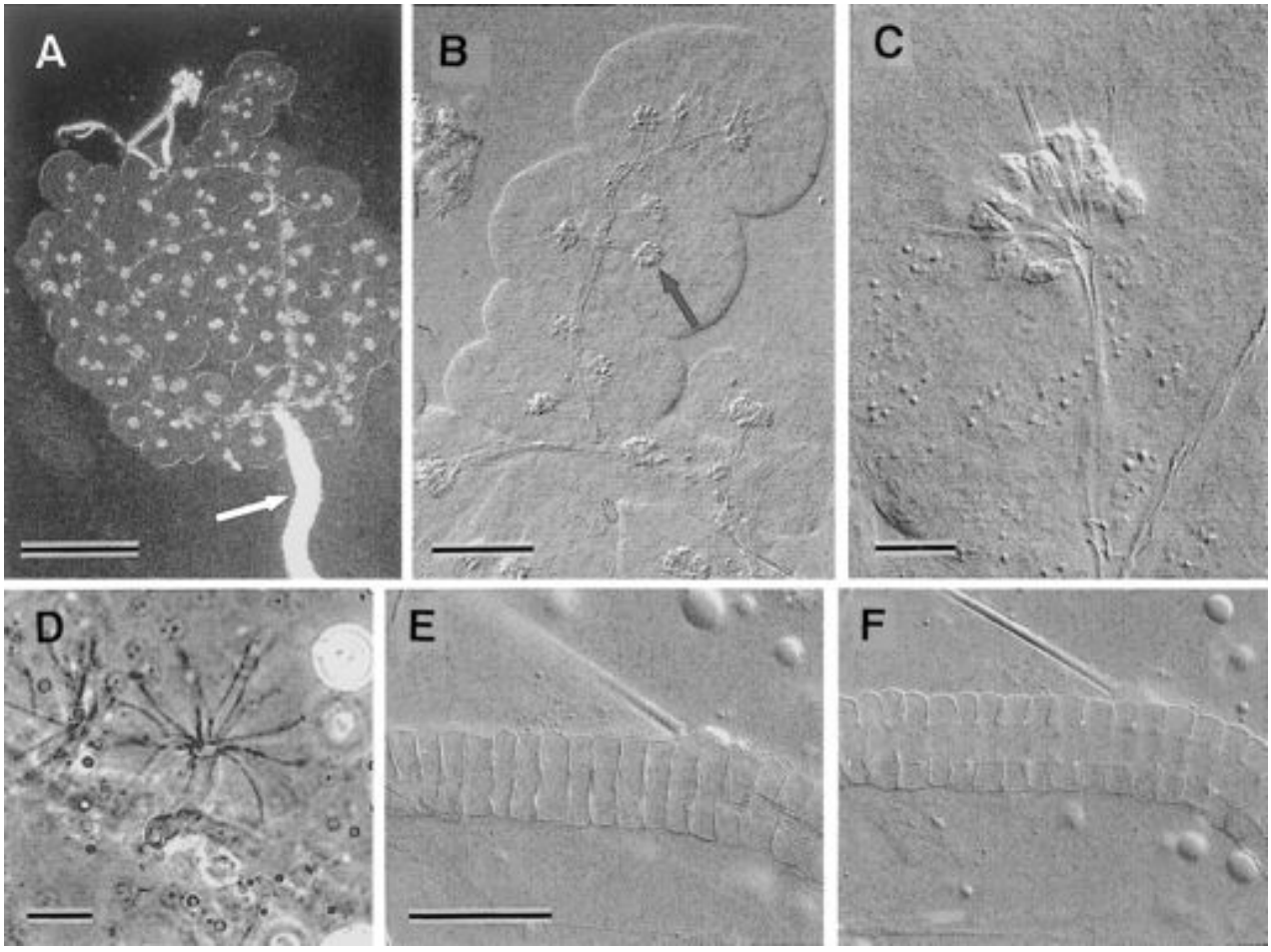

Fig. 6 *Crepidogaster atrata*. **A**. Glandular tissue, with efferent duct (arrow). The cuticular drainage system is clearly outlined. **B**. Detail of preceding, showing a number of cuticular "florets" (arrow) linked to a drainage channel. **C**. Enlarged view of a floret, showing its microtubules and associated granules. **D**. KOH-treated floret, from which the granules have been disolved away. **E.** Efferent duct in tangential optical section, showing the ringed appearance of the duct wall. **F**. Same, in optical mid-section showing the hollow core of the duct. (**A**, dark field; **B**, **C**, **E**, **F**, Nomarski interference contrast; **D**, phase contrast). Bars **(A)** 0.2 mm; (**B**, **E**) 50 μm; (**C**, **D**) 10 μm

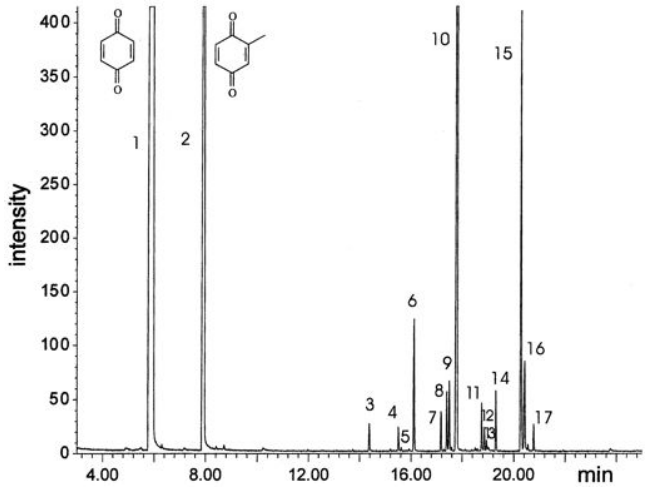

Fig. 7 Reconstructed gas chromatogram from GC-MS analysis of a dichloromethane extract of a defensive secretion sample of *Crepidogaster atrata* (see Table 1 for peak identifications). A fused silica column (30 m × 0.22 mm) coated with HP-1 was used. The oven temperature was held at 40°C for 2 min and raised 8°C/min to 270°C

Table 2 Differences and similarities of glandular apparatus of brachinine, crepidogastrine, and paussine beetles. Based on data presented herein: Eisner unpublished; and Schildknecht 1957, Eisner & Aneshansley 1982, Aneshansley *et al.* 1983, Dean *et al.* 1990, and Eisner *et al.* 1992, 2000

	Brachinine	Crepidogastrine	Paussoid
Gland	Bicompartmented		
Discharge	Quinonoid, hot, audible		
Elytra	Stop short of abdominal tip		Full length
Gland openings	Adjacent, on abdominal tip		Separate
Aiming	By rotation of abdominal tip		By way of elytral flanges*
Reaction chamber	Indented		Not indented
Efferent Duct	Ring-wrapped		Bubble-wrapped
Microtubular arrangement	Filiform	Floral	
Discharge sound	Pulsed	Non-pulsed	
Glandular tissue	Lobular	Globular	Compact
Form of discharge	Jet	Mist	Froth or jet**

* Except *Metrius*

** Froth in *Metrius* only

Discussion

In Table 2 we provide a summary of how the crepidogastrine defensive system compares with that of its fellow brachinoids, the Brachinini, and with that of the other major grouping of bombardiers, the paussoids.

It is clear, first, that crepdidogastrines are indeed bombardiers. Taxonomically they had been grouped with bombardiers (Erwin & Sims 1984) and their formal name alluded to the fact that they have a "detonating rear end," but neither the chemistry of their secretion nor the anatomy of their glands had been studied. Crepidogastrines are bombardier-like in every major respect. Their glands are two-chambered, and their discharges quinonoid, hot, and audible. The presence of hydrocarbons in the secretion, together with 1,4-benzo-

Table 1 Analytical data for components of a spray sample of *C. ambreana*

No.[a]	Compound	Relative amount[b]	EI-Mass spectral data (*m*/*z*)	GC-IR data (cm^{-1})
1	1,4-benzoquinone	100.00	108	1679, 1299, 1067, 1057, 880
2	2-methyl-1,4-benzoquinone	37.97	122	1673, 1283, 1085, 903
3	tridecane	0.57	184	c
4	2-methyltridecane	0.48	198, 155, 154	c
5	3-methyltridecane	trace	198, 169, 168	c
6	tetradecane	2.59	198	c
7	2-methyltetradecane	0.75	212, 169,168	c
8	a pentadecene[d]	1.26	210	c
9	a pentadecene[d]	1.44	210	c
10	pentadecane	36.81	212	2933, 2865, 1461
11	2-methylpentadecane	0.91	226, 183, 182	c
12	3-methylpentadecane	0.43	226, 197,196	c
13	hexadecene[d]	0.22	224	c
14	hexadecane	1.23	226	c
15	heptadecadiene[d]	10.17	236	c
16	heptadecene[d]	2.48	238	c
17	heptadecane	0.56	240	c

[a] Numbers refer to chromatographic peaks in Fig. 7.
[b] There was only minor quantitative and no qualitative variation in the composition of the two spray samples analyzed. The values listed were obtained by integrating GC peak areas, assuming all compounds respond similarly in the mass spectrometer. The amounts of components are given relative to benzoquinone, which is listed as 100.
[c] material insufficient for IR data.
[d] double bond position(s) undetermined.

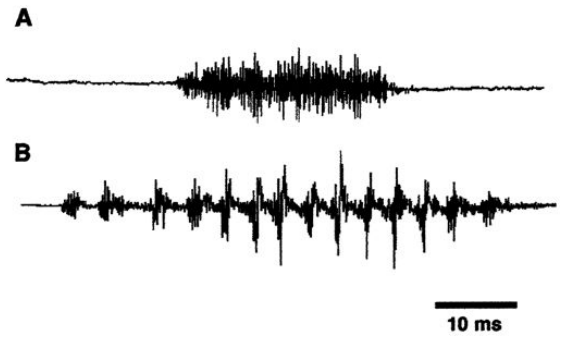

Fig. 8 Oscillograms of sound accompanying single discharges of *Crepidogaster atrata* (**A**) and *Brachinus (?) elongatulus* (**B**)

quinones, is also typical for bombardiers (Eisner *et al.* 1977, 1992; Roach *et al.* 1979; Aneshansley *et al.* 1983).

It is clear further that the crepidogastrines share more defensive features with brachinines than with paussoids, thereby bolstering the view that they are in fact close relatives of the brachinines. Thus, crepidogastrines and brachinines both aim their discharges by revolving the abdominal tip. In both the glands open close together on the abdominal tip and the tip is free to rotate because it projects beyond the shortened elytra. Paussoids are fundamentally different in these regards. Their elytra cover the entire abdomen and their glands open laterally at some distance from the abdominal tip. Moreover they aim their discharges by a combination of downward deflection of the abdominal rear and the use of special elytral flanges that serve as launching guides for the ejections (Eisner & Aneshansley 1982; Eisner *et al.* 1992, 2000).

The glands of crepidogastrines bear close similarity to those of brachinines. Their reaction chambers, for instance, are merely asymmetrical versions of those of brachinines. And their efferent ducts are identical to those of brachinines. Somewhat mystifying is the fact that the cuticular drainage system of the glandular tissue should be paussoid-like in crepidogastrines rather than brachinine-like. Whether the possession by both groups of cuticular "florets" as part of their drainage system is due to analogy or homology remains open to question and will probably not be established until the relationship of brachinoids and paussoids is definitively settled. We are reluctant to claim that homology is involved and that brachinines and paussoids shared a recent common ancestry. For all we know, cuticular drainage systems in Carabidae are highly variable in their own right, and ill-suited as phyletic indicators.

Most interesting, perhaps, was the finding that the discharge of crepidogastrines is not pulsed. Since the ability to pulse, as evidenced by brachinines, is in all likelihood a derived condition, crepidogastrines can probably be viewed as being ancestral, in the sense of possessing what is essentially a primitive version of the brachinoid ejection system. We find it impossible to speculate on how the inability to pulse might relate to the observed structural peculiarities of the crepidogastrine glands – to the asymmetry of the reaction chambers, for instance. We suspect, however, that what makes the difference in brachinines is a specialization of the valves that control access to the reaction chambers. As we have argued before (Dean *et al.* 1990) the pulsed secretory delivery in brachinines is a function of the passive oscillation of these valves, induced by pressure fluctuations in the chambers themselves. In crepidogastrines the valves might be improperly shaped, or might not project far enough into the reaction chambers, to flutter in response to pressure oscillations. No major difference was discernable in the parts of the valves of brachinines and crepidogastrines visible from outside the glands, in the regions where the reservoirs connect to the reaction chambers. The differences that matter, however, could be differences in the construction of the nozzles of the valves, such as would be apparent only by laying open the reaction chambers. A similar argument might be applied to the venting channels by which the secretion is expelled from the reaction chambers. These too could be specialized in brachinines for the

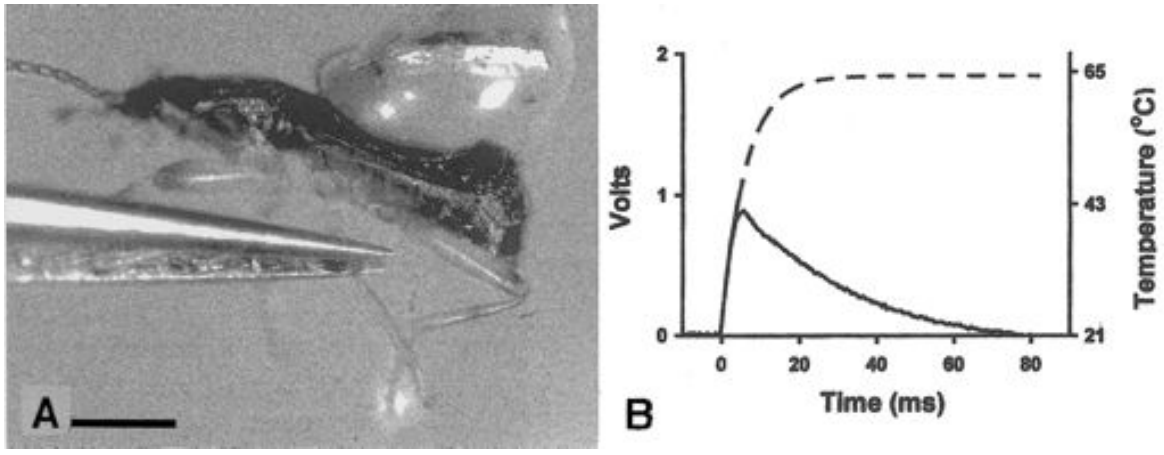

Fig. 9 **A**. Tethered *Crepidogaster atrata* being pinched so as to cause it to discharge on a thermocouple (visible just to the right and slightly below the tip of the forceps). **B**. Actual tracing (solid line) of the thermocouple output elicited by a *C. atrata* discharge. The dotted line gives theoretical projection of the voltage output

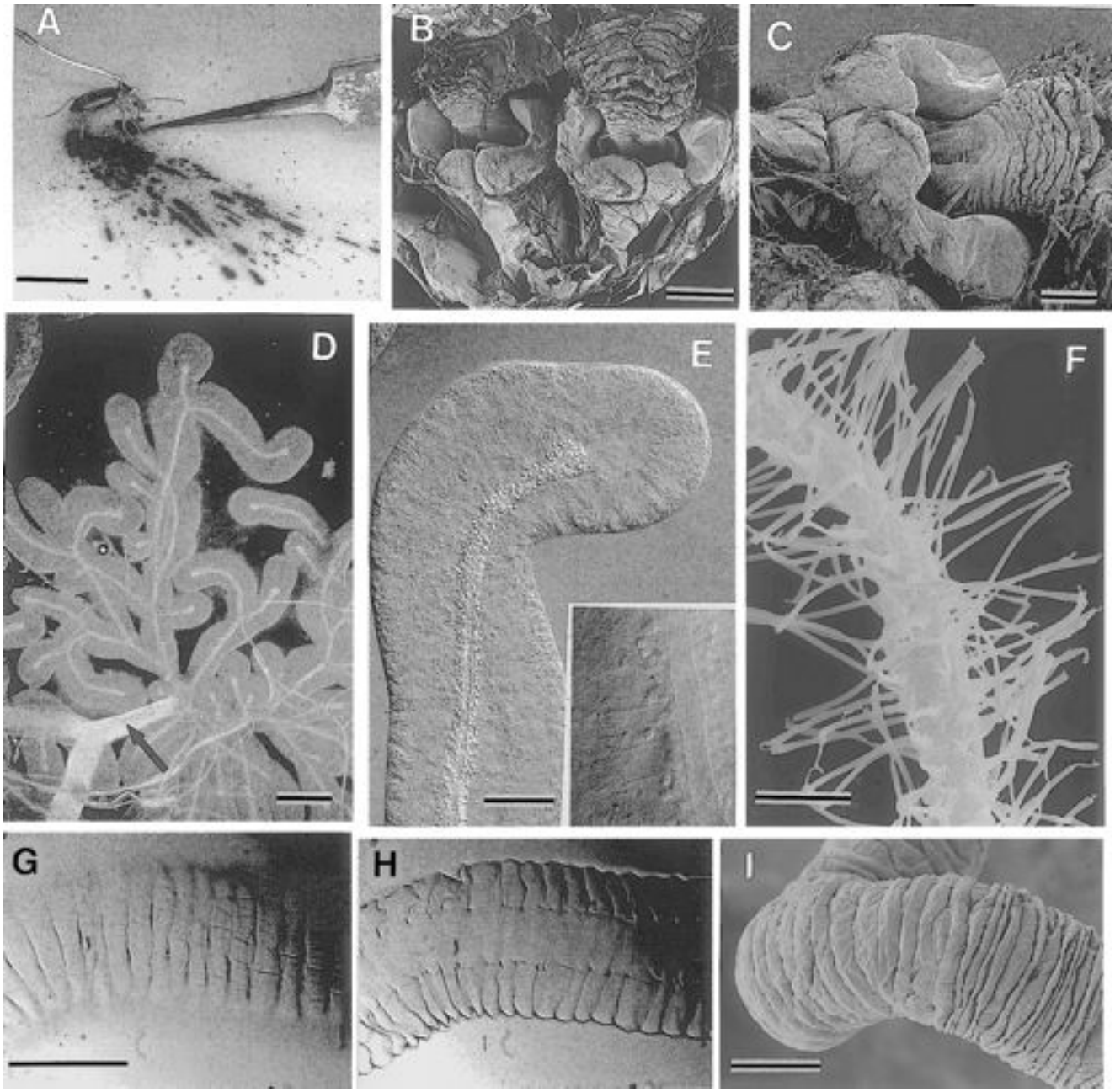

Fig. 10 *Brachinus* spp. **A**. Tethered beetle showing its discharge pattern on indicator paper. **B**. Reaction chambers (compare with Fig. 3B). **C**. Oblique view of junction of reservoir and reaction chamber. **D**. Portion of glandular tissue with efferent duct (arrow). The cuticular drainage system is clearly apparent. Note lobular arrangement of tissue. **E**. Detail of preceding. The microtubules that serve for the uptake of secretion project outward from the drainage channel. They are ordinarily obscured by granules and only partly visible (inset). **F.** Scanning electronmicrograph of portion of drainage channel showing microtubules (KOH-treated preparation, with granules dissolved). **G**. Efferent duct in tangential optical section. **H**. Same, in midsection, showing hollow inner core. **I.** Same, scanning electronmicrograph. (**D**, dark field; **E**, **G**, **H**, Nomarski interference contrast). Bars (**A**) 1 cm; (**B**, **D**) 0.2 mm; (**C**) 0.1 mm; (**E**, **G**) 50 μm; (**F**) 10 μm; (**I**) 20 μm

pulsed ejection of secretion, but we lack data altogether, for both brachinines and crepidogastrines, on the structure of these channels.

By not being able to pulse, crepidogastrines lose out on several advantages. The first is that pulsation provides for high secretory discharge velocity, without requiring muscles to supply the necessary pressure (Dean *et al*. 1990). The chemical precursors delivered by the resevoir can be supplied at low pressure; the ensuing chemical reaction generates the high pressure that propels the discharge. The second advantage, related to the first, concerns the separation of control and propulsion functions. As we have argued previously (Dean *et al*. 1990), muscle force shows low-pass characteristics; providing high forces involves a reduction in the precision of temporal control. Because brachinines do not use muscles to provide propulsion, they can exercise more precise control over their deliveries. They

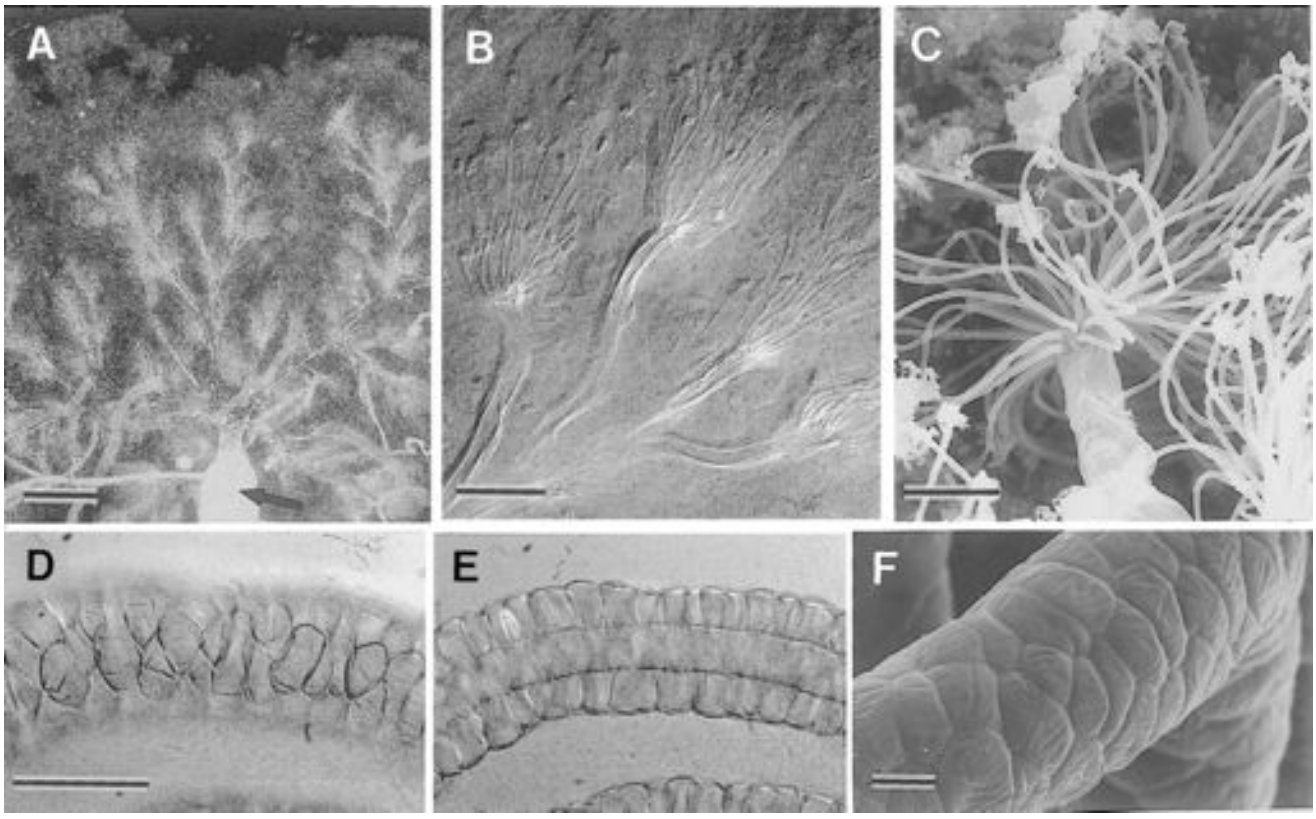

Fig. 11 *Metrius contractus*. **A**. Glandular tissue with efferent duct (arrow). The drainage system with its "florets" is clearly outlined. **B**. Enlarged view of a group of florets. Note microtubules and associated granules. **C**. Scanning electronmicrograph of floret (brief KOH treatment; some granules remain undissolved). **D**. Efferent duct in tangential optical section showing the densely packed bubbles that make up the outer wall of the duct. **E**. Same, in mid-section, showing the hollow core. **F**. Same, scanning electronmicrograph. (**A**, dark field; **B**, **D**, **E**, Nomarski interference contrast). Bars (**A**) 0.1 mm; (**B**) 10 μm; (**C**) 5 μm

can produce short discharges without reduction in spray velocity, with the result that they can maintain a constancy of spray delivery over time and adjust the length of the pulse train to the magnitude of the attack. A third advantage is that the discontinuity of the chemical events in the reaction chamber, triggered by the periodic infusion of the reactants, provides for the repetitive cooling of the chamber, and the protection (from thermal denaturation) of the enzymes within (Dean *et al.* 1990).

We envision a glandular discharge in crepidogastrines to proceed essentially as a single explosive event, the consequence of an initial infusion of a quantity of reservoir fluid into the reaction chamber. The fluid heats up as it interacts with enzymes in the chamber and fizzes out as a quinonoid mist. Duration of the event is probably determined by the quantity of fluid initially fed into the reaction chamber. The diffuse quality of the emission may be a reflection of the crepidogastrine's inability to generate the expulsion pressures that are the concomitants of pulsation. Taken together these differences could indeed be considered to indicate that crepidogastrines are living fossils, embodying what is essentially an early version of the brachinine defensive apparatus. Unfortunately, there is nothing that we learned about crepidogastrines that helps solve the vexing mystery of whether brachinoids and paussoids evolved their bombarding apparatuses independently or from a common ancestor.

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References

Aneshansley DJ, Eisner T, Widom JM, Widom B (1969) Biochemistry at 100°C: explosive secretory discharge of bombardier beetles (*Brachinus*). Science 165:61–63

Aneshansley DJ, Jones TH, Alsop D, Meinwald J, Eisner T (1983) Thermal concomitants and biochemistry of the explosive discharge mechanism of some little known bombardier beetles. Experientia 39:366–368

Ball GE, McCleve S (1990) The middle American genera of the tribe Ozaenini, with notes about the species in southwestern United States and selected species from Mexico. Questiones Entomologicae 26:30–116

Bousquet Y (1986) Description of first instar larva of *Metrius contractus* Escholtz (Coleoptera: Carabidae) with remarks about phylogenetic relationships and ranking of the genus *Metrius* Escholtz. Can Entomol 118:373–388

Charif RA, Mitchell S, Clark CW (1995) Canary 1.2 User's Manual, Cornell Laboratory of Ornithology, Ithaca, NY

Crowson RA (1981) The Biology of the Coleoptera. New York: Academic Press

Dean J, Aneshansley DJ, Edgerton HE, Eisner T (1990) Defensive spray of the bombardier beetle: a biological pulse jet. Science 248:1219–1221

Deuve T (1988) Étude phylogenetique des Coléoptères Adephaga: rédefinition de la famille Harpalidae, sensu novo, et position systematique des Pseudomorphinae et Brachinidae. Bull Soc Entomol France 92:161–182

Eisner T (1958) The protective role of the spray mechanism of the bombardier beetle, *Brachynus ballistarius* Lec. J Insect Physiol 2:215–220

Eisner T, Aneshansley DJ (1982) Spray aiming in bombardier beetles: jet deflection by the Coanda effect. Science 215:83–85

Eisner T, Jones TH, Aneshansley DJ, Tschinkel WR, Silberglied RE, Meinwald J (1977) Chemistry of defensive secretions of bombardier beetles (Brachinini, Metriini, Ozaenini, Paussini). J Ins Physiol 23:1383–1386

Eisner T, Ball GE, Roach B, Aneshansley DJ, Eisner M, Blankespoor CL, Meinwald J (1989) Chemical defense of an ozaenine bombardier beetle from New Guinea. Psyche 96:153–160

Eisner T, Attygalle AB, Eisner M, Aneshansley DJ, Meinwald J (1992) Chemical defense of a primitive Australian bombardier beetle: *Mystropomus regularis*. Chemoecol 2:29–34

Eisner T, Aneshansley DJ, Eisner M, Attygalle AB, Alsop DW, Meinwald J (2000) Spray mechanism of the most primitive bombardier beetle (*Metrius contractus*). J Exp Biol 203:1265–1275

Erwin T, Sims LL (1984) Carabid beetles of the West Indies (Insects: Coleoptera): a synopsis of the genera and checklists of tribes of Caraboidea, and of the West Indian species. Questiones Entomologicae 20:351–466

Forsyth DJ (1972) The structure of the pygidial defence glands of Carabidae (Coleoptera). Proc Zool Soc London 32:253–309

Moore BP, Wallbank BE (1968) Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. Proc R Entomol Soc London B 37:62–72

Moore BP, Weir TA, Pyke JE (1987) Rhysodidae and Carabidae. Pp 20–239 *in* Walton DW (ed.) Zoological Catalogue of Australia, vol. 4 (Coleoptera: Archostemata, Myxophaga and Adephaga). Canberra: Australian Government Publishing Service

Roach B, Dodge KR, Aneshansley DJ, Wiemer D, Meinwald J, Eisner T (1979) Chemistry of defensive secretions of ozaenine and paussine bombardier beetles (Coleoptera: Carabidae). Coleopterists Bull 33:17–20

Schildknecht H (1957) Zur Chemie des Bombardierkäfers. Angew Chem 69:62

Schildknecht H, Holubek K (1961) Die Bombardierkäfer und ihre Explosionschemie. Angew Chem 73:1–7

Schildknecht H, Maschwitz E, Maschwitz U (1968) Die Explosionschemie der Bombardierkäfer (Coleoptera, Carabidae) III. Mitteilung: Isolierung and Charakterisierung der Explosionskatalisatoren. Z Naturforsch 23b:1213–1218

Schildknecht H, Maschwitz E, Maschwitz U (1970) Die Explosionschemie der Bombardierkäfer: Struktur und Eigenschaften der Brennkammerenzyme. J Insect Physiol 16:749–789

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