Description of Defensive Glands from Cardinal Beetles (Coleoptera, Pyrochroidae) – Their phylogenetic Significance as compared with other heteromeran Defensive Glands

by **K. Dettner**

Abstract: Three species of cardinal beetles (Coleoptera: Pyrochroidae) have been shown for the first time to possess paired abdominal defensive glands which are situated between sternits 5 and 6. Small amounts of nonquinoid secretion are exuded from two wrinkled glandular areas of the intersegmental membrane on molestation. Here defensive glands of *Pyrochroa coccinea* L. are described in detail with respect to their morphology and histology. The new found glands are characterized by both primitive and advanced characters when compared with other defensive glands of Heteromera.

Due to the new found glands which seem morphologically homologous with other heteromeran glands, Pyrochroidae can be phylogenetically connected with this stock of heteromeran beetles. On the contrary abdominal glands within both beetle groups could have originated by convergence at the same morphological structure. This would support the suggestion that there is a high probability that defensive glands independently can originate in the same exposed body positions of different beetle groups.

When molested specimens of Pyrochroidae bend their abdominal tips ventrally and immediately emit a penetrating, somewhat unpleasant, sweetish odour. By carefully observating the abdominal tip of cardinal beetles during a molestation procedure a pair of enlarged exocrine glands could be discovered for the first time. The morphology and histology of these complex gland systems of firecolored beetles (Pyrochroidae) is now described below. This seems of special interest since homologous defensive glands as found in Pyrochroidae only have a limited distribution within heteromeran beetles and probably are of great taxonomic and phylogenetic importance. The pyrochroid abdominal glands certainly represent defensive glands for they have been found to be present in both sexes and only respond on molestations. It has been shown that these glands are present in both sexes of the european Pyrochroidae *Pyrochroa coccinea* L., *P. serraticornis* Scop. and *Schizotus pectinicornis* L.

Although most species of the worldwide known 100 Pyrochroids are characterized by distinct, often red warning colorations it is astonishing that no toxic principle or defensive behaviour has been observed till now in fire-colored beetles.

Description of the Pyrochroidae glands

On molestation *Pyrochroa coccinea* L. slightly bends its abdominal tip ventrally (Fig. 1) and the sternite 7 (visible sternite 5, S7) is clapped downward which results in the protrusion of the intersegmental membrane (IM) between sternites 7 and 8 by the aid of hemolymph pressure (Fig. 2, 3). In *Pyrochroa* there is found a soft thin structure representing the posterior margin of the seventh sternite which is folded against its main sternite (Fig. 4). Two muscles are associated with the glandular
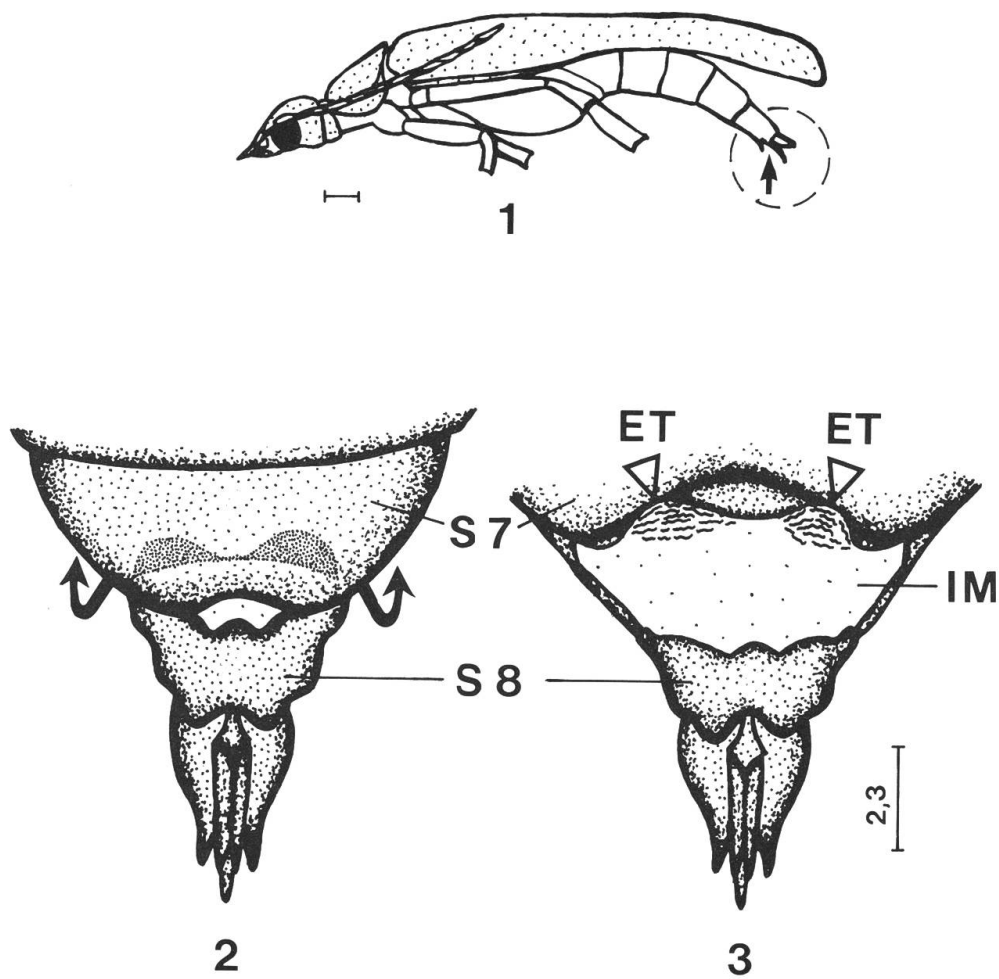
Figs 1–3: 1. Lateral view of *Pyrochroa coccinea L.* to show the abdominal tip (circle) with the glandular area (arrow). 2 and 3. Ventral view of abdominal tips of male *P. coccinea L.* before (2) and after (3) a molestation to show the exposure of the intersegmental membrane (IM). S7, S8 = sternites 7 and 8; ET = evaporation tissue; dotted area in 2 = position of evaporation tissues; arrows = tipping downward of sternite 7.
Bars 1–3: 1 mm.
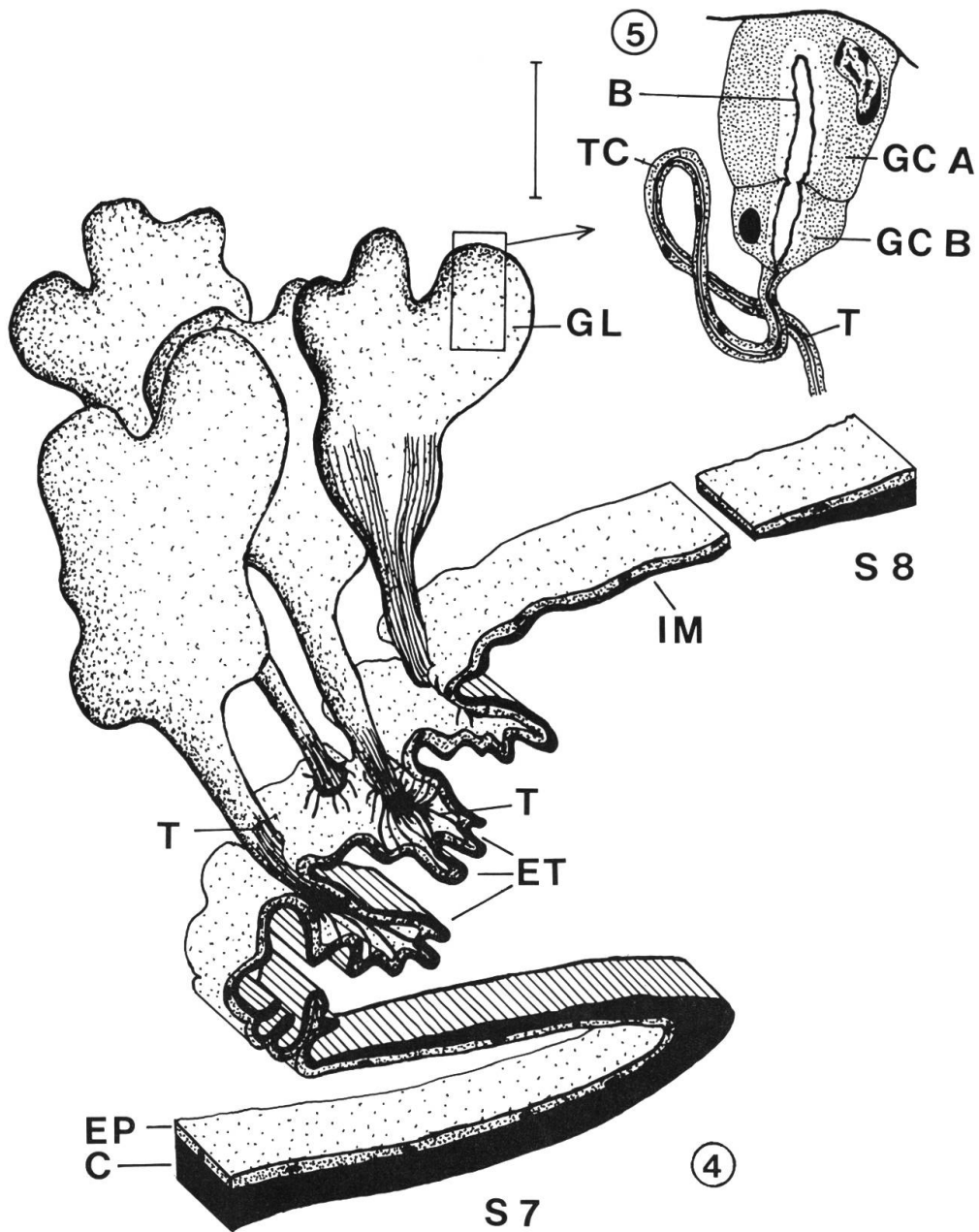
Figs 4–5: Semischematic longitudinal section through sternites 7 and 8 (S7, S8), the inter-segmental membrane (IM) and evaporation tissue (ET) of *Pyrochroa coccinea* L. (4). EP = epidermis, C = cuticle, GL = gland cells. Gland cells A and B (GC A, GC B) and the tubule (T) are demonstrated by the inset figure 5 (TC = tubule carrying cells; B = bulb). Bar: 20 μm.

intersegmental membrane, each of them extending dorsally from the seventh tergite to the posteriolateral part of the intersegmental membrane between sternites 7 and 8. The intersegmental membrane anteriolaterally towards the posterior margin of the seventh abdominal sternite is characterized by two distinct ovoid areas where an enlargement and wrinkling of the cuticle surface is obvious. As seen in figure 6–8 those wrinkled parts (ET) of the intersegmental membrane (IM) actually represent cuticle enlargements and are not caused by the desiccating procedure. As shown in figure 7, 9 the surface of the wrinkled areas consists of several transversal ridges characterized by a secondary wrinkling (Fig. 8). Due to this considerable enlargement of the membrane surface these areas were named evaporation tissue (ET). The space between both the ridges and the secondary wrinkles probably acts as reservoir for the highly volatile, liquid glandular products. This is obvious when the beetles are handled: After an irritation the odour fades out immediately but can be perceived again on molestation about one to several hours later. Obviously in the meantime a small fraction of the volatile, intensively smelling glandular material has to be resynthesized by the beetle.

When wrinkled areas of the intersegmental membrane were mazerated a lot of tubules from glandular cells are seen from the interior view (Fig. 11). Approximately 15–20 single tubules are collected to form a bundle (Fig. 4). At the inner wall of the evaporation tissue the bundles form no discrete pore plates as found within the defensive glands of the darkling beetle genera *Latheticus* or *Blaps* (KENDALL, 1974). On the contrary the tubules of the bundles disaggregate to reach the diversity of secondary wrinklings individually within the ridges of the evaporation tissue. From the evaporation tissue towards the glandular cells the approximately 17 bundles of tubules per evaporation tissue diverge to form two or three branches where few tubules are united (Fig. 4). Each tubule is enclosed by several tubule-carrying cells before reaching the secretory cells (Fig. 5). Here the cuticular tubule (T) loops back and forth before entering an enlarged chitinous bulb (B) (Fig. 5, Fig. 10). The wavy surface of this bulb is seen after treatment with KOH (Fig. 10). Squash preparations of fresh glandular tissue reveal that Pyrochroidae possess two kinds of glandular cells (A, B) surrounding the bulb as found within the secretory tissue of the heteromeran beetles (Fig. 5). The distally situated secretory cell (GC A) is characterized by a crescent-shaped granulated nucleus. The proximally situated secretory cell (GC B) is smaller and shows an ovoid, darkened nucleus.
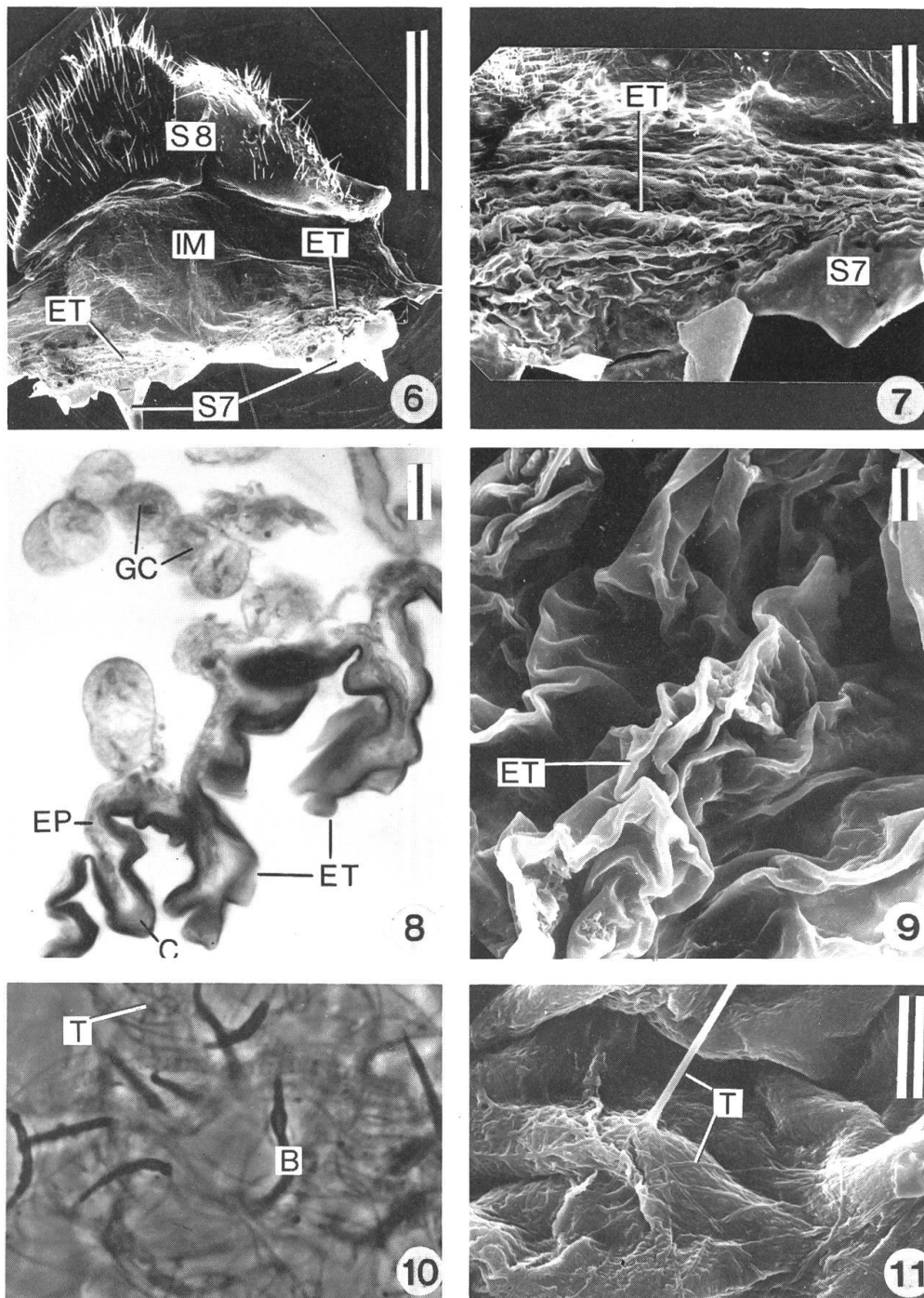
Figs 6–11: Abdominal defensive glands of *Pyrochroa coccinea* L.: 6, Surface view (SEM-photograph) of abdominal segment 8 (S8) and parts of segment 7 (S7) with intersegmental membrane (IM) and evaporation tissues (ET). 7, Enlargement of an evaporation tissue, surface view (SEM). 8, Microscopical cross section of the evaporation tissue (ET) with epidermis (EP), cuticle (C) and gland cells (GC). 9, Further enlargement of an evaporation
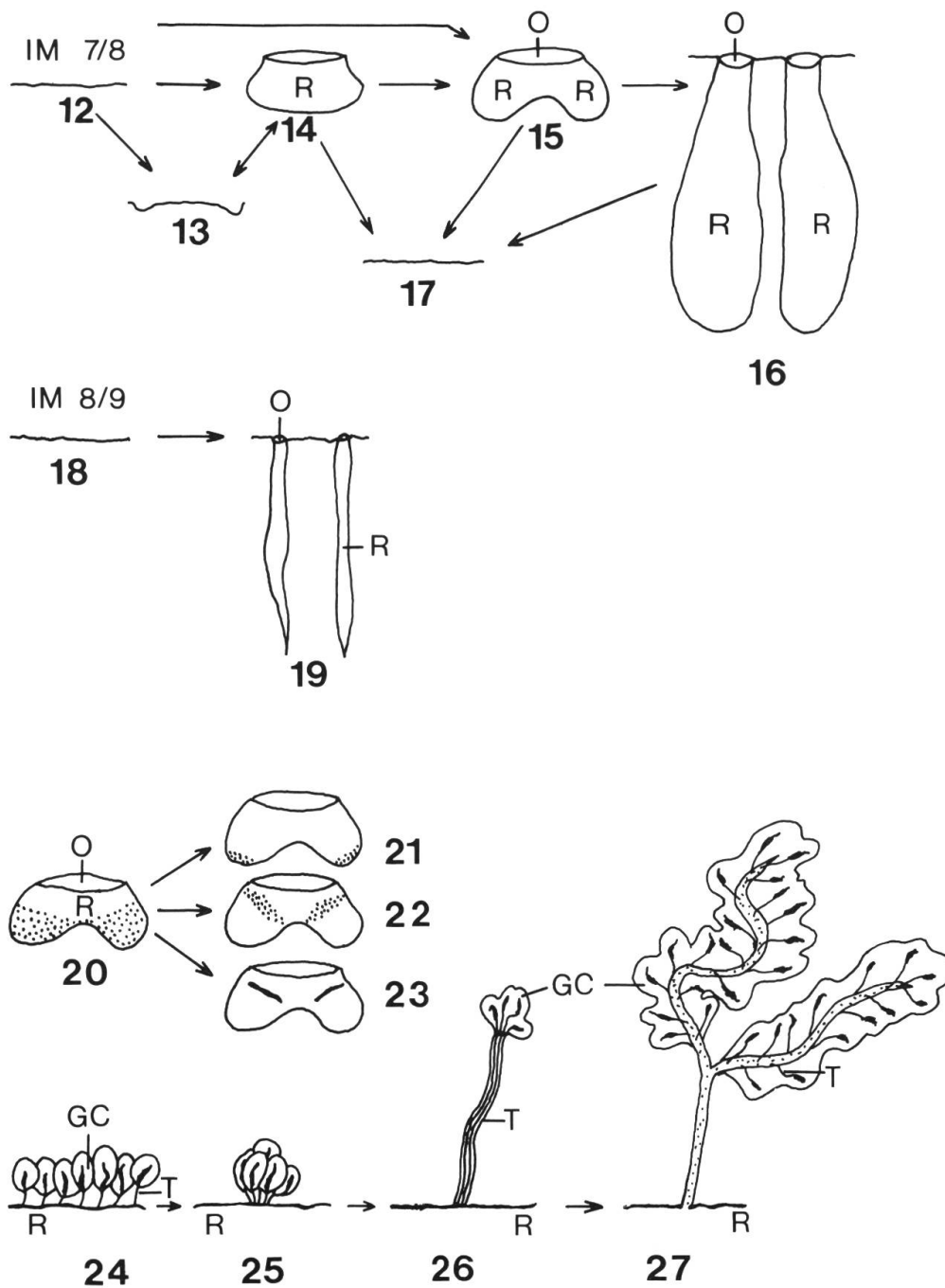
Evolution of abdominal defensive glands of Heteromera and taxonomic importance of the Pyrochroidae glands

A narrow fraction of heteromeran beetles is characterized by abdominal defensive glands which apparently represent very important characters to define these groups phylogenetically (e. g. DOYEN 1972; DOYEN & LAWRENCE, 1979). To conceive the phylogenetic importance of such gland systems and to include the new found pyrochroid glands within other heteromeran gland systems it is primarily necessary to discuss the known evolutionary trends on the glandular level of heteromeran beetles in detail. To avoid confusing discussions on the taxonomic rank of families, subfamilies or tribes in the following the tribal arrangement given by DOYEN & TSCHINKEL (1982) in their appendix X is used. Here the separation of heteromeran beetles is adopted according to CROWSON (1967), LAWRENCE (1982) and LAWRENCE & NEWTON (1982). As far as known till now Zopheridae and Boridae possess no abdominal defensive glands (KENDALL, 1964) whereas a distinct fraction of Tenebrionidae (DOYEN & TSCHINKEL, 1982) shows different kinds of abdominal gland systems.

The absence of abdominal glands has been supposed to represent the primary and plesiomorphic constitution as found within the following Tenebrionidae subgroups: Tentyriinae, Zolodinini and Belopini (both Tenebrioninae) (see Fig. 12, 18). Independently intersegmental membranes either between sternites 7 and 8 (Fig. 13–17) or between sternites 8 and 9 (Fig. 19) became glandular or were lost secondarily as shown in figure 17. It seems principally difficult to distinguish between a primary absence or a secondary loss of the gland systems mentioned. DOYEN & TSCHINKEL (1982), however, showed that heteromeran beetle species with abdominal glands always are characterized by external membranes between sternites 5–7 which become internalized quickly if glands are lost. They assumed that «non-defended» external membranes can be exploited by predators particularly piercing feeders. If there are species without glands but characterized by those external membranes a secondary loss of the glands seems probable as found within Goniaderini (Tenebrioninae) and probably in Cossyphini (Tene-

Figs 12–27: Schematic drawings demonstrating evolutionary trends within defensive glands of heteromeran beetles (according to Tschinkel 1975, Tschinkel & Doyen 1980): 12–23, Dorsal views of intersegmental membranes (IM) or gland reservoirs (R) with reservoir openings (0). 24–27, Enlarged cross sections of gland reservoirs (R) with glandular cells (GC) and tubules (T). 12–17, Formation of defensive glands from the intersegmental membrane between sterna 7 and 8 (12); glands of Pyrochroidae (13); unpaired pouch (14) with progressive lateralization (15); complete separation of the gland reservoirs (16) and

brioninae) (Fig. 17). The primary absence of abdominal defensive glands within Tentyriinae and probable in Zolodini (Tenebrioninae) and Belopini (Tenebrioninae) (Fig. 12) was suggested on grounds of internalized intersegmental membranes (Doyen & Tschinkel, 1982).

The first type of paired abdominal glands evolved between sterna 8 and 9 (Fig. 19) and was found in Adeliini (Tenebrioninae; Doyen & Tschinkel, 1982).Tschinkel & Doyen (1980) supposed that this type of gland might be homologous to the large colleterial glands of *Lagria* and *Derolagria* (Tenebrionidae, Lagriinae).

All remaining heteromeran abdominal glands evolved between sterna 7 and 8 (Fig. 12–16) and I can see no reason to discuss their independent origin in Pycnocerini (Tenebrioninae), most Tenebrioninae (paired reservoirs) or Lagriinae (paired reservoirs with musculature). Primarily there probably evolved a small medial pouch (Fig. 14) with glandular cells scattered randomly on the dorsal surface of this reservoir (Tenebrioninae: Pycnocerini and genus Phrenapates within Phrenapatini; Fig. 20). As seen in other beetle groups with medium gland reservoirs (e. g. Staphylinidae; Klinger, 1979) there is a strong tendency to divide the pouch to lateralize both gland reservoirs in order to enlarge the reservoir volume by evading centrally situated voluminous organs as intestine or internal sexual organs. This evolutionary stage is seen within several tribes of Tenebrioninae. As in *Tenebrio molitor* (Fig. 28, 29) the primitive state is represented by two reservoirs with a still common reservoir volume close by the reservoir opening (Fig. 15) whereas two distinct sac like reservoirs without a common volume represent the advanced state (Fig. 16). Further evolutionary trends were found with respect to the termination and morphology of the secretory tubules, size and shape of reservoir and reservoir walls, quantity and quality of the secretions and modi of delivering secretions (Tschinkel, 1975). By comparing a lot of Tenebrioninae genera secretory tubules on the one hand were found to be randomly scattered on the dorsum of the reservoir (plesiomorphic; Fig. 20, 24). In the derived condition the secretory tubules are confined to certain reservoir areas

secondary loss of the glands (17). 18–19, Formation of separated defensive gland reservoirs (19) from the intersegmental membrane between sterna 8 and 9 (18). 20–23, Termination of secretory tubules on dorsum of reservoir; 20, tubules scattered randomly (dotted). 21–23, Tubules terminate in limited specific areas. 24–27, cross sections of heteromeran defensive glands; tubules scattered randomly on dorsum reservoir (24); tubules terminate in limited specific areas (25); tubules are distinctly lengthened (26); origin of secondary collecting ducts (dotted, 27).
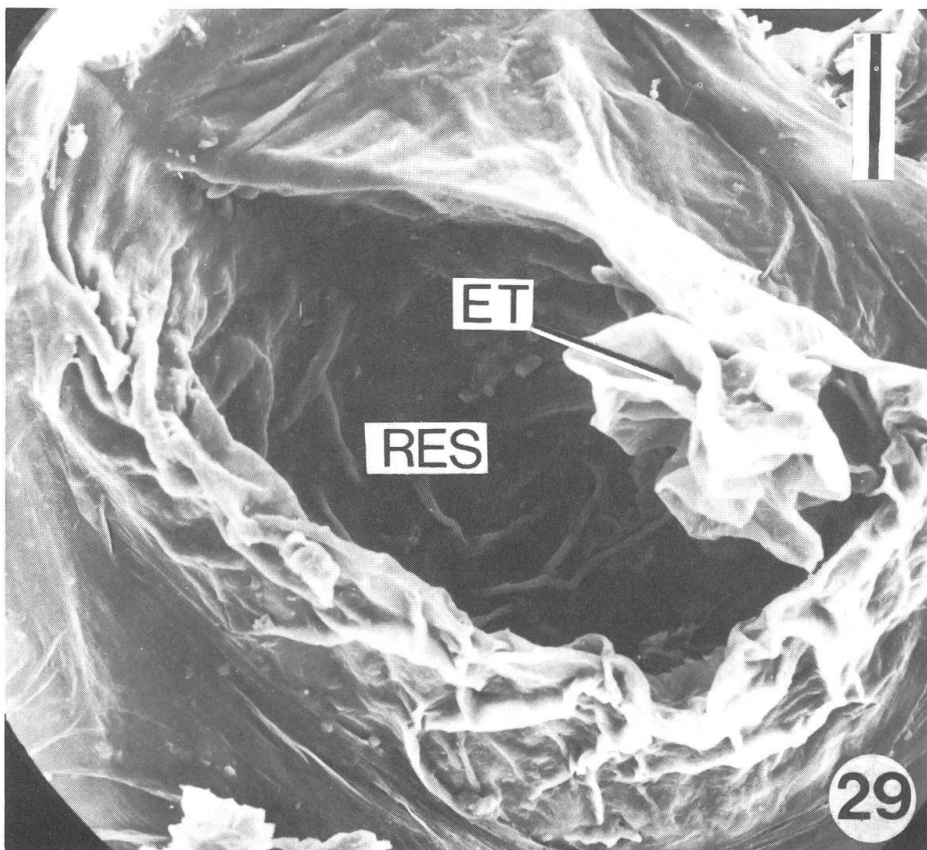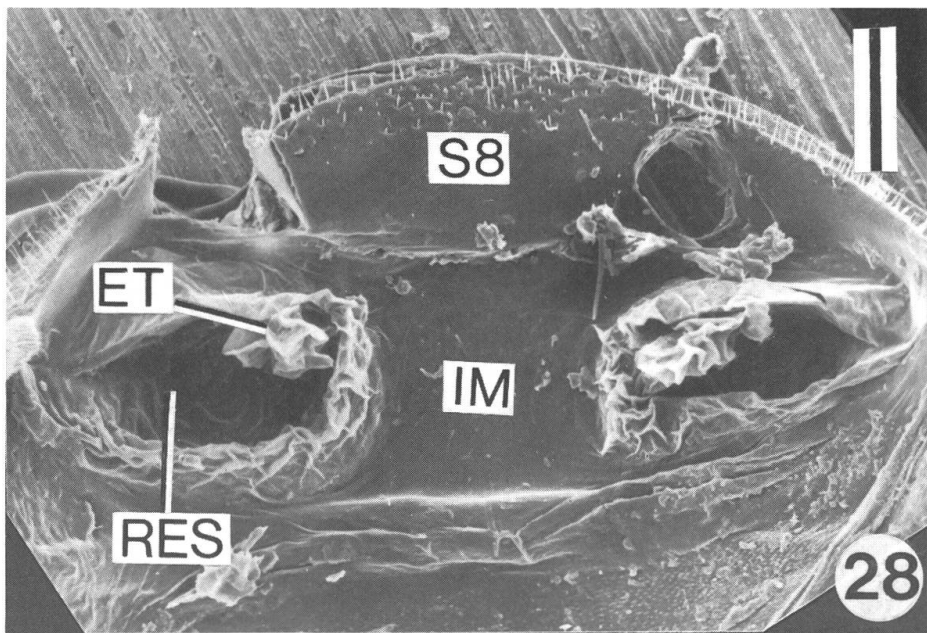
Figs 28–29: Abdominal defensive glands of *Tenebrio molitor* L. (SEM-photographs): 28, Surface view of segment 8 (S8), intersegmental membrane (IM) gland reservoirs (RES) and evaporation tissue (ET). 29, Enlargement of a gland reservoir.
Bars: 28: 0.3 mm, 29: 0.1 mm.

(Fig. 21–23, 25), the tubules are lengthened (Fig. 26) and secondary efferent ducts appear (Fig. 27).

Apart from the lateralization and separation of the gland reservoirs a distinct enlargement of the reservoir volumes and an increased wrinkling of the reservoir walls from primitive towards advanced species is obvious both in Tenebrioninae (Tschinkel, 1975) and Staphylinidae (Dettner & al., in prep.). This tendency seems advantageous in order to exude defensive secretions on repeated molestations. Moreover Tschinkel (1975) recognized that primitive species of darkling beetles secreted small amounts of quinones whereas advanced genera sequestered large amounts of chemically diverse defensive secretions. With respect to the delivery of the secretions finally it has been found that primitive species evert gland reservoirs whereas most advanced genera exude their secretions or still use it as a spray.

If the new found pyrochroid defensive glands are compared with the abdominal glands of other heteromeran beetles it seems clear that the defensive glands of cardinal beetles are homologous to other heteromeran gland systems with respect to their morphological position between sterna 7 and 8 (Fig. 13). The pyrochroid gland therefore could originate either from an intersegmental membrane (Fig. 12) or from a medium situated pouch as demonstrated in figure 14. As compared with the mentioned evolutionary trends the pyrochroid glands are primitive by the absence of a true gland reservoir, the small amounts of produced secretions and by the mere everting of the glandular tissue on molestation. Advanced characters of the pyrochroid glands are the lengthened tubules, their collection as bundles and termination in specific areas of the evaporation tissue (Fig. 4). As to the gland chemistry several trials failed in collecting and elucidating the defensive principle of the Pyrochroidae on grounds of the small amounts of the highly volatile secretion. Certainly quinones as characteristic defensive chemicals of heteromeran beetles are absent in the cardinal beetles investigated and a chemical comparison seems therefore not possible.

According to Crowson (1967) or Lawrence & Newton (1982) Pyrochroidae show no affinities to Tenebrionidae but might be phylogenetically placed in the neighbourhood either of the Pythidae or the Anthicidae (Abdullah, 1964). Therefore freshly killed specimens of *Anthicus floralis* (L.) (Anthicidae) and *Pytho depressus* L. (Pythidae) were studied in order to look for abdominal glands. In both cases no trace of a glandular tissue could be detected between sterna 7 and 8 of the two species.

To understand the development of the Pyrochroidae abdominal glands several alternatives are now conceivable. There might be the possibility that a great stock of diverse heteromeran beetles (Tenebrionidae, Pyrochroidae) possessed homologous abdominal glands between sterna 7 and 8. Later these plesiomorphic characters were reduced within some taxa and now there is a mosaic like distribution of those glands originally characteristic in the heteromeran ground plan. If the Pyrochroidae gland is homologous with the Tenebrioninae glands and if these gland systems arose from glandless conditions as found in Tentyriinae (DOYEN & TSCHINKEL, 1982) it would be interesting if the now found phylogenetic connections between Pyrochroidae and Tenebrioninae in future would be supported by other characters.

Nevertheless it is interesting that there exist several genera of completely uncertain taxonomic position which were placed within Pyrochroidae, Oedemeridae, Pythidae or Anthicidae (PAULUS, 1971) and that there might exist affinities between Tenebrionidae and Pythidae (neighbourgroup of Pyrochroidae) when the tenebrionid genera *Perimylops*, *Chanopterus* or the genus *Boros* (Boridae) are taken into consideration (CROWSON, 1967).

As third alternative finally an independent origin of the pyrochroid glands might be plausible. This could be supported by the absence of quinones within cardinals, the characteristic toxic principles of the remaining tenebrioninae glands. This alternative would be highly interesting since glandular homologisations based on pure morphological data would be questionable especially if exposed body positions (at last abdominal segments) are involved in the genesis of defensive glands. Therefore the probability of a convergent origin of defensive glands at exposed body regions seems considerably enlarged.

Acknowledgements

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