MORPHOLOGICAL STUDY OF THE PYGIDIAL DEFENSIVE SYSTEMS IN CARABID BEETLES

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Pygidial defensive systems are present in all carabid beetles at the posterior end of the abdomen. The structures are paired and can spray a mixture of chemicals that are employed in escaping from attackers, such as predators. Research has recently progressed on carabid defensive systems. In the previous report (Kanehisa and Murase 1977), it was shown that components so far detected from 132 species in 60 genera may be divided into six groups : (1) formic acid, (2) methacrylic, tiglic and/or ethacrylic acids, (3) other acids, such as *n*-butyric, *iso*-butyric, *iso*-valeric, *iso*-crotonic, angelic and few other acids, (4) *meta*-cresol, (5) benzoquinones with one chamber, (6) benzoquinones with two chambers. (6) group is the bombardier beetle. And the gland types observed so far may be divided into three groups : (1) fatty acid producing spherical lobes, (2) *meta*-cresol producing elongated lobes and (3) benzoquinone producing long thick lobes.

This report describes more details on gland morphology, especially the synthetic lobes observed mainly by the scanning electron microscope, and discusses the relationship between the morphology and components.

MATERIALS AND METHODS

Carabid beetles. Beetles tested were collected from fields year around since 1970 in the Chūgoku district, mostly Okayama prefecture. All living beetles were immersed in ether and stored in a freezer (−20℃) until observation.

Observation. The external morphology of the abdomen was initially observed under the dissection microscope. The abdomen was dissected along the midline of tergite in a small quantity of water, and the whole structure of the gland system was observed. Careful observations were performed on the shape of the reservoirs and the shape and number of synthetic lobes. Reservoirs and lobes were connected with slender flexible connecting canals. The entering points of the connecting canals into the reservoirs were also examined.

Scanning electron microscope. The whole gland system was removed, immersed in 1 % glutaraldehyde solved in 1/15 M phosphate buffer (pH 7.2), and stored in a refregerator for at least several days. The samples were directly or after critical point drying treatment observed under the scanning electron microscope (JSOL JSM-50A). All specimens were treated with gold coating. In order to reduce surface deformation produced by surface tension in the immersed liquid, some specimens were treated by the critical point drying method. In this method, specimens were transferred from glutaraldehyde solution to *iso*-amyl-

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acetate or *n*-amylacetate, and further transferred to liquid carbon dioxide and dried gradually.

RESULTS

The pygidial gland systems were dorsally paired, cuticular invaginations of the body wall that opened to the outside at the last intersternite membrane. Glands were composed of a pair of large reservoirs and many synthetic lobes connected with the reservoirs by long flexible cannals, and from the posterior end of the reservoirs efferent ducts passed to the opening valves immediately anterior to the external opening (Fig. 1 and Plate Ⅰ).

The shape and size of reservoirs, the entering points of the collecting canals, the shape and number of the synthetic lobes were quite diverse among the tribes. Four examples of reservoirs are shown in Plate Ⅰ-1～4. Schematic drawings of the diversity are shown in Fig. 1. The reservoirs were made of chitinous substances, as shown in Plate Ⅰ-5, 6. Plate Ⅰ-5 illustrates the fresh reservoir and Plate Ⅰ-6 shows the dessicated reservoir. It was observed the arrangement of chitinous threads.

NOTES ON INDIVIDUAL TRIBES

The tested beetles and their main components were described in the previous report (Kanehisa and Murase 1977).

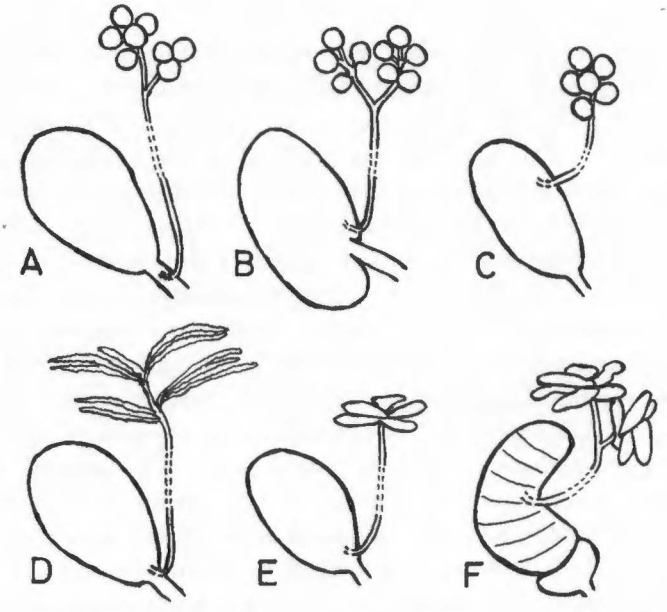

Fig. 1. Schematic figures of the pygidial gland systems of carabid beetles. A,B and C illustrate fatty acid producing spherical lobes. D shows *meta*-cresol producing elongated lobes. E and F show benzoquinone producing long thick lobes.

Carabini : *Campalita chinense* Kirby, *Carabus* 2 species, *Apotomopterus* 3 species and *Damaster blaptoides* Koller. Half opaque, large oval reservoirs with more than 100 spherical lobes (Fig. 1-A, Plate Ⅱ -1 ~ 6) were observed. The entering point of the collecting canal was just above the entry of the efferent duct. The main components were methacrylic, tiglic and/or ethacrylic acids, only *Campalita* contained additional salicylaldehyde.

Nebrini : *Nebria lewisi* Bates and other 2 *Nebria*. Semi-transparent rather small ovoid reservoirs with 20-40 spherical lobes (Fig. 1-A, Plate Ⅱ -7,8) were observed. The entering point of the collecting canal was similar to Carabini. The main components were methacrylic and tiglic acids.

Scaritini : *Scarites aterrimus* Morawitz and 3 other *Scarites*. Semi-transparent oval reservoirs with 40-50 spherical lobes were observed with some lobes apparently fused (Fig.1-C, Plate Ⅲ -1.2). Collecting canals entered the middle part of the reservoir. The main components were *iso*-crotonic, angelic, methacrylic and tiglic acids.

Clivini : Tested 2 species. Brownish, small oval reservoirs and fused long thick synthetic lobes (Fig. 1-E) were observed. The main components were benzoquinone and toluquinone.

Broscini : *Broscosoma doenitzi* Halord and *Craspedonotus tibialis* Schaum. Semi-transparent large oval reservoirs with 40-60 spherical lobes were observed with some lobes seemly fused. The entering points of the collecting canal were middle part of the reservoir (Fig. 1-C, Plate Ⅲ -3,4). The main components were *iso*-valeric and few fatty acids.

Patrobini : *Patrobus flavipes* Motschulsky, *Diplous caligatus* Bates and *D. depressus* Gebler. Semi-transparent oval large reservoirs with 40-50 fused spherical lobes (Fig. 1-A, Plate Ⅲ -5,6) were observed with the entering point of the collecting canal similar to Carabini. The main components were methacrylic, tiglic acids, only *Patrobus* contained additional two other unsaturated acids.

Pterostichini : *Trigonognatha cuprescens* Motschulsky, *Lesticus magnus* Motschulsky, *Pterostichus fortis* Morawitz and 15 other species, mostly *Pterostichus*. Semi-transparent large oval reservoirs with 20-50 spherical lobes (Fig. 1-A, Plate Ⅰ -2, Plate Ⅲ -7,8 and Plate Ⅳ -1,2) were observed. The main components were methacrylic and tiglic acids.

Amarini : *Amara chalcites* Dejean, *Curtonotus giganteus* Motschulsky and 3 other species. Semi-transparent faint yellowish large oval reservoirs with 20-40 spherical lobes (Fig. 1-A, Plate Ⅳ -3,4) were observed. The main components were mathacrylic, tiglic and ethacrylic acids.

Agonini : *Synuchus cycloderus* Bates, *Calathus halensis* Schaller, *Agonum daimio* Bates, *Colpodes japonicus* Motschulsky and 11 other species. Semi-transparent oval reservoirs with 30-80 spherical lobes (Fig. 1-B, Plate Ⅳ -5,6) were observed. The main component was formic acid.

Harpalini : *Anisodactylus signatus* Panzer, *Harpalus capito* Morawitz, *Stenolophus iridicolor* Redtenbacher and 15 other species. Semi-transparent oval

reservoirs with 20-100 (almost related with body size) spherical lobes (Fig. 1-B, Plate Ⅰ-3,5,6, Plate Ⅳ-7,8) were observed. The main component was formic aicd.

Licinini : *Diplocheila zeelandica* Redtenbacher and *D. elongata* Bates. Semi-transparent large oval reservoir with 60-80 spherical lobes (Fig. 1-B) were observed. The main component was formic acid.

Chlaenini : *Macrochlaenites costiger* Chaudoir, *Chlaenius pallipes* Gebler, *Callistoides deliciolus* Bates and 15 other species mostly *Chlaenius*. Yellowish-brown ovoid reservoirs with 30-50 elongated lobes (Fig. 1-D, Plate Ⅴ-3,4) were observed. The main component was *meta*-cresol.

Three species of subgenus *Chlaenius Chlaeniellus prostenus* Bates, *C. C. circumductus* Morawitz, *C. C. inops* Chaudoir had brownish oval reservoirs with 30-40 long thick lobes (Fig. 1-E, Plate Ⅴ-5,6). The main components were benzoquinone and toluquinone.

Panagaeini : *Panagaeus japonicus* Chaudoir, *Dischissus mirandus* Bates and 3 other species. Yellowish-brown ovoid reservoirs with 30-50 elongated lobes (Fig. 1-D, Plate Ⅴ-7,8) were observed. The main component was *meta*-cresol.

Lebini : *Lebia retrofasciata* Motschulsky, *Coptoderina japonica* Bates, *Lebidia octoguttata* Morawitz and 5 other species. Semi-transparent oval reservoirs with 40-80 spherical lobes (Fig. 1-B, Plate Ⅴ-1,2). The main component was formic acid.

Dryptini : *Drypta japonica* Bates. Semi-transparent oval reservoirs with about 50 spherical lobes (Fig. 1-B, Plate Ⅵ-1,2) were observed. The main component was formic acid.

Zuphini : *Planetes puncticeps* Andrewes and *Galeritura japonica* Bates. Semi-transparent large oval reservoirs with 70-100 spherical lobes (Fig. 1-B, Plate Ⅵ-3,4) were observed. The main component was formic acid.

Brachini : *Pheropsophus jessoensis* Morawitz, *Brachinus scotomedes* Bates and 2 other *Brachinus*. This group is the bombardier beetles with two chambers. Secretion was made with sound production and heat. The upper chamber was half opaque and the lower chamber was brown with 30-50 long thick lobes (Fig. 1-F, Plate Ⅵ-5 ~ 8). The main components were benzoquinone and toluquinone. Sometimes swelling of the upper chamber apparently caused by the backward flowing of reaction gas.

DISCUSSION

The pygidial defensive gland systems, especially reservoirs, of carabid beetles were described by Eisner et al. (1963, 1968), Schildknecht et al. (1963, 1968) and Moore and Wallbank (1968). Forsyth (1970, 1972) described the whole gland systems of 71 species from 34 tribes using histological and transparent electron microscopical observations. He showed that the gland systems had considerable diversity in shape, size and structure, and dicussed some of the relationships between the gland forms and main components.

In the present study for the detailed observations and comparisons were made, especially of the synthetic lobes by the scanning electron microscopy. The shape, size and number of synthetic lobes were specific among species, with the shape quite distinctive in accordance with biologically synthetic materials which were transported through the collecting canals and stored into reservoirs and secreted by the stimulation of attackers.

Fatty acid synthetizing lobes were mostly spherical, the *meta*-cresol synthetizing lobes were elongated, and benzoquinone synthetizing lobes were long and thick. Brachini, Clivini and subgenus *Chlaenius Chlaeniellus* secreted benzoquinones. Even though Brachini is known as a bombardier beetle having two chambers with the capacity for spraying long distance, but two other beetles having one chamber and rather oozing secretion. They all had white opaque long and thick synthetic lobes with little differences evident in size and fusion manner. Panagaenini and most of Chlaenini secreted *meta*-cresol, these beetles had white elongated lobes. Many other beetles secreted several kinds of fatty acids and they had generally spherical lobes.

Some regularity appeares evident in the relation of spherical shapes and kinds of fatty acids. The formic acid secreting tribes, Agonini, Harpalini, Licini, Lebini, Dryptini and Zuphini, had rather perfectly rounded shapes with small regular tubercles on the surfaces. The methacrylic, tiglic and/or ethacrylic acid secreting tribes, Carabini, Nebrini, Pterostichini and Amarini had rather oval shapes with small regular tubercles on the surfaces. Scaritini, Broscini and *Patrobus* they secreted mixtures of more than three kinds of fatty acids, had rather fused round shape lobes. Synthetized and secreted compounds were related with the shape of the lobes.

Secreted compounds are also related to the shape of reservoirs and the entering points of the collecting canals as shown in Figure 1. Type A in Figure 1 secreted methacrylic, tiglic and/or ethacrylic acids, typically observed in Pterostichini and Amarini. But Carabini and Nebrini had collecting canals entering just above the entry of the efferent duct. Type B secreted formic acid and had rather large number of lobes in comparison with other types.

The size of the lobes was almost the same in similar compound producing beetles but number of lobes was rather related to body size. Large beetles had the many lobes and small beetles had fewer lobes. The size of reservoirs was independent of the compounds produced but was rather related to body size with large beetles having large reservoirs.

SUMMARY

Morphological variations were investigated in the pygidial defensive systems of carabid beetles by scanning electron microscopy.

1. All beetles had a pair of homologous glands, but variations were observed in the shape and size of reservoirs, shape and number of synthetic lobes and in the entering point of the collecting canal into the reservoirs. The glands

were roughly divided into six types, as shown in Fig. 1.

2. A relationship was evident between the shape of the synthetic lobes and the main components. Spherical lobes produced fatty acids, elongated lobes produced *meta*-cresol and long thick lobes produced benzoquinones.

3. Some regularity was observed in the fatty acid producing spherical lobes. Formic acid producing lobes were rather perfectly round. Methacrylic, tiglic and/or ethacrylic acids producing lobes were rather oval shaped. Some species had more than three kinds of fatty acid producing lobes those were fused and round shaped.

4. Species in same genus, at least same subgenus, had same gland morphology as observed in main components.

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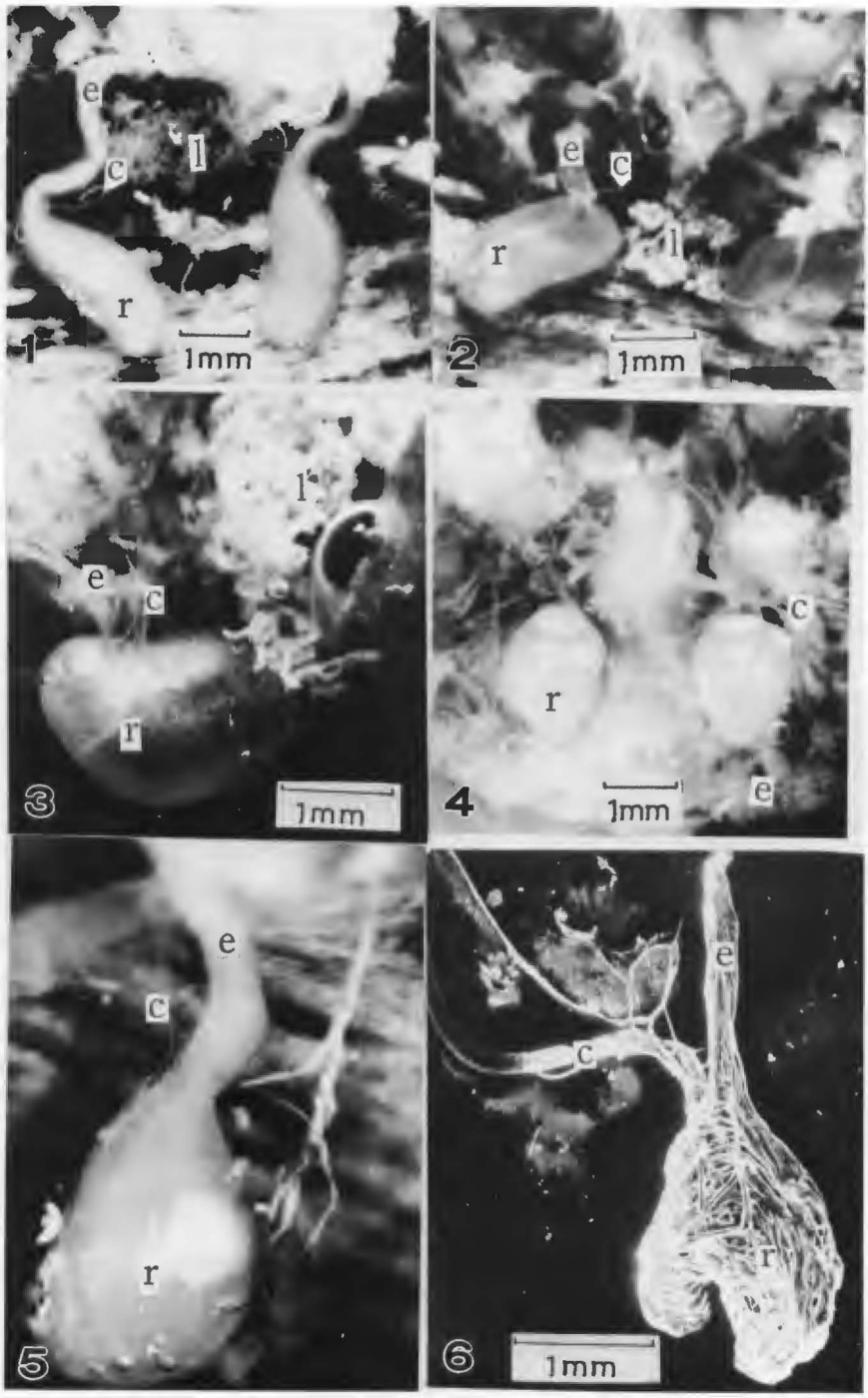

Plate I. Gland systems, c : collecting canal, e : efferent duct, l : synthetic lobes, r : reservoir. 1 : *Apotomopterus yaconinus*. 2 : *Pterostichus fortis*. 3 : *Harpalus capito*. 4 : *Chlaenius pallipes*. 5 and 6 : *Harpalus tridens* (5 is fresh and 6 is dessicated).

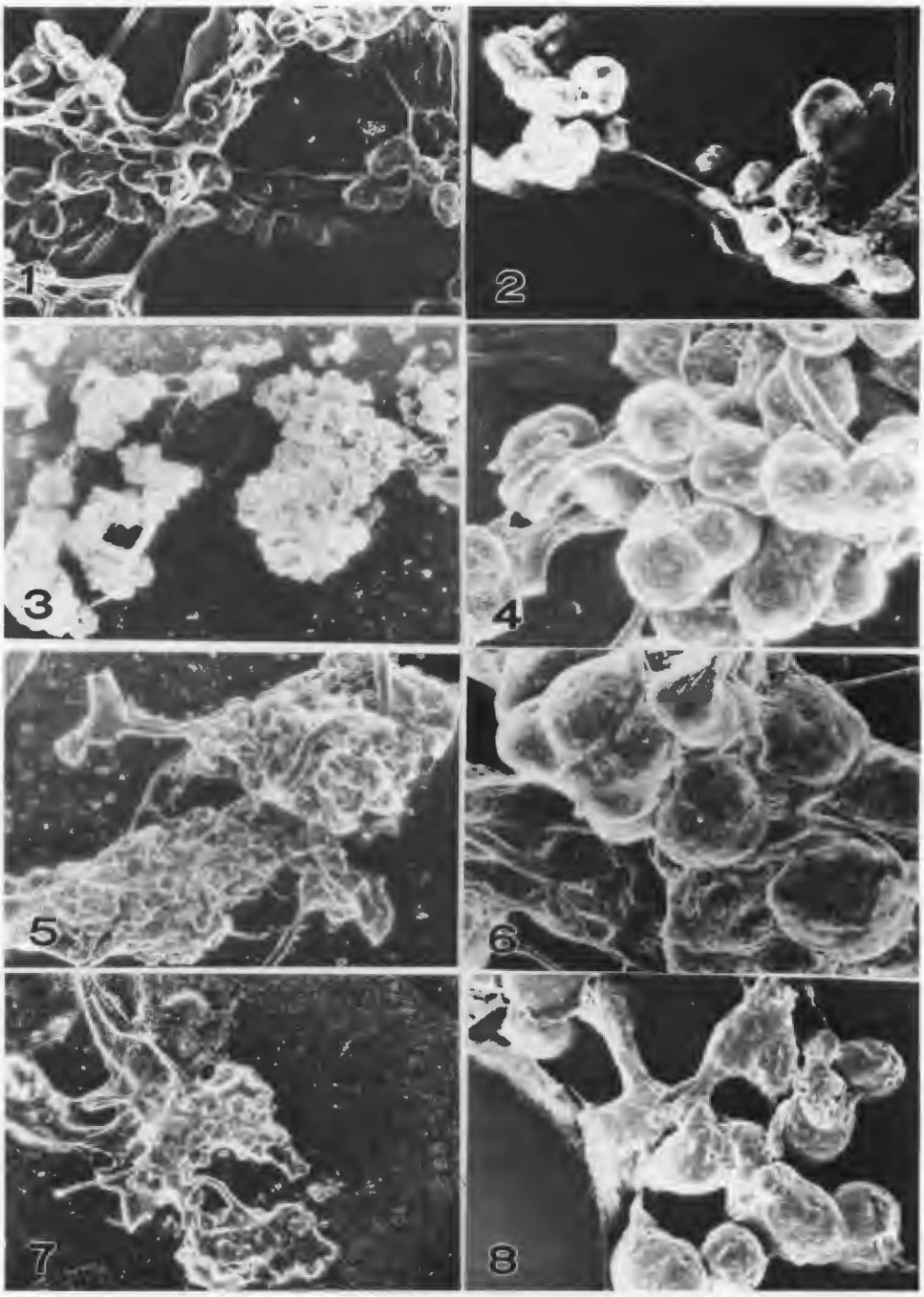

Plate II. Scanning electron micrographs. 1 (x 80) and 2(x 200) of *Campalita chinense*. 3(x 40) and 4(x 300) of *Apotomopterus yaconinus*. 5(x 50) and 6(x 300) of *Damaster blaptoides*. 7(x 40) and 8(x 300) of *Nebria lewisi*.

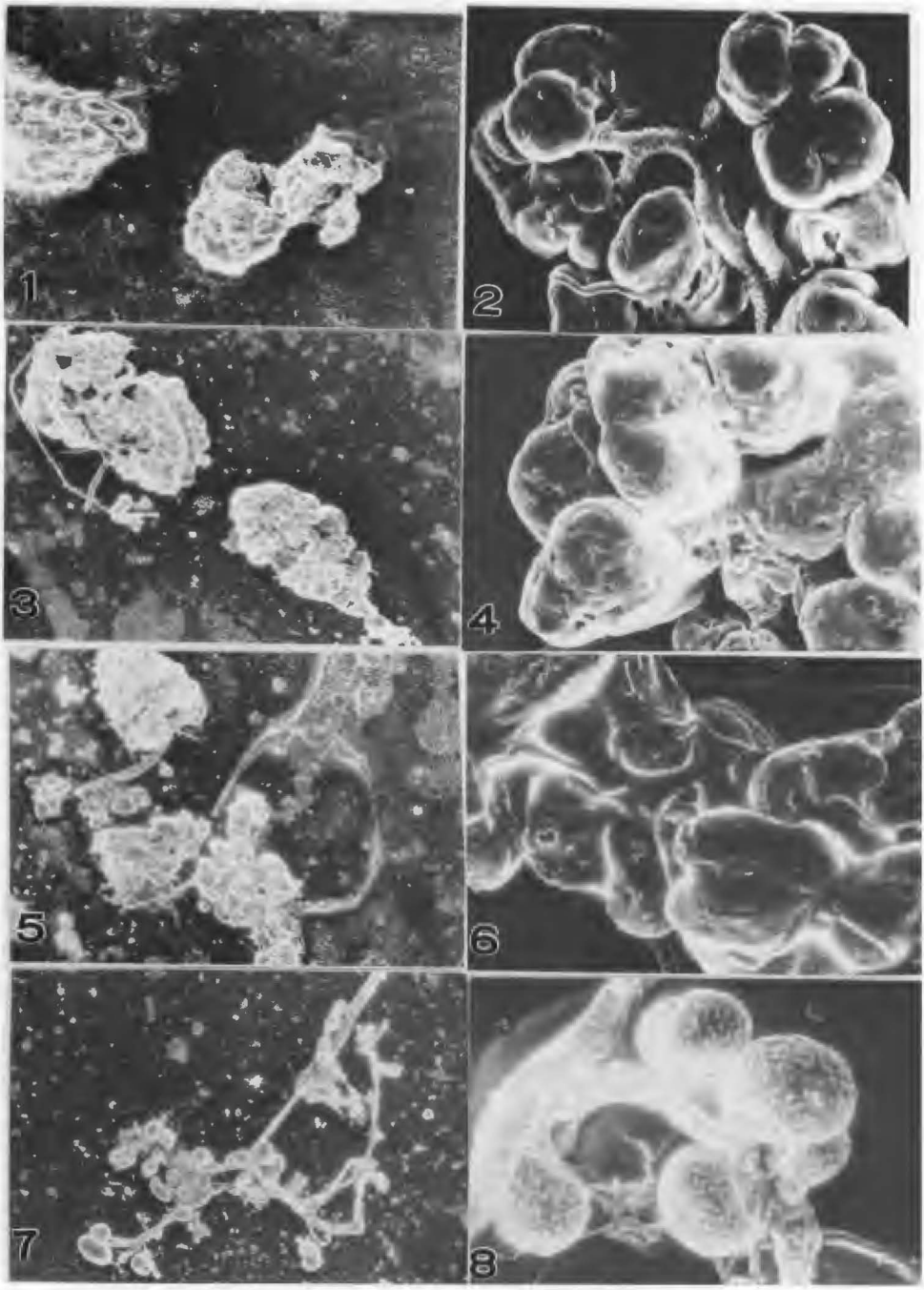

Plate III. Scanning electron micrographs. 1(x50) and 2(x 300) of *Scarites aterrimus*. 3(x 40) and 4(x 300) of *Craspedonotus tibialis*. 5(x 50) and 6(x 300) of *Diplous caligatus*. 7(x 50) and 8(x 300) of *Pterostichus fortis*.

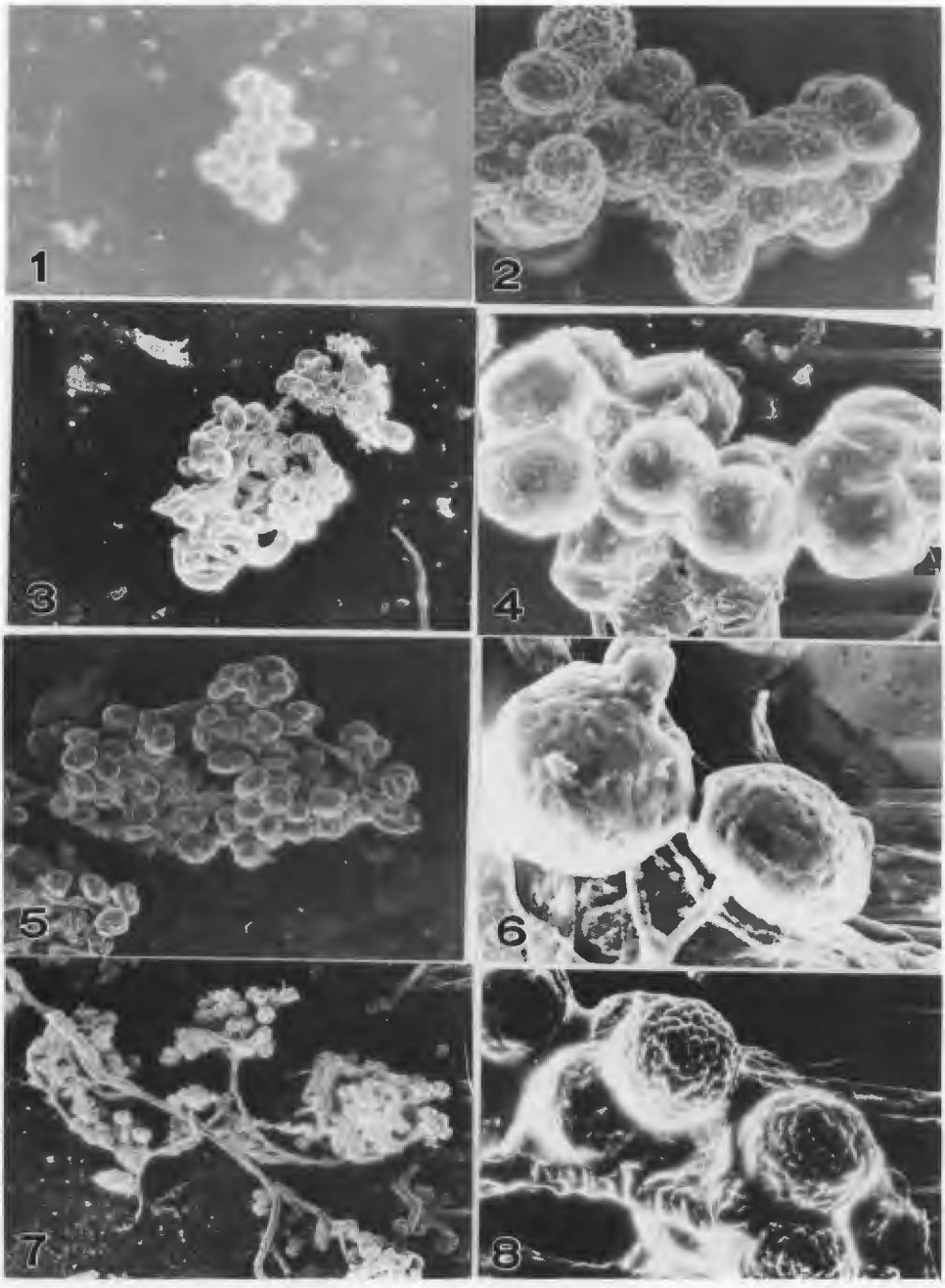

Plate IV. Scanning electron micrographs. 1(x 50) and 2(x 300) of *Pterostichus yoritomonus*. 3(x 50) and 4(x 300) of *Curtonotus giganteus*. 5(x 50) and 6(x 500) of *Calathus halensis*. 7(x 50) and 8(x 300) of *Harpalus capito*.

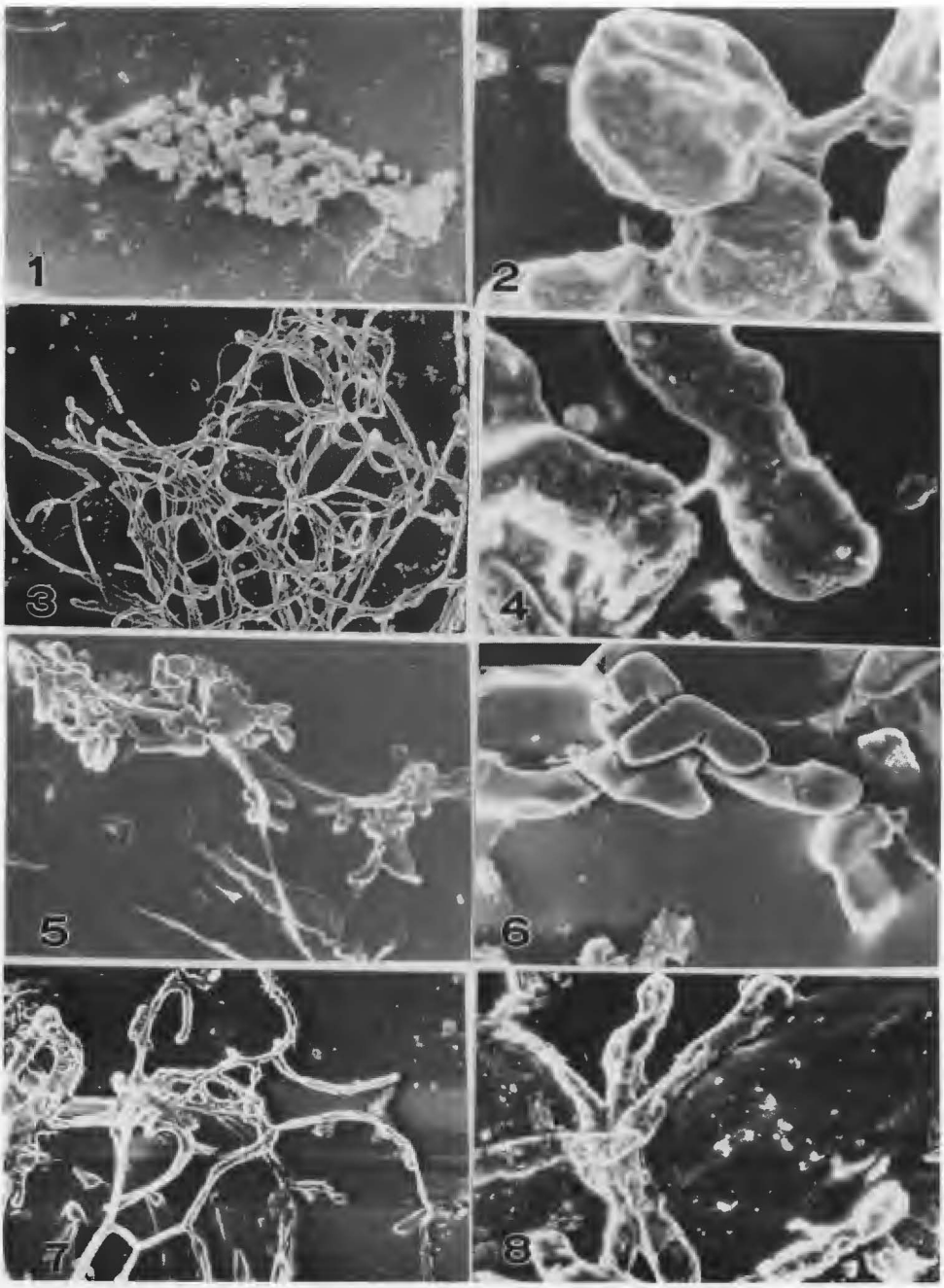

Plate V. Scanning electron micrographs. 1(x 40) and 2(x 500) of *Lebidia octoguttata*. 3(x 100) and 4(x 1000) of *Chlaenius pallipes*. 5(x 100) and 6(x 350) of *Chlaenius Chlaeniellus inops*. 7(x 100) and 8(x 600) of *Dischissus mirandus*.

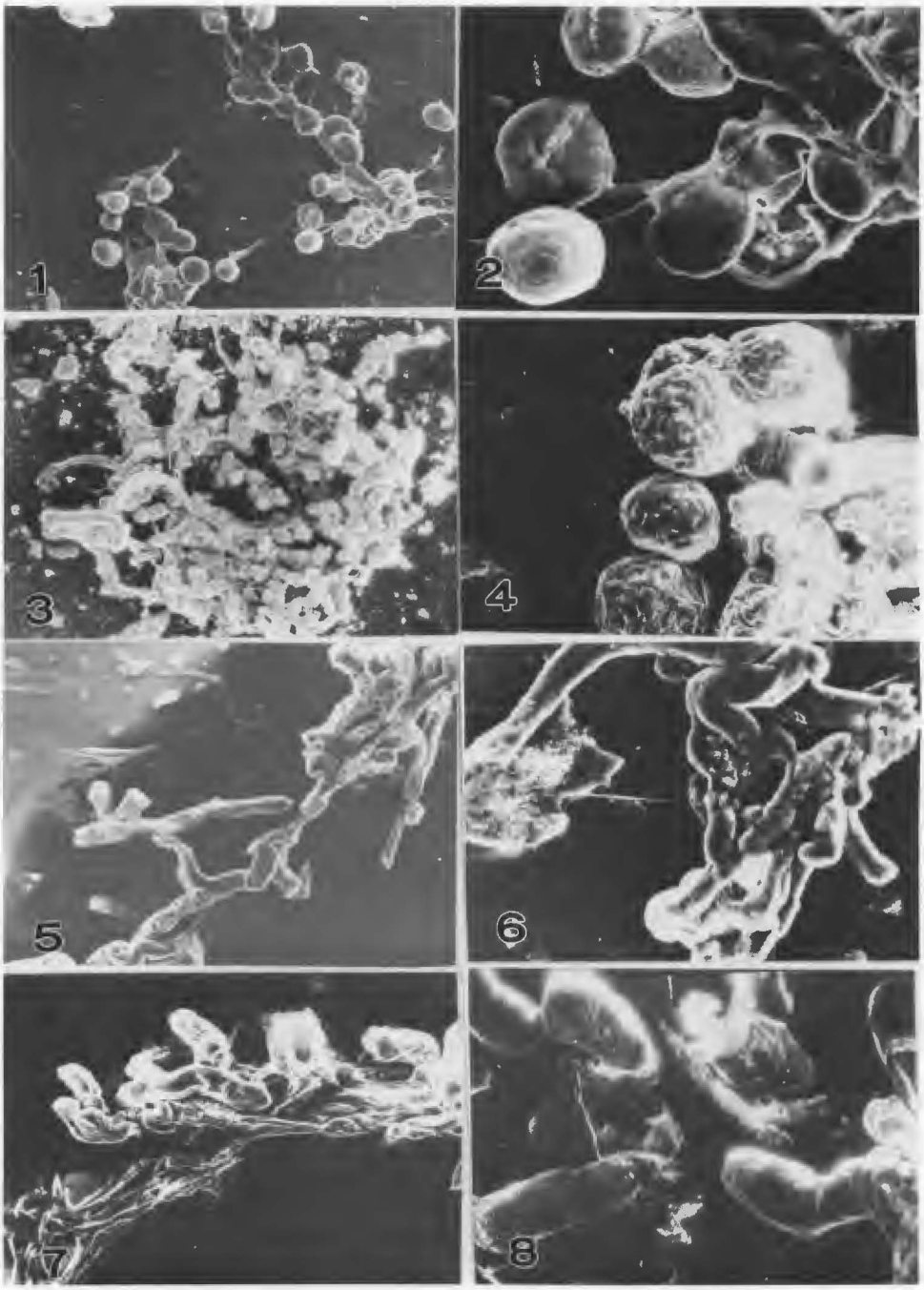

Plate VI. Scanning electron micrographs. 1(x 50) and 2(x 300) of *Planetus puncticeps*. 3(x 40) and 4(x 250) of *Galeritura japonica*. 5(x 100) and 6(x 250) of *Brachinus scotomedes*. 7(x 100) and 8(x 300) of *Pheropsophus jessoensis*.