Int. J. Insect Morphol. & Embryol., Vol. 22, Nos 2–4, pp. 417–446, 1993
Printed in Great Britain

0020-7322/93 $6.00+.00
Pergamon Press Ltd

ULTRASTRUCTURAL DIVERSITY IN THE EGG CHORION OF HAWAIIAN *DROSOPHILA* AND *SCAPTOMYZA*: ECOLOGICAL AND PHYLOGENETIC CONSIDERATIONS

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Abstract — Formation of the egg shell (chorion) in *Drosophila* and *Scaptomyza* (Diptera : Drosophilidae) is a complex developmental process involving coordinated synthesis and secretion of multiple proteins by the monolayer of follicle cells surrounding the egg. Using scanning electron microscopy, the ultrastructure of the chorion in 37 endemic Hawaiian drosophilids, representing the genera *Drosophila* and *Scaptomyza*, were analyzed and compared with 7 representative species of continental *Drosophila*. The detailed structure of the chorion was described for 8 chorionic regions: the respiratory filaments, follicle imprints, operculum, micropyle, dorsal ridge, ventral rim, posterior pole, and the chorion cross-section. The morphology of each region is similar among related species, but strikingly different among groups. The main functions of the chorion are to protect the developing embryo from the vicissitudes of the environment and to provide channels for gas exchange during embryogenesis. Adaptation to the diverse ovipositional substrates used by *Drosophila* in general, and the Hawaiian species in particular, has resulted in extraordinary diversity in the various chorionic structures. The respiratory filaments differ in number and have evolved to different lengths and degrees of porosity. Furthermore, other regions also involved in respiratory exchange (the operculum, follicle imprints, the pole region, and the dorsal ridge) have diverged in parallel to the ecological divergence. The thickness and complexity of the outer endochorion are dramatically different in various groups, providing varying degrees of mechanical strength to the eggshell, which promotes embryonic survival in the diverse microenvironments. These varied chorionic structures have been found to provide useful morphological characters for phylogenetic analyses of the drosophilids.

Index descriptors (in addition to those in title): Evolution, oogenesis, reproductive strategies, ecological adaptation.

INTRODUCTION

THE KEY to the success of insect life cycles lies in their reproductive strategies. Egg production is a very critical event, and production of an egg mass of a size appropriate to the available resources is a measure of the species' level of adaptation to its ecological niche.

The *Drosophila* species endemic to the Hawaiian Islands provide a prime example of the diversification of reproductive strategies correlated with adaptive radiation into a wide array of ecological niches. On a land mass of only 6420 square miles, more than 700

Drosophila species are found (Hardy, 1965; Hardy and Kaneshiro, 1981), comprising a quarter of the world's known *Drosophila* fauna. These species have evolved in the recent geological past as a result of a series of interisland migrations, and successful founding of new populations in each new habitat (Carson *et al.*, 1970; Carson, 1971; Carson and Templeton, 1984). Sexual selection has acted on male courtship and female preference behaviors, often leading to altered acoustic signals (Hoy *et al.*, 1988), to provide the requisite sexual isolation between derived populations (Kaneshiro, 1976, 1983).

Ecological diversification is a major feature of speciation in these flies which primarily utilize decaying plants as substrates for oviposition (Heed, 1968). Various parts of the plant, such as the bark of tree trunk, stems, leaves, fruits, or flowers, are utilized by different *Drosophila* species, and different plant families are used by different groups of flies (Heed, 1968, 1971; Montgomery, 1975). The nutritional reserves available in these sources vary dramatically and the *Drosophila* species have adapted to these diverse nutritional reserves by regulating their reproductive strategies, specifically the size of the egg mass produced (Kambysellis and Heed, 1971). Flower breeders, for example, mature and oviposit only one egg at a time, whereas bark breeders mature and oviposit several hundred eggs at a time. Besides this adaptation to optimize larval development, other adaptations have evolved to promote survival during embryogenesis, involving changes in the amounts of nutritional resources (yolk) and in the degree of protection for the embryo (chorion).

Commitment to the adaptively appropriate developmental pathway begins during larval stages, when the number of ovarioles per ovary is determined (King, 1970), and culminates in the female adult during the complex developmental process of oogenesis. This process involves the interaction of 3 cell types, the oocyte, nurse cells, and follicle cells (Fig. 1), which collectively contribute to the development of the egg (for a review, see Mahowald and Kambysellis, 1980). The functions of the surrounding follicle cells are of particular importance to the differentiation and ecological adaptation of the egg. Initially, these cells express the vitellogenin genes to produce yolk proteins (Brennan *et al.*, 1982), which, together with those synthesized by the fat body (Gelti-Douka *et al.*, 1974), constitute the major nutrient source supporting macromolecular synthesis during embryogenesis. During later stages of oocyte development, follicular transcription of the vitellogenin genes is turned off, and a different set of genes is switched on, leading to the synthesis of new proteins, which form the protective vitelline membrane and the chorionic layers of the egg (Kafatos *et al.*, 1977; Margaritis *et al.*, 1980). The variable functions of these follicle cells are responsible for much of the diversity in the reproductive strategies and ecological adaptation of the endemic Hawaiian *Drosophila* species. To accomplish the observed diversity, evolutionary changes must have occurred in both gene systems. Firstly, mutations in the vitellogenin genes have generated modified yolk proteins, as well as different quantities of each vitellogenin protein in different species (Craddock and Kambysellis, 1990). The latter partially accounts for the variability in egg production and the changes necessary to establish the appropriate egg mass for each particular ecological niche. Secondly, mutational changes in the chorion genes have been fixed, producing variations in structure and morphology of the egg shell, which ensure adaptation of the egg to diverse and often hostile ecological situations, and ultimately survival of the embryo.

The focus of this article is on comparing the chorion morphology from a variety of *Drosophila* species. First, the chorionic structures, which are relevant to the ecological

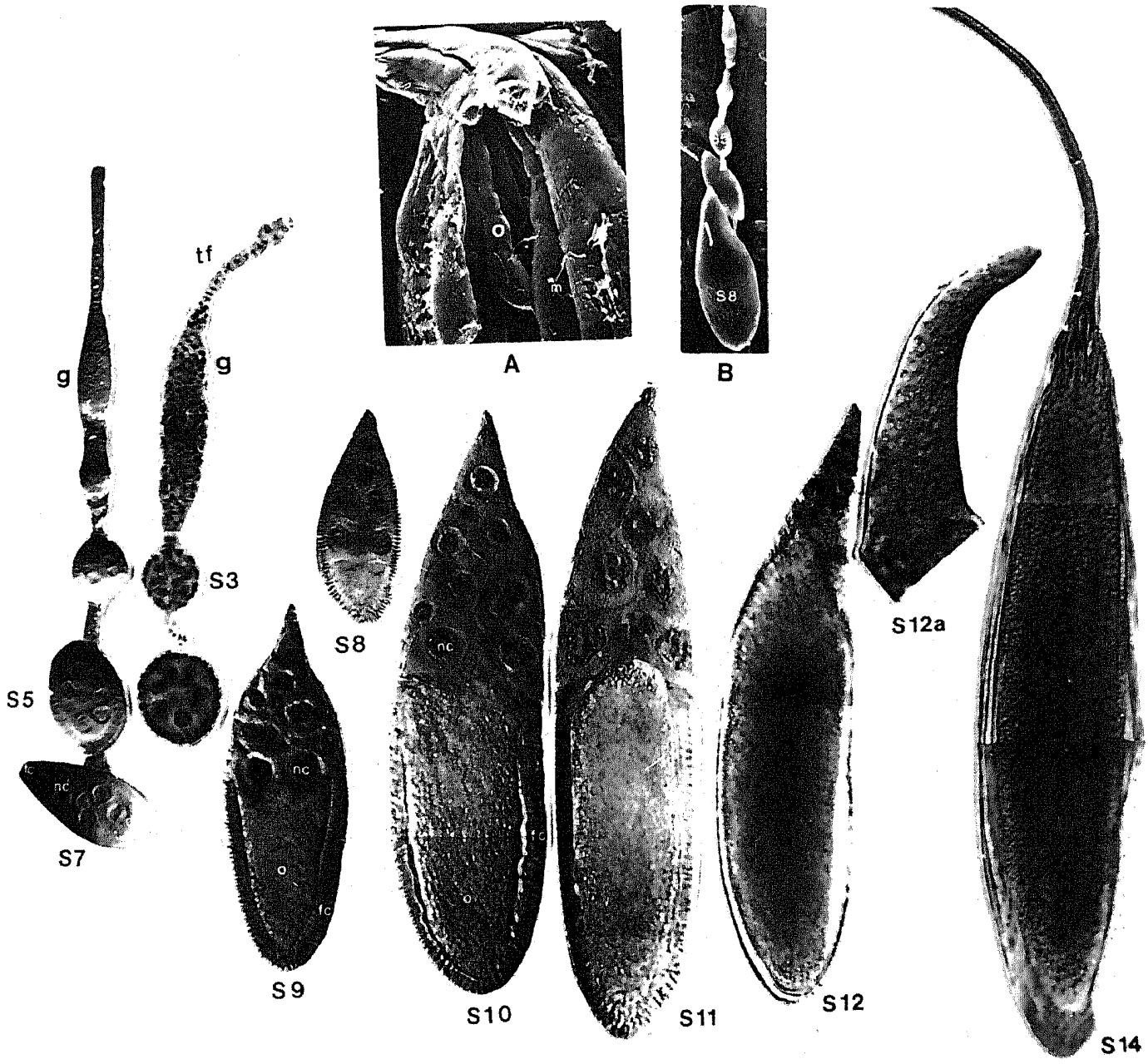

FIG. 1. Stages of egg development in the Hawaiian species *Drosophila grimshawi*. (A) Scanning electron micrograph of anterior portion of an ovary. Some ovarioles (o) have been removed to show interior organization of ovary. (B) Follicles from one ovariole with peritoneal sheath and epithelial sheath removed to show bead-like arrangement of follicles at consecutively more advanced developmental stages. Lower portion of figure shows individual follicles at various developmental stages according to King's (1970) terminology, photographed with Nomarski optics. From left to right are: germarium, g, with more terminal filament cells, tf, than in continental species; previtellogenic (S3–S7), vitellogenic (S8–S12), and chorionic stages (S12–S14). S12a shows follicle cell migration to anterior end to form long respiratory filaments of mature egg (S14). Previtellogenic stages were photographed at a magnification of × 400; all other stages at a magnification of × 250. Follicle cells, fc, nurse cells, nc, and oocyte, o, are indicated.

adaptation of the eggs to their oviposition substrates, will be identified, and then it will be demonstrated that we can use the observed interspecific diversity in chorionic structures to trace the history of their evolution and thus help determine phylogenetic relationships among the species analyzed.

MATERIALS AND METHODS

Eggs from species of the genera *Drosophila* and *Scaptomyza* were analyzed. The eggs were obtained from females collected in various locations on the Hawaiian Islands, primarily Waikamoi on the island of Maui, and Kipuki Ki, Kipuka Puaulu, Olaa Forest and Pohakuloa on the island of Hawaii. Females of additional species were obtained from established laboratory stocks maintained at the University of Hawaii or the National *Drosophila* Species Resource Center at Bowling Green, Ohio, U.S.A. Oviposited eggs or mature eggs dissected from the ovaries were processed for scanning electron microscopy as described by Margaritis *et al.* (1980), with minor modifications.

RESULTS AND DISCUSSION

Eggs of 37 species endemic to Hawaii, and 7 continental species, representing the genera *Drosophila* (subgenera *Drosophila, Sophophora, Scaptodrosophila,* and *Hirtodrosophila*), and *Scaptomyza* (subgenera *Tantalia* and *Exalloscaptomyza*) were examined (Table 1). The general structure of the chorion in Hawaiian *Drosophila* eggs is shown in Fig. 2. The most prominent structures are the follicle cell imprints covering the main body of the egg, the respiratory filaments protruding from the dorsal anterior end, the posterior pole region, the operculum, the micropyle, the collar at the anterior ventral end of the egg, and the dorsal ridge. The morphology of these structures is consistent among the eggs of all females of a species, yet shows extensive interspecific variability. The morphology of some characters, as for example the follicle imprints or the dorsal ridge, varies in different regions of the egg surface, and thus consistency in the region selected for scoring is important. The 7 chorionic regions shown in Fig. 2, and an additional one, namely the cross-section of the chorion, were analyzed for all species, and are shown, by way of example, for the egg of the Hawaiian species, *D. bostrycha* (Fig. 3). In the following, examples are presented which demonstrate the diversity in these characters among the taxa analyzed, and their value in the adaptation of the species to their particular ecological habitats is discussed. It is inferred that these morphological characters can be used to derive phylogenetic relationships. In this article, only selected representative species or characters will be illustrated; a more complete atlas of the chorionic characters in each species will be presented elsewhere.

Egg morphology

In the overall egg morphology, the most striking feature which differentiates the various *Drosophila* species is the number and size of the respiratory filaments (Fig. 4). All of the Hawaiian species of the genus *Drosophila* examined are consistent in having 4 respiratory filaments, one pair positioned somewhat more anteriorly than the other. The one exception is *D. mulli* (Fig. 4F), which only has the 2 more anterior filaments. By contrast, among the non-Hawaiian species of the genus *Drosophila*, there is extensive variation in filament numbers 1–11 (Sturtevant, 1921; Wheeler, 1949; Throckmorton, 1962; Kambysellis, 1968; Okada, 1968), some of which are illustrated in Fig. 4H–J. It is noteworthy that species of the genus *Drosophila* endemic to Hawaii, are exclusively

TABLE 1. DROSOPHILID SPECIES ANALYZED, THEIR OVIPOSITIONAL SUBSTRATES, AND GEOGRAPHIC SOURCES

Taxonomic grouping	Oviposition substrate* Primary	Secondary	Hawaiian island or stock source
I. Species endemic to Hawaii			
Genus *Drosophila*			
Subgenus *Drosophila*			
hawaiiensis subgroup			
D. silvarentis	tree flux		Hawaii
D. heedi	soil flux		Hawaii
D. gradata	tree flux		Oahu
D. formella	tree flux		Hawaii
grimshawi subgroup			
D. bostrycha	bark		Molokai
D. sproati	bark		Hawaii
D. disjuncta	stems	bark	Maui
D. murphyi	bark		Hawaii
D. engyochracea	bark		Hawaii
D. claytonae	?		Hawaii
D. crucigera	stems	bark	Kauai
D. grimshawi	stems	bark	Maui
D. mulli	?		Hawaii
glabriapex subgroup			
D. macrothrix	stems		Hawaii
D. fasciculisetae	?		Maui
planitibia subgroup			
D. silvestris	bark		Hawaii
D. heteroneura	stems	bark	Hawaii
D. planitibia	stems		Maui
D. cyrtoloma	bark		Maui
D. hanaulae	?		Maui
adiastola subgroup			
D. setosimentum	stems	bark	Hawaii
D. clavisetae	stems		Maui
D. spectabilis	?		Maui, Molokai
D. adiastola	stems	bark	Maui, Lanai
D. truncipenna	?		Maui
D. ornata	stems		Kauai
primaeva subgroup			
D. primaeva	stems	bark	Kauai
modified mouthparts			
D. mimica	fruits		Hawaii
D. infuscata	?		

Table continued on next page.

Taxonomic grouping	Oviposition substrate* Primary	Secondary	Hawaiian island or stock source
antopocerus species group			
D. aduncα	leaves		Maui
D. diamphidiapoda	leaves		Maui
white tip scutellum			
D. dolichotarsus	fungi		
D. fungiperda	fungi		Hawaii
D. longiperdis	fungi		
D. nigra	fungi		Maui
Genus *Scaptomyza*			
Subgenus *Tantalia*			
S. albovittata	?		Oahu
Subgenus *Exalloscaptomyza*			
S. oahuensis	flowers		Oahu
II. Continental *Drosophila*			
Subgenus *Drosophila*			
D. immigrans	—		Bowling Green
D. mojavensis	cacti		Bowling Green
D. virilis	—		Kent, CT
Subgenus *Sophophora*			
D. paulistorum	—		Lee Ehrman
D. willistoni	—		Lee Ehrman
Subgenus *Scaptodrosophila*			
D. pattersoni	—		Bowling Green
Subgenus *Hirtodrosophila*			
D. pictiventris	—		Bowling Green

*Data from Heed (1968) and Montgomery (1975).

members of the subgenus *Drosophila*, whereas several subgenera are included among the continental *Drosophila* (Throckmorton, 1962, 1975; Okada, 1968). The variability in number of respiratory filaments is substantially less within each subgenus. In the subgenus *Sophophora*, all species reported have 2 respiratory filaments, as in *D. willistoni* (Fig. 4H). In the subgenus *Drosophila*, the majority of species have 4 filaments, as represented by *D. virilis* (Fig. 4I), but some species (the *quinaria* group) have 3, the 2 more posterior ones appearing to be fused to form a single, much thicker, filament. Others (the *melanica*, *bromeliae*, and *nannoptera* groups) have 2 filaments, and in the *tripunctata* group, there are 1–4 filaments. Variability in the subgenus *Scaptodrosophila* is more extensive, the number of filaments varying from 2 to 11. In fact, variability is observed even among individuals of a species, as for example in *D. pattersoni*, which has 6 or 7 filaments (Fig. 4J).

Species in the related genus *Scaptomyza*, which is well represented among the

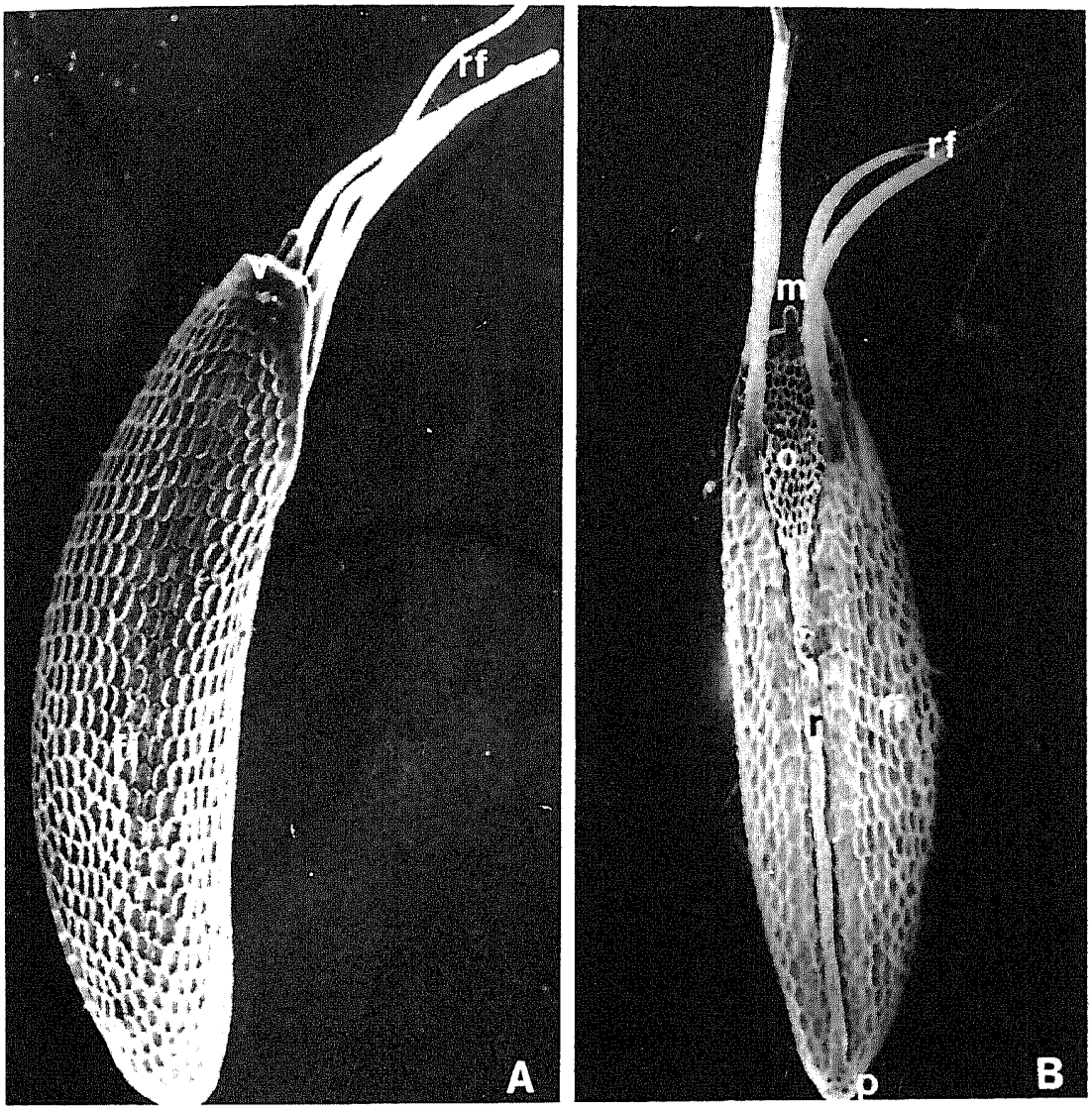

FIG. 2. Scanning electron micrograph (SEM) of eggs from modified mouthparts species *D. mimica* (A) and *D. infuscata* (B), depicting major chorionic structures analyzed in this study. Respiratory filaments, rf, follicle imprints, fi, operculum, o, micropyle, m, posterior pole, p, and dorsal ridge, r, are indicated.

Hawaiian endemics by several subgenera (Throckmorton, 1966; Hardy and Kaneshiro, 1981), have rudimentary respiratory filaments or alternatively, highly modified filaments, the normal forms being replaced by 2 structures running longitudinally along the dorsal side of the egg (Fig. 4L) parallel to the site of the dorsal ridge in *Drosophila* (Fig. 2). In the subgenus *Exalloscaptomyza*, the filaments are totally missing (Fig. 4K).

Although the number of respiratory filaments is relatively conserved within subgenera, the filament length varies enormously among species of the Hawaiian *Drosophila* (Throckmorton, 1966; Kambysellis and Heed, 1971). In some species, the filaments are shorter than the egg length (Fig. 4D, E, G), and in others, 3–4 times the egg length (Fig. 4A–C).

The relative lengths of the 2 pairs of filaments also vary. In species with short filaments, both pairs tend to be of equal lengths (Fig. 4D, E, G), whereas in species with long filaments their lengths are highly variable (Fig. 4A–C). Among the continental

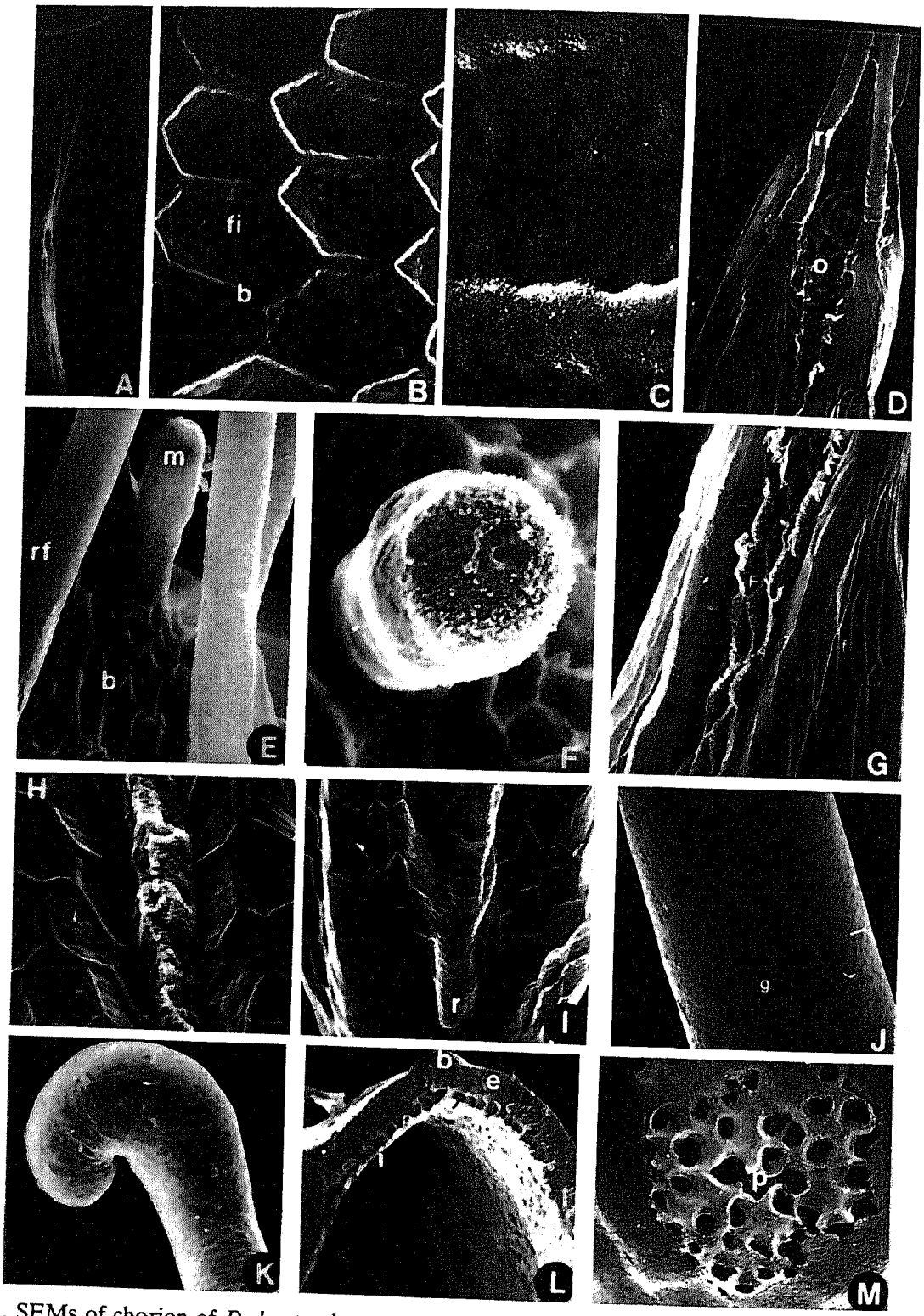

FIG. 3. SEMs of chorion of *D. bostrycha* eggs, showing regions analyzed. (A) Lateral view of egg, × 57. (B) Follicle cell imprints on anterior ventral region of egg. Note floor of imprints, fl, with distinct aeropyles and follicle cell borders, b, × 1420. (C) Detailed structure of follicle cell imprints and borders, × 7100. (D) Operculum region, o, within bases of respiratory filaments, rf, × 710. (E) Micropyle, m, located at most anterior part of operculum. Note tall follicle imprint borders in operculum region, b, and base of respiratory filaments, rf, × 1136. (F) Tip of micropyle. Note point of sperm entry, s, × 3550. (G) Anterior part of dorsal ridge. Note different morphology of follicle cell imprints within ridge, F, and at periphery, f, × 5110. (H) Middle part of ridge. Note that follicle imprints within ridge, F, and at periphery, f, have changed by comparison to anterior region, × 1420. (I) Posterior part of ridge showing modified morphology of imprints at end of ridge, r, × 710. (J) Respiratory filaments, middle portion, showing solid surface with ghost, g, imprints or pores, × 3550. (K) Tip of respiratory filament, × 3550. (L) Cross-section of chorion showing thin inner endochorion, i, pillars, p, and thick outer endochorion, e. At border of follicle imprints, b, outer endochorion is thicker, × 3550. (M) Posterior pole region. Note asymmetrical organization of large pores, some of which are fused, p. Borders of follicle cell imprints are not distinct, × 2020.

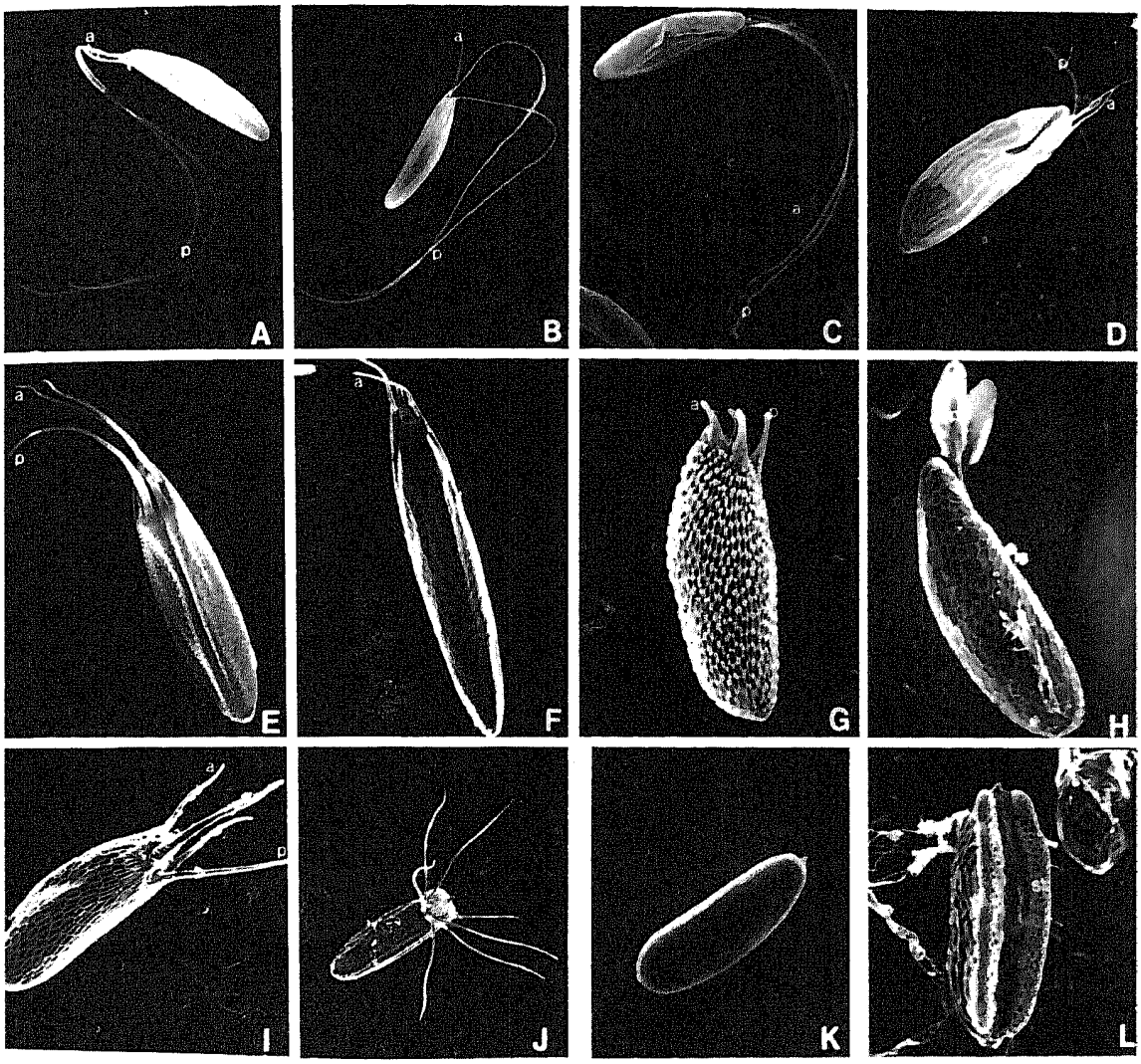

FIG. 4. Overall view of various drosophilid eggs showing variability in egg shape, number of filaments, length of anterior, a, versus posterior, p, filaments, and relation of egg length and filament length. (A) *D. murphyi*, × 56. (B) *D. claytonae*, × 35. (C) *D. heteroneura*, × 52. (D) *D. formella*, × 78. (E) *D. truncipenna*, × 71. (F) *D. mulli*, × 89. (G) *D. longiperda*; note prominent follicle imprints, f, × 128. (H) *D. willistoni*, note 2 oar-shaped posterior filaments, p, × 156. (I) *D. virilis*, × 102. (J) *D. pattersoni*, × 73. (K) *Scaptomyza* (*Exalloscaptomyza*) *oahuensis*, note smooth surface of chorion and absence of respiratory filaments, × 71. (L) *Scaptomyza* (*Tantalia*) *albovittata*, respiratory filaments have apparently been replaced by 2 porous respiratory stripes, st, × 109.

species, there is only limited variation in filament length. The majority of species have filaments no longer than the egg itself, but one out of 71 species reported by Okada (1968) has filaments 3 times the egg length. Why do we find such diversity in the filaments of drosophilid eggs?

At least among the Hawaiian species, the respiratory filaments have evolved in response to the nature of the ovipositional substrate in order to anchor the egg near the surface (Kambysellis and Heed, 1971). Eggs oviposited in deep cracks in rotting tree trunks have long filaments (Fig. 5A–D). On the other hand, eggs oviposited in leaves (Fig. 5F) or fruits (Fig. 5G, H) have only short filaments, and those oviposited in flowers, such as those of *Exalloscaptomyza* (Fig. 5E), have no filaments at all. The length variation observed between the 2 pairs of filaments also appears to be an adaptation to the variety of ovipositional substrates. Eggs oviposited in decaying tree

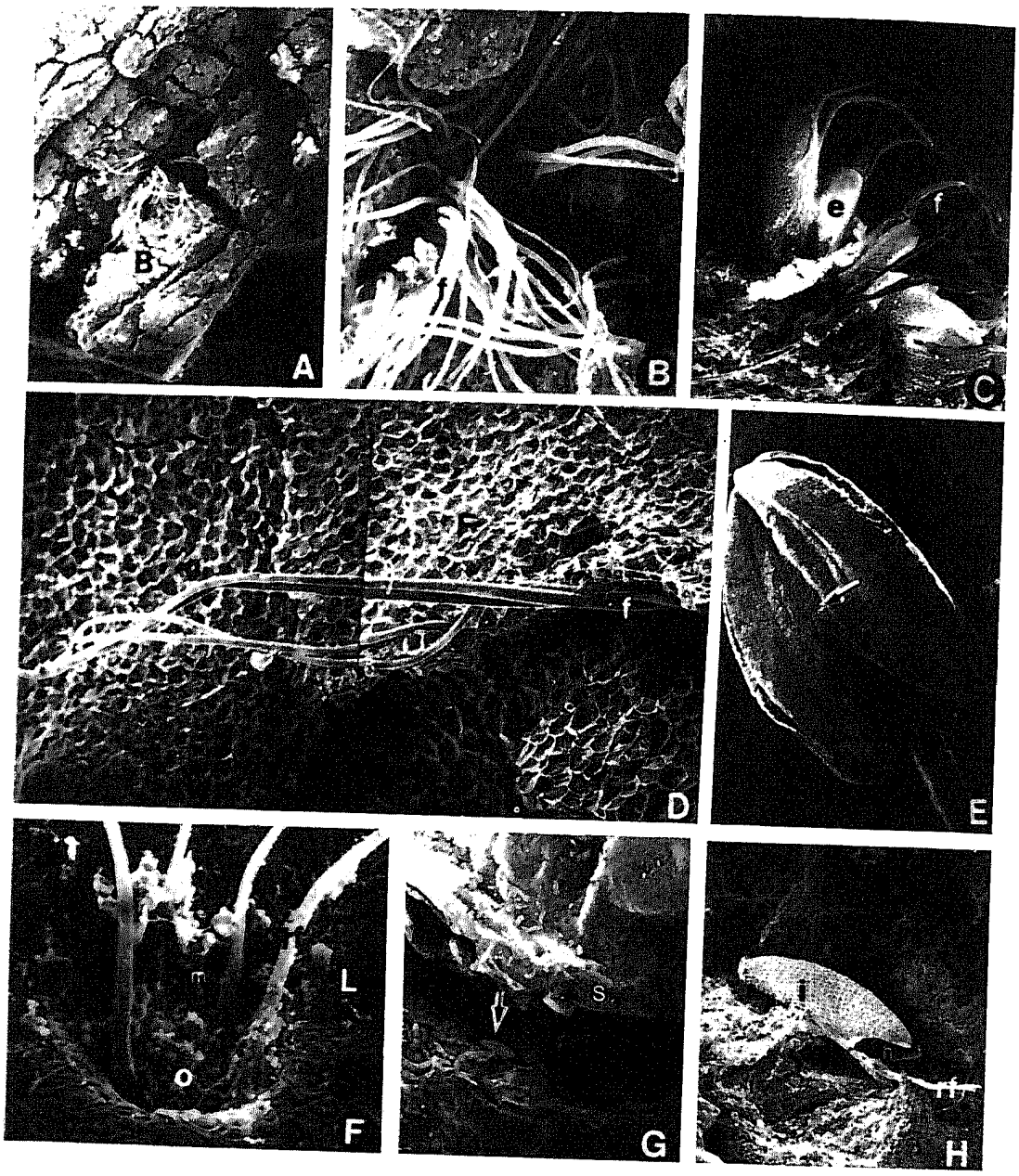

FIG. 5. Representative examples of *Drosophila* eggs oviposited in natural substrates. (A) *D. silvarentis* eggs on *Myoporum* bark, B. Note long respiratory filaments, f, × 15. (B) Higher magnification of oviposited eggs, × 74. (C) *D. heedi* eggs in flux drippings on soil. Note orientation of oviposited eggs, e, and respiratory filaments, f, × 32. (D) Egg of unknown species oviposited in decaying fern, F. Note 4 long respiratory filaments, f, resting on substrate. Region near bases of filaments is shown in (a) × 146 and filament tips in (b) × 149. (E) Anther of *Convolvulus* flower showing position where *Exalloscaptomyza* egg was dropped, arrow. Egg was lost in processing. (F) *D. adunca* egg oviposited in *Cheirodendron* leaf, L. Note 4 respiratory filaments, rf, micropyle, m, and operculum, o, × 256. (G) *D. mimica* egg, arrow, in a decaying *Sapindus* fruit, S, × 12. (H) Higher magnification showing details of oviposited egg. Note that hatching area, h, respiratory filaments, rf, ventral rim, r, and follicle imprints, i, are well preserved, × 60.

trunks may utilize primarily the more posterior pair of filaments for anchoring the egg, with a degree of variation depending on the particular substrate; eggs oviposited in leaves appear to use all 4 filaments (Fig. 5F). In addition to anchoring the eggs near the surface of the ovipositional substrate, in some niches the respiratory filaments are the only exposed structures available for gas exchange. This applies especially in the case of the fern breeders, in which all 4 respiratory filaments appear to be of equal length (Fig.

5D). The *Scaptomyza*, which have no filaments, oviposit similarly to the *Exalloscaptomyza*, dropping their eggs on an appropriate ovipositional substrate (Heed, 1968).

Fine structure of the respiratory filaments

In addition to the number and length of the filaments, their fine surface structure varies from solid to porous, to open with stripes, or plaque-like structures (Fig. 6). This is likely to be an adaptation to environmental constraints and particularly to the humidity. Species whose egg filaments have a solid surface tend to be found in wet forests where humidity is high, as, for example, *D. planitibia* and *D. adunca* (Fig. 6A, F). Those with very porous filaments, as, for example, *D. silvarentis* (Fig. 6D), are found in very dry and even desert-like environments. Species with filaments having a plaque-like surface, as, for example, *D. mimica*, occupy moderately dry areas and the eggs are oviposited in open spaces (Fig. 5G). Not all our findings can be explained in these terms. For example, species with plaque-like structures on their filaments are found not only in dry areas, but also in forests of high humidity, e.g. *D. fasciculisetae* (Fig. 6E), or *D. adiastola*. The latter species primarily uses decaying stems, but also leaves and fruits of *Clermontia* (Heed, 1968; Montgomery, 1975), and is sympatric with *D. planitibia* (a *Clermontia* stem breeder) and *D. adunca* (a leaf breeder), both of which have solid filaments (Fig. 6A, F, respectively). The plaque-like surface of the respiratory filaments is conserved in all 6 members of the *adiastola* subgroup analysed (Table 1), as well as in *D. primaeva*. All these species inhabit rainforests on the major Hawaiian islands (Table 1) and utilize decaying stems of various endemic plants as oviposition substrates (Montgomery, 1975).

The number, size and fine structure of the respiratory filaments undoubtedly play an important role in establishing physiological adaptation of the eggs to the diverse ecological habitats used by these species, an adaptation which remains to be fully understood. From a practical point of view, they can be used as diagnostic characters to indicate the nature of the oviposition substrate where it is unknown. Furthermore, they are valuable taxonomic characters for determining phylogenetic relationships.

Operculum

A prominent chorionic structure is the operculum region, located anteriorly and usually surrounded by the bases of the respiratory filaments and the micropyle (Fig. 7). The morphology of the operculum area is extremely diverse among Hawaiian drosophilids. In some species, as, for example, *D. grimshawi* (Fig. 7A) and *D. silvestris* (Fig. 7B), the operculum area extends far beyond the respiratory filament region, connecting to the dorsal ridge, while in others it is rather restricted, as in *D. mulli* (Fig. 7D), or rudimentary as in *Scaptomyza* (Fig. 4L) or even missing as in *Exalloscaptomyza* (not shown).

This region, similarly to other chorionic regions, is synthesized by a particular subpopulation of follicle cells (Margaritis *et al.*, 1983), whose borders are well defined (Fig. 7) and which are present in both the Hawaiian and continental *Drosophila*. The morphology of these follicle imprint borders varies extensively among species groups (Figs 7, 8). In some species, the imprint borders are tall, thin or lacy (Figs 7A, B, 8E), while in others they are short and thick (Figs 7C, E, 8L), and in exceptional species, like *D. mulli*, they are rudimentary, restricted only to the extreme anterior end near the micropyle (Figs 7D, 8K). In some species the operculum area appears to be divided into

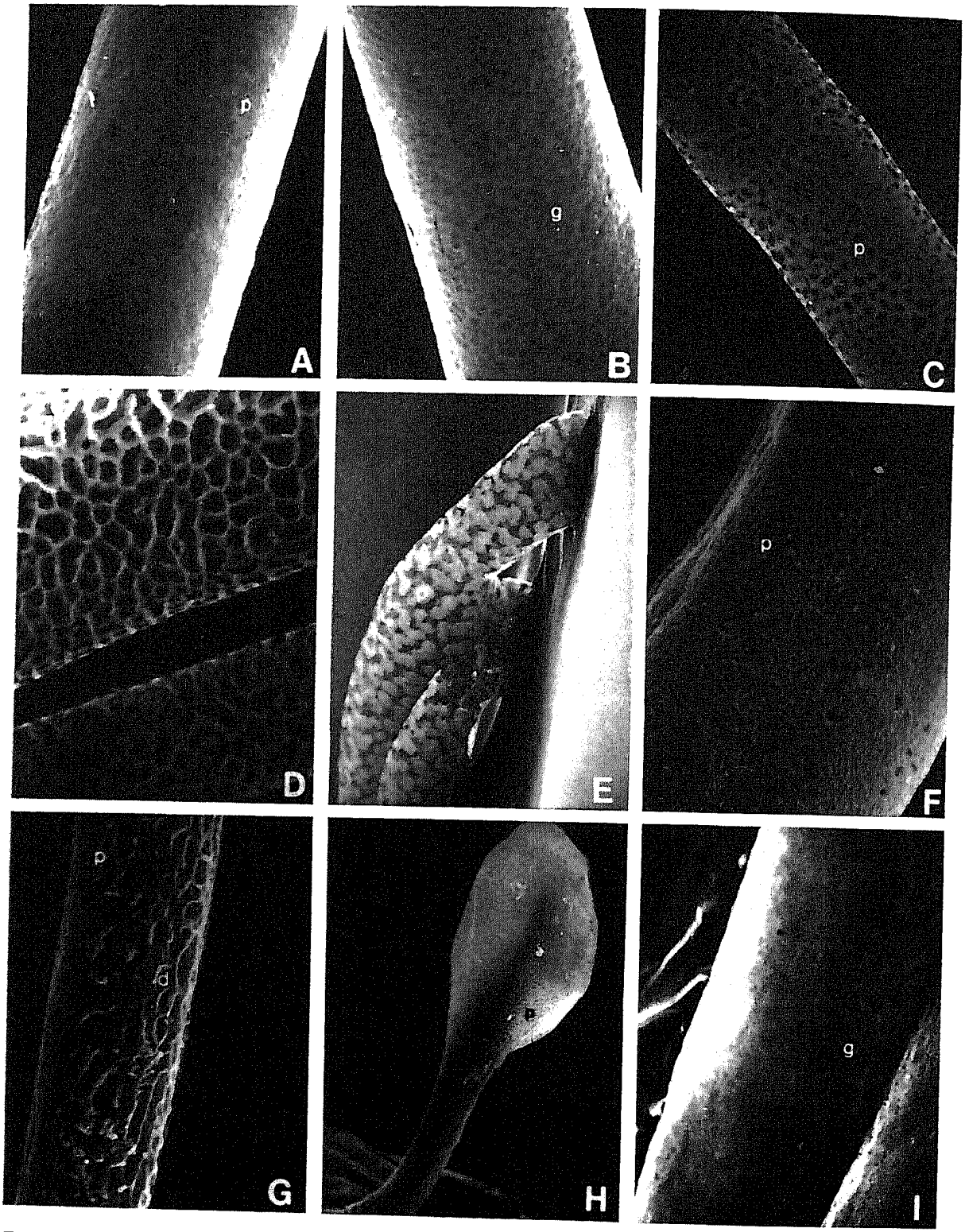

FIG. 6. SEMs of middle region of respiratory filaments in representative *Drosophila* species. (A) *D. planitibia*, solid surface with numerous small pores, p, × 355. (B) *D. bostrycha*, solid surface with ghost imprints or pores, g, × 3550. (C) *D. crucigera*, solid surface with many large pores, p, × 3550. (D) *D. silvarentis*, very open respiratory filaments, × 3550. (E) *D. fasciculisetae*, plaque-like structures, p, covering filaments, × 2840. (F) *D. adunca*, surface solid with a granular appearance and several medium size pores, p, × 3550. (G) *D. mojavensis*, filament surface with regional depressions, d, or protrusions, p, × 3550. (H) *D. paulistorum*, oar-like filament with a plaque-like region, p, × 1420. (I) *D. pictiventris*, filament surface solid with ghost imprints or pores, g, × 6390.

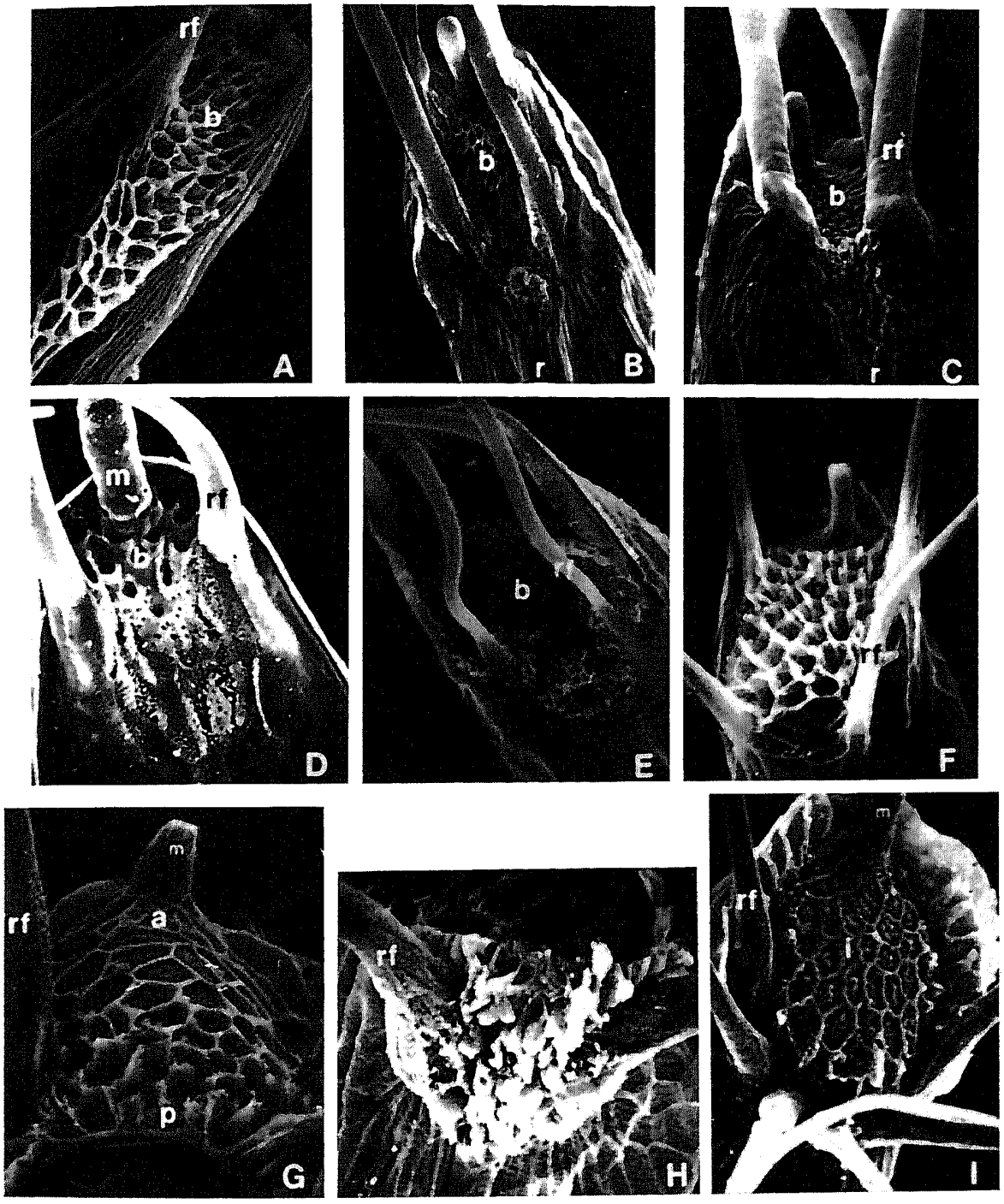

FIG. 7. SEMs of representative *Drosophila* species depicting morphological diversity in operculum region. (A) *D. grimshawi*, broad operculum extending well below bases of respiratory filaments, rf, and characterized by tall thin follicle imprint borders, b, × 355. (B) *D. silvestris*, operculum as in (A) but more restricted. Note beginning of dorsal ridge, r, × 355. (C) *D. clavisetae*, operculum restricted within respiratory filaments, rf, with short thick imprint borders, b. Dorsal ridge, r, connects to operculum, × 372. (D) *D. mulli*, modified operculum with few imprints, b, near micropyle, m, above respiratory filaments, rf, × 816. (E) *D. adunca*, broad operculum extending beyond respiratory filaments, rf, with short thick imprint borders, b, × 227. (F) *D. mojavensis*, broad operculum but restricted within respiratory filaments, rf, tall thin follicle imprint borders, b, × 568. (G) *D. virilis*, broad operculum yet restricted within respiratory filaments, rf, follicle imprints well marked with porous floor and borders short and thick anteriorly, a, but long and thin posteriorly, p, × 745. (H) *D. paulistorum*, extensively modified follicle cell imprint borders, b, below respiratory filaments, rf, × 710. (I) *D. pattersoni*, well-defined operculum within respiratory filaments, rf. Follicle cell imprints, i, well formed with porous floor and short borders which extend and cover micropyle, m, × 588.

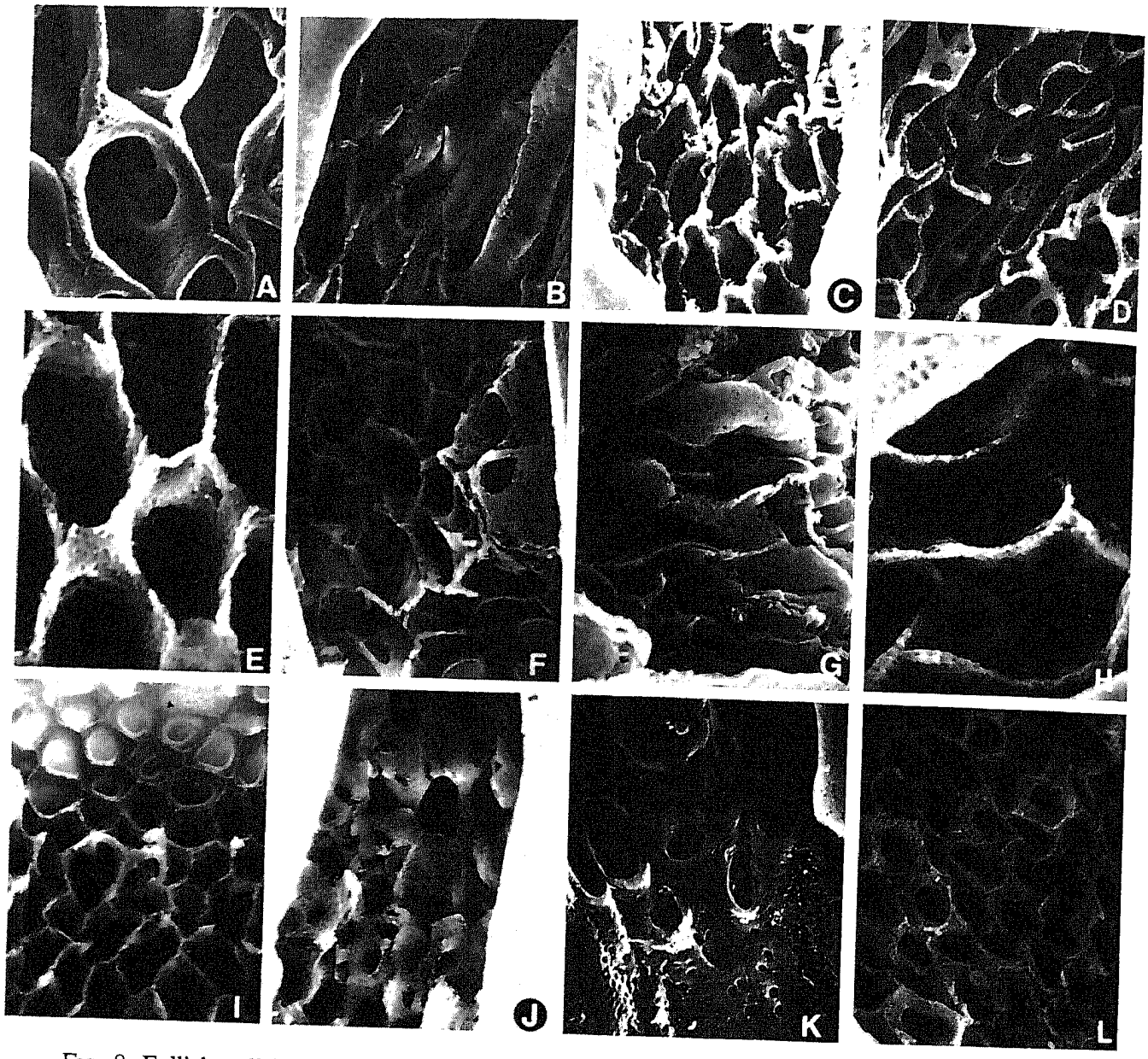

FIG. 8. Follicle cell imprints within operculum region. (A) *D. disjuncta*, tall imprint borders with struts, × 3550. (B) *D. crucigera*, tall and spatulate borders, × 1491. (C) *D. bostrycha*, tall and thin borders, × 1420. (D) *D. fasciculisetae*, tall borders with struts, × 2130. (E) *D. silvestris*, thin, lacy, porous borders, × 3550. (F) *D. heteroneura*, tall, convoluted borders, × 2556. (G) *D. planitibia*, tall, convoluted, porous borders, × 2272. (H) *D. silvarentis*, tall, lacy, porous borders, × 3727. (I) *D. ornata*, note different anterior and posterior morphology, × 1420. (J) *D. primaeva*, tall, thick, spatulate borders, × 2556. (K) *D. mulli*, very short borders and porous floor, × 1633. (L) *D. adunca*, short, thick borders, × 1420.

anterior and posterior regions, with the follicle imprints having distinct morphologies, as, for example, in *D. ornata* (Fig. 8I). In the anterior region near the micropyle, the imprint borders are short and thick, whereas in the posterior region near the dorsal ridge, they are thin and tall. Similarly, in the continental species, the operculum area is rather prominent (Figs 7F–I), and in some species, such as *D. virilis* (Fig. 7G) and *D. paulistorum* (Fig. 7H), the anterior and posterior follicle imprints have distinct morphologies, suggesting that 2 subpopulations of follicle cells are synthesizing the operculum region rather than one, as previously proposed (Margaritis *et al.*, 1983).

There also appears to be extensive differentiation among species and species groups in the fine structure of the imprint borders (Fig. 8), providing additional taxonomic characters.

The function of the operculum area can be inferred from observations of oviposited eggs in their natural substrates, a sample of which is shown in Fig. 5. In the species ovipositing on leaves, the 4 short chorionic filaments retain the egg near the surface of the substrate (Fig. 5F), thus exposing the operculum region to the atmosphere, and consequently making it one of the major areas for gas exchange during embryogenesis, in addition to the respiratory filaments. In such species, e.g. *D. adunca* (Fig. 7E), the operculum area is broad and the borders of the follicle imprints are short and broad (Fig. 8L). In species which oviposit deep within the tree bark (Fig. 5A–D), the operculum is not visible and does not appear to represent a major area for respiratory exchange, that function being carried out mostly by the long respiratory filaments. The reasons for the interspecific variability in the fine structure of the follicle imprint borders of the operculum are not known. However, a correlation appears to exist between the fine structure of the operculum and the respiratory filaments. In species in which the respiratory filaments display a plaque-like surface, the operculum is restricted to within the area of the filament bases, and the borders of their follicle imprints are short and broad. This collection of features is found in all members of the *adiastola* species subgroup (Figs 7C, 8I), and in *D. mimica* and *D. primaeva*. On the other hand, in species with solid respiratory filaments, the operculum has thin and tall follicle imprint borders, as, for example, in members of the *grimshawi* species subgroup (Figs 7A, 8A–C) and the *planitibia* species subgroup (Figs 7B, 8E–G). Undoubtedly, these fine ultrastructural characteristics have evolved to enhance respiration during embryogenesis. Whereas the solid respiratory filaments of the 2nd group (*grimshawi* and *planitibia* species subgroups) provide surfaces suitable for underwater plastron respiration (Hinton, 1969; Margaritis, 1983; Margaritis *et al.*, 1983), the operculum area does not. On the other hand, the plaque-like structures on the respiratory filaments of the first group (the *adiastola* species subgroup) do not facilitate plastron respiration, because they would be flooded when the egg is submerged, as suggested for *D. mimica* (Margaritis, 1983). In these cases, however, the anterior portion of the operculum may permit plastron respiration. It is interesting to note that the oviposition sites used by these 2 groups of species are different (Table 1). These observations seem to suggest that both the operculum and the filaments are part of the embryonic respiratory system, and that the combination of the 2 structures provides more flexibility for adaptation to diverse microhabitats by selective use of one of the structures, or both, to varying degrees. Although the proposed physiological adaptiveness of the operculum/respiratory filaments to particular oviposition substrates will require experimental confirmation, the taxonomic value of operculum characters for tracing phylogenies can readily be demonstrated.

Micropyle

At the anterior end of the operculum is the protruding micropyle (Figs 2, 9), which consists of 2 morphologically distinct regions: the main body which in *D. grimshawi* is 40–50 μm long and 10 μm thick (Margaritis *et al.*, 1983), and the tip with its opening, which actually provides the sperms' entrance into the egg. Although the general micropyle structure is conserved among drosophilids, some interspecific diversity exists in the fine structure of both the main body and the tip of the micropyle (Fig. 9). In both

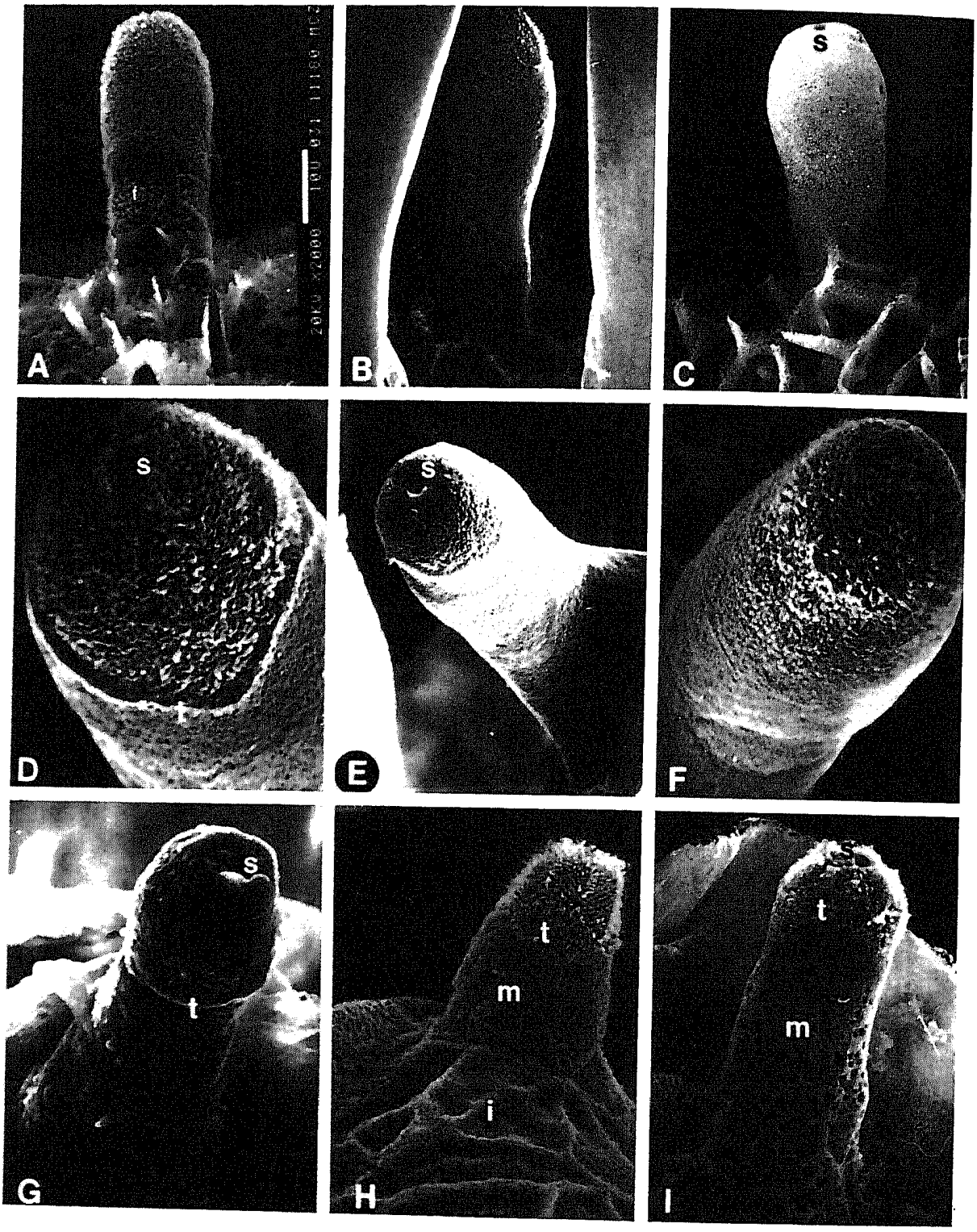

FIG. 9. SEMs of micropyle region from representative *Drosophila* species. (A) *D. gradata*, distinct follicle cell imprints, f, × 1420. (B) *D. sproati*, short fibrils at tip, × 1491. (C) *D. fasciculisetae*, note sperm opening, s, × 2272. (D) *D. sproati*, note boundaries of tip, t, and sperm opening, s, × 5964. (E) *D. spectabilis*, solid surface and distinct sperm opening, s, × 2499. (F) *D. adunca*, short fibrils at tip, × 3195. (G) *D. immigrans*, distinct formation of tip, t, with clear sperm opening, s, × 3727. (H) *D. virilis*, short, porous main body, m, resembling follicle imprint floor, i. Tip, t, with fibrils, × 1789. (I) *D. pattersoni*, very porous main body, m, and distinct tip, t, with short fibrils at sperm opening, s, × 2090.

Hawaiian (Fig. 9A–F) and continental species (Fig. 9G–I), the micropyle is tall. In some species follicle imprints are clearly distinct on the main body (Fig. 9A), while in others, small (Fig. 9D) or large (Fig. 9I) aeropyles are present. The tip region is distinctly demarcated in some species (Fig. 9B, D, G–I), frequently being covered with short or long fibrils (Fig. 9A, B, H, I).

Dorsal ridge

The operculum region in most Hawaiian species is connected to the posterior pole by the dorsal ridge (Fig. 10A). Anteriorly, the ridge is usually broad and convoluted (Fig. 10A–C), while posteriorly from about the middle of the egg, it becomes narrower and protruding (Fig. 10F). Toward the posterior pole in some species, the ridge again becomes broader and fuses with the posterior pole region, while in others the ridge disappears, there being no visible connection to the posterior pole. The dorsal ridge is one of the most variable chorionic structures. In addition to the morphological variation within different regions of the ridge, extensive variation exists among species and species groups. Initially, it was believed that the ridge is only present in Hawaiian *Drosophila* (Throckmorton, 1966; Margaritis *et al.*, 1983). However, our present work (also Piano and Kambysellis, unpublished) shows that not all Hawaiian species of the subgenus *Drosophila* have a dorsal ridge, and the extent of its formation varies among the species groups, as summarized diagrammatically in Fig. 11. The ridge is more prominently displayed in the picture-winged and modified-mouthpart species (Figs 10A–F, 11). The *Antopocerus* species group has a simple restricted ridge that is slightly protruding, starting from the operculum region and terminating just before the posterior pole region (Fig. 11, and unpublished data). No ridge has been observed in the white-tip scutellum flies (Fig. 11), which are also members of the subgenus *Drosophila*, or in the *Scaptomyza*. The data for the few continental *Drosophila* representative of the various subgenera analyzed demonstrate that the dorsal ridge is not present in continental species of the subgenera *Drosophila* (*D. virilis, D. immigrans*), *Scaptodrosophila* (*D. pattersoni*, Fig. 10G) or *Sophophora* (*D. melanogaster*, Margaritis (1983)). Similarly, no ridge was observed in the Sophophorans *D. willistoni* or *D. paulistorum* (Fig. 10H), although anteriorly, just at the end of the operculum region where the dorsal ridge usually starts, there are a few modified follicle imprints. A similar modification was also found in *D. pictiventris* of the subgenus *Hirtodrosophila* (Fig. 11). Whether this structure represents a rudimentary dorsal ridge remains to be verified. The ridge has evolved in the Hawaiian members of the subgenus *Drosophila*, presumably as an adaptation to the ecological constraints imposed by the particular oviposition substrates. The species groups which oviposit in decaying plants have a prominent dorsal ridge, conceivably to provide surface for underwater plastron respiration (Hinton, 1969; Margaritis *et al.*, 1983), ensuring embryonic survival in their typically wet microenvironments. The white-tip scutellum flies, which oviposit on fungi, may have evolved an altogether different respiratory system to accommodate the gases released by the rotting fungi. The subgenus *Exalloscaptomyza*, which oviposits on flowers, also lacks a dorsal ridge.

Posterior pole

In all eggs analyzed of both genera *Drosophila* and *Scaptomyza*, a distinct posterior pole region is present. The detailed morphology of the region, however, varies among taxa. In continental *Drosophila* (e.g. *D. virilis*, Fig. 12G, and *D. paulistorum*, Fig. 12H)

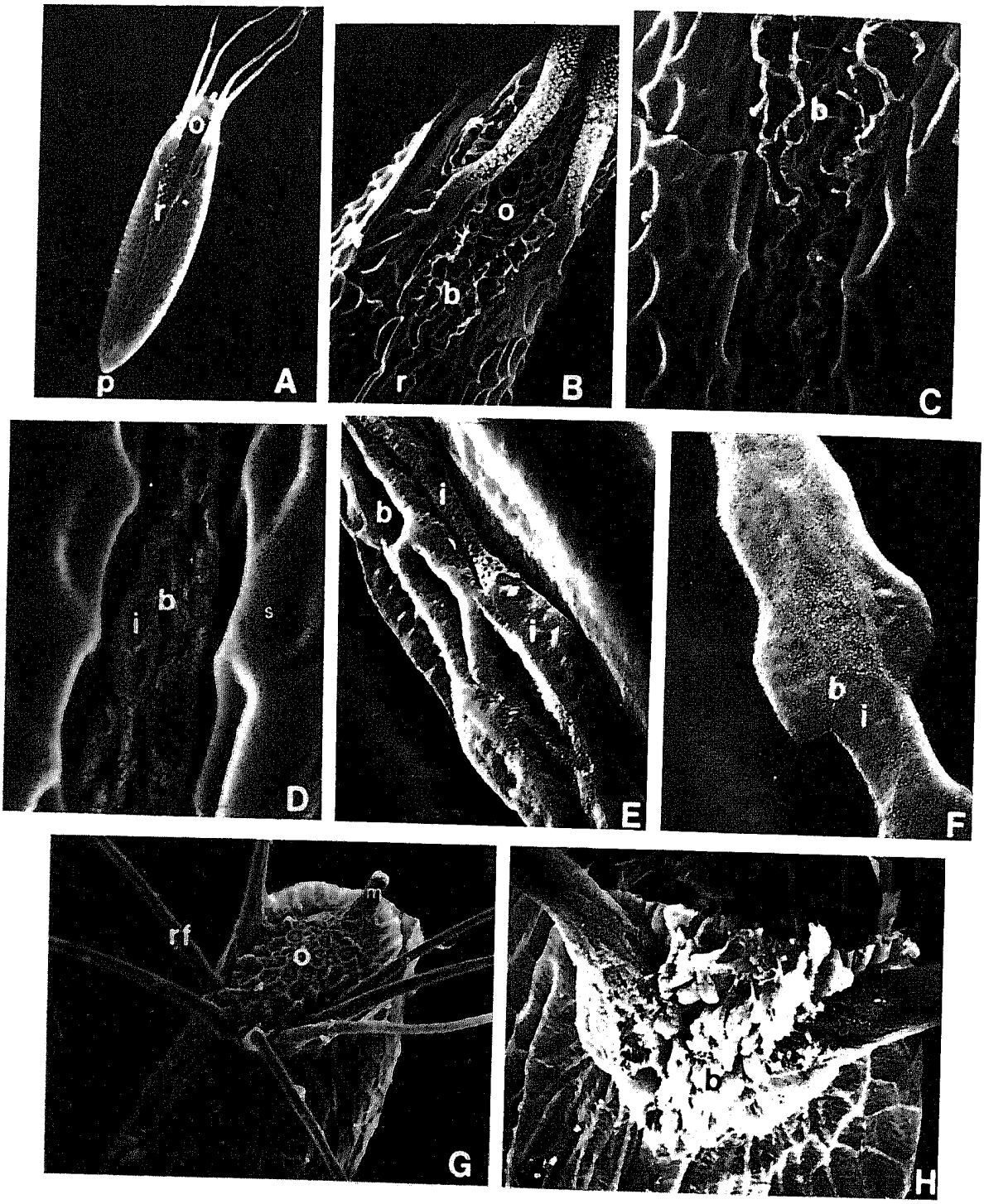

FIG. 10. SEMs of dorsal view of *Drosophila* eggs showing dorsal ridge. (A) *D. mimica*, dorsal ridge, r, connecting operculum, o, and posterior pole, p, region. (B)–(D) Various regions of dorsal ridge in *D. fasciculisetae*. (B) Anterior portion of ridge, r, connected to operculum, o, × 710. (C) Anterior middle region, × 1420. (D) Middle region of ridge, × 1420. (E) *D. grimshawi*, middle region of ridge, × 1420. (F) *D. grimshawi*, posterior region of ridge, × 1420. (G) *D. pattersoni*, dorsal view of egg showing operculum, o, micropyle, m, and respiratory filaments, rf, no dorsal ridge is present, × 340. (H) *D. paulistorum*, dorsal view showing rudimentary dorsal ridge, r, just below operculum region where follicle imprints have elaborate borders, b, × 710. Throughout, follicle imprints are indicated by i, and their borders by b.

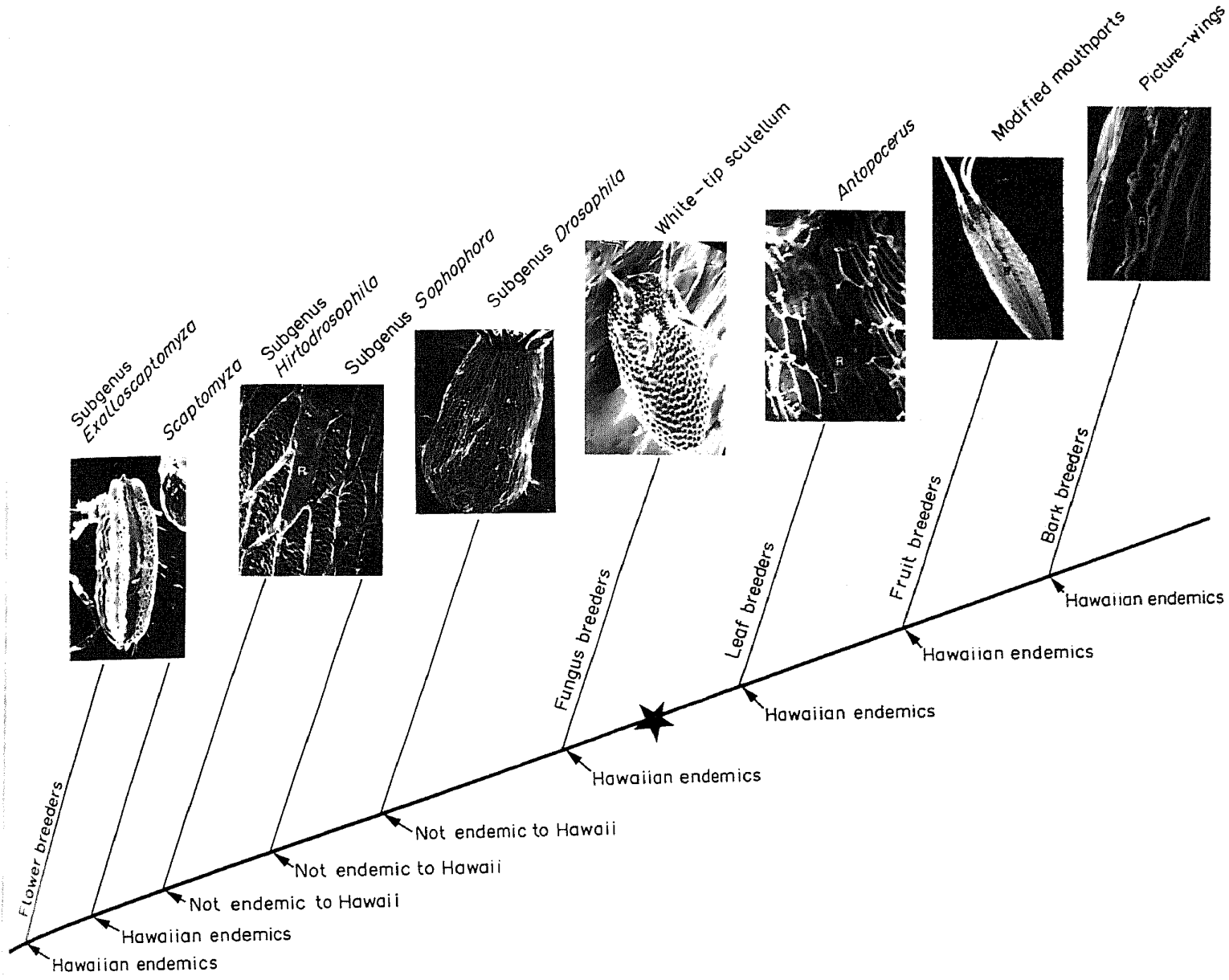

FIG. 11. Summary of phylogenetic variation in form of dorsal ridge among members of genera *Drosophila* and *Scaptomyza*. SEMs from representative species are as follows from left to right: *Scaptomyza* (*Tantalia*) *albovittata*, *D.* (*Hirtodrosophila*) *pictiventris*, *D.* (*Scaptodrosophila*) *pattersoni*, *D.* (*Drosophila*) *dolichotarsus*, *D.* (*Drosophila*) *diamphidiapoda*, *D.* (*Drosophila*) *infuscata*, *D.* (*Drosophila*) *bostrycha*. Taxonomic grouping of Hawaiian endemics is indicated above micrographs, and their breeding substrates, where known, are shown below. In groups to right of asterisk, dorsal ridge, R, is well formed; in those to left, it is rudimentary, R, or not formed at all.

a small group of follicle cells (8–15) appears to form the chorion of the pole region, with distinctive follicle cell imprints, and a small or large pore in the centre. In the *Scaptodrosophila* (*D. pattersoni*; Fig. 12I), 2 rows of imprints are present, one centrally located, having no distinct borders, with one or more large pores and numerous smaller ones. Peripheral to these, is a 2nd row of imprints with distinct borders and only the small pores.

In the Hawaiian *Drosophila*, the posterior pole region varies extensively in the number of cells forming the region, their distribution and their morphology (Fig. 12A–F). In

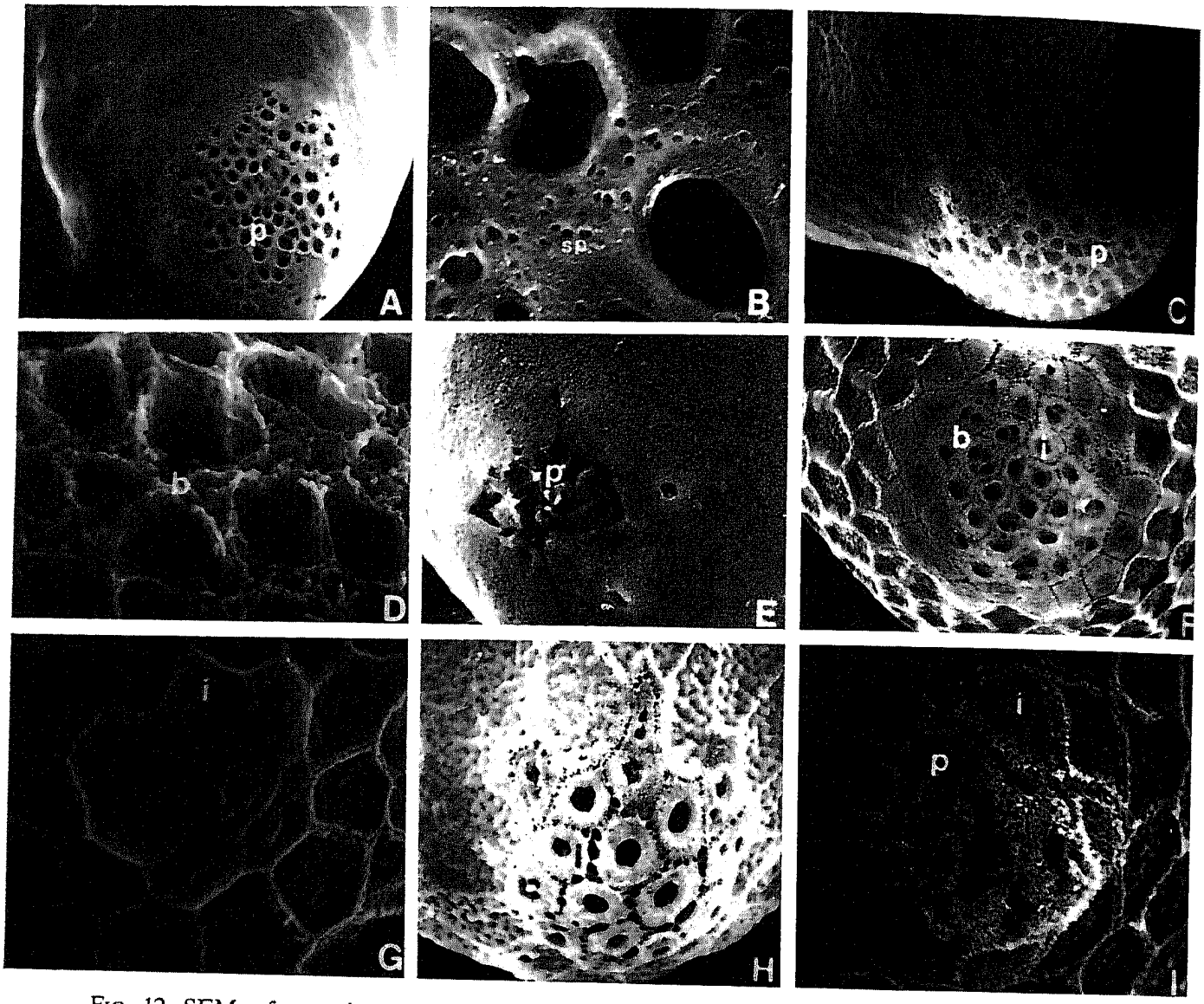

FIG. 12. SEMs of posterior pole region. (A) *D. murphyi*, note asymmetrical position of pores, p, × 710. (B) *D. murphyi*, high magnification of pores showing numerous smaller ones, sp, × 7100. (C) *D. formella*, note protruding region, p, with follicle cell imprints covering entire area, × 1342. (D) *D. formella*, high magnification of posterior pole region showing tall, thin, porous follicle imprint borders, b, × 6710. (E) *D. truncipenna*, note restricted region with fused pores, p, × 1136. (F) *D. primaeva*, note distinct follicle imprints, i, most with one or 2 large pores and porous imprint borders, b, × 710. (G) *D. virilis*, few distinct posterior follicle imprints, i, with a small central pore, × 1207. (H) *D. paulistorum*, distinct follicle cell imprints, i, with a large central pore and porous borders, × 1420. (I) *D. pattersoni*, note distinct peripheral follicle cell imprints with very porous floor and borders, i, and fused central imprints with few large pores, p, × 1427.

some species, they are asymmetrically located with no distinct follicle imprint borders (Fig. 12A); in others, they are protruding (Fig. 12C), or fused (Fig. 12E), or the imprint borders are marked by a series of small pores with one or more larger pores in the center of the imprint (Fig. 12F). The total number of large central pores in the pole region varies among species from 3 in *D. adunca* (Fig. 13L) to more than 200 in *D. formella* (Fig. 12C). In the latter species, the posterior pole is rather complex with tall and lacy porous imprint borders (Fig. 12C, D). At higher magnification (Fig. 13), it appears that the pores are rather deep, sometimes arranged in a circular manner (Fig. 13B, C), with some exhibiting complex internal structures such as multilayer pillars (Fig. 13D–I, K).

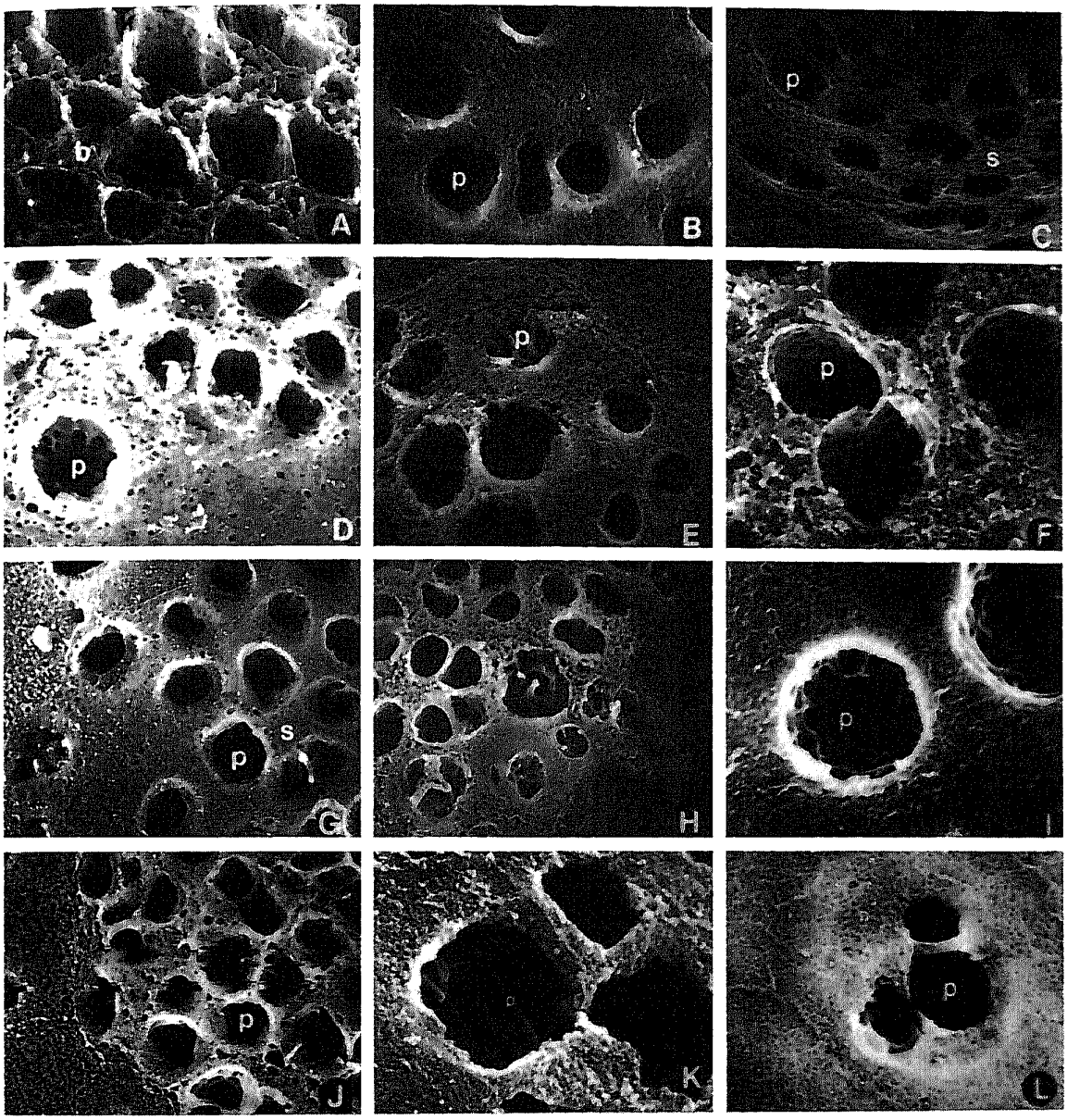

FIG. 13. SEMs of posterior pole region showing detailed structure of pores. (A) *D. formella*, tall, thin, porous imprint borders, b, × 6710. (B) *D. sproati*, smooth surface with large deep pores, p, × 5680. (C) *D. engyochracea*, numerous large pores, p, floor surface, s, with many small pores, × 3692. (D) *D. claytonae*, note internal structure of large pores, p, and numerous small pores, × 3550. (E) *D. crucigera*, note variable internal structure of large pores, p, × 3550. (F) *D. fasciculisetae*, note rather porous surface, s, between large pores, p, × 5325. (G) *D. silvestris*, smooth porous surface, s, between large deep pores, p, × 3550. (H) *D. heteroneura*, similar to *D. silvestris*, × 2840. (I) *D. planitibia*, note internal pillars within large pores, p, × 7100. (J) *D. cyrtoloma*, numerous large pores, p, × 3372. (K) *D. ornata*, pillars, p, within large pores are distinct, × 7100. (L) *D. adunca*, pores fused to form only 3 large deep pores, P, in the center, with many very small pores, p, in surrounding area, × 6035.

The posterior pole region protects a vital region of the embryo, the site of formation of the prospective germ cells, and this portion of the embryo may have higher respiratory needs during the dynamic events of early embryogenesis. Since this portion of the egg (at least in the Hawaiian species) is continuously subject to rather moist or wet environments, the respiratory mechanism probably resembles that of aquatic insects (Hinton, 1969).

Follicle cell imprints of the main body

The follicle cell imprints covering the main body of the eggshell constitute one of the most variable characters. The basic pattern in Hawaiian species is a series of hexagonally-shaped cells covering the entire egg surface (Fig. 2). In some species, however, as in members of the *planitibia* subgroup, the imprints are regionally distinct, present only in the ventral (both anterior and posterior) region and at the boundaries of the dorsal ridge (data not shown, Kambysellis *et al.*, unpublished). In others, the imprints are lacking altogether, as in *D. grimshawi* and *D. crucigera* (Margaritis *et al.*, 1983; Kambysellis, unpublished). The morphology of the imprints is consistent among individuals of a species, but varies among species.

This divergence is exemplified by the island populations of *D. grimshawi*, an unusual Hawaiian species in that it is not endemic to just a single island, as in the case of most Hawaiians, but has populations on each of the islands. The exception is the island of Hawaii where the homosequential species *D. pullipes* is found, and even this form was initially thought to belong to the species *D. grimshawi* (Hardy and Kaneshiro, 1972). Analyses of the chorion of these 6 populations, have shown that the follicle imprints and some other chorionic structures are distinctive among populations. The East Maui population initially analyzed has no follicle imprints (Margaritis *et al.*, 1983). The Oahu and Kauai populations have well-formed imprints (Piano *et al.*, 1988), while eggs of the West Maui, Lanai, and Molokai populations have intermediate forms (unpublished). In addition to the diverse chorionic structures, behavioural differences and diverse ecology (Ohta, 1978), and differences in DNA sequence of the vitellogenin genes (Kambysellis, unpublished data) suggest extensive evolutionary divergence of these populations, requiring reevaluation of their taxonomic status.

Follicle imprints were scored from the ventral, middle–anterior region of the egg. The floor of the imprints varies in structure, and in the presence, number and size of pores or aeropyles (Fig. 14A, E–H). In some species, as in *D. macrothrix*, the entire surface of the chorion is covered with spike-like structures (Fig. 14C, D). The precise morphology of these protrusions varies among the species from spikes to spheres; these appear to have been independently derived in members of diverse taxonomic groups.

The borders of the imprints are also variable (Kambysellis, 1973). In some species, these are tall and lacy (Fig. 14A, B), while in others they are short and narrow (Fig. 14G). The variability is so extensive that we have not seen any 2 *Drosophila* species sharing the same follicle cell imprint pattern. The morphology of follicle imprints from hybrids between related species in the *planitibia* subgroup indicates multigenic inheritance (Kambysellis, 1974).

The continental *Drosophila* have a similar but less variable follicle imprint pattern (Fig. 14H). This is particularly so for the imprint borders, which, unlike the elaborate structures in Hawaiian species, tend to be short and narrow, resembling the *antopocerus*

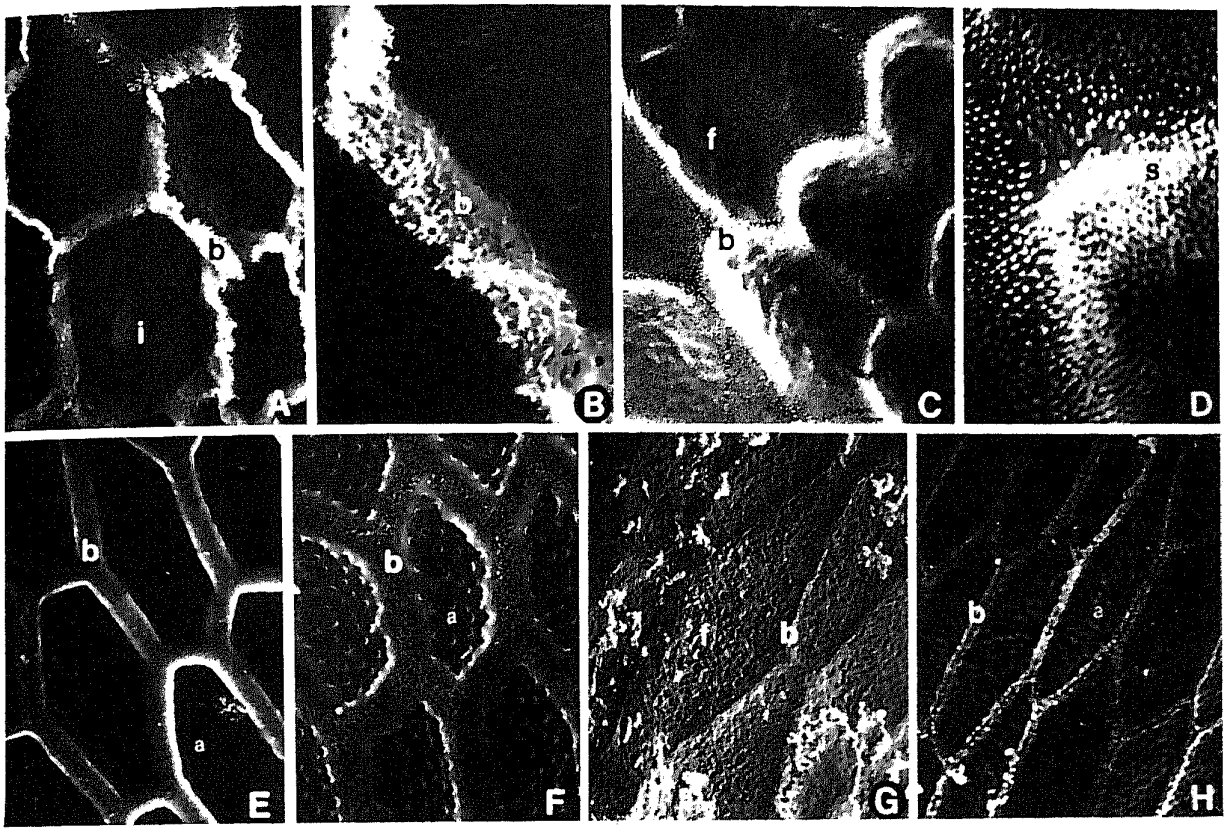

FIG. 14. SEMs of follicle imprints from *Drosophila* species. (A) *D. silvarentis* egg showing imprints, i, with solid floor and tall, thin, lacy cell borders, b, × 1728. (B) Higher magnification of lacy borders, b, in *D. silvarentis*, × 6000. (C) *D. macrothrix*, note that floor, f, and borders, b, of follicle imprints are covered with spike-like structures, × 1980. (D) Higher magnification of follicle imprint borders showing distinct spike-like structures, s, × 6600. (E) *D. silvestris*, follicle imprints showing solid borders, b, and floor with few small aeropyles, a, × 1854. (F) *D. primaeva*, note short and broad imprint borders, b, and floor with many large aeropyles, a, × 1200. (G) *D. adunca*, imprints with narrow short borders, b, and textured floor, f, × 1440. (H) *D. pattersoni*, narrow short borders, b, and numerous aeropyles, a, on floor, × 1140.

species group of Hawaiian *Drosophila* (Fig. 14G). This appears to be the primitive state of the character for the genus *Drosophila*. The *Scaptomyza* have not been extensively analyzed, yet from the few species studied, it appears that the imprints are not elaborate, resembling the primitive state for the character. The *Exalloscaptomyza* eggs have very faint follicle cell imprints with narrow and very short borders, almost invisible at lower magnifications (data not shown).

Ventral rim

The hatching region or "collar" presents another useful chorionic character for identifying *Drosophila* species groups. In the complex hatching region from which the larva escapes, several structures have been recognized (Margaritis, 1983). Here, we only describe one of these at the ventral anterior end of the chorion, a morphologically variable region that we call the "ventral rim" of the collar (Fig. 15). Among the Hawaiian species of the picture-winged, the modified mouthparts and the *antopocerus* groups, the ventral rim is a clear, compact, semicircular area devoid of follicle imprints or other structures (Fig. 15A, B). It extends from the ventral anterior tip of the egg, runs

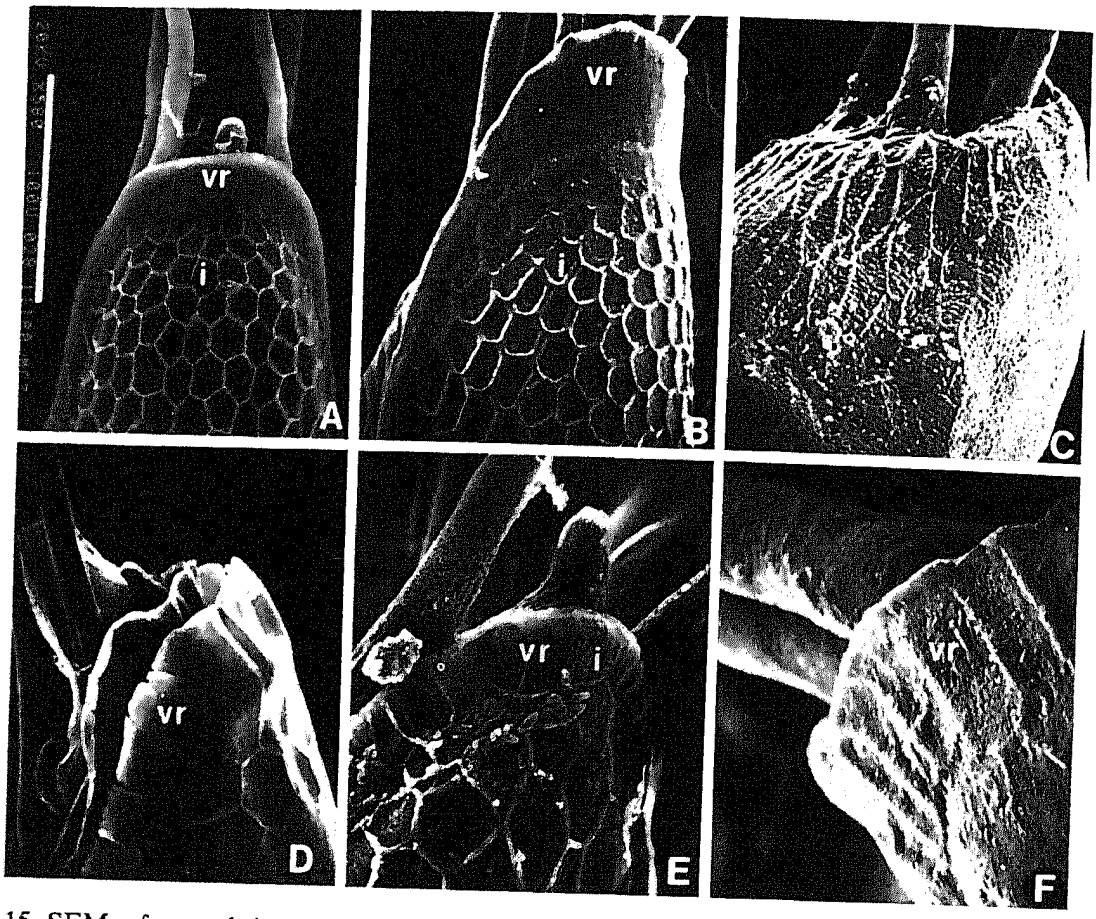

FIG. 15. SEMs of ventral rim of larval hatching region. (A) *D. gradata,* note broad, smooth surface of ventral rim, vr, with no follicle imprints, i, × 348. (B) *D. hanaulae*, oversized ventral rim, vr, × 528. (C) *D. pictiventris*, ventral rim is absent and follicle imprints, i, extend up to anterior edge, × 540. (D) *D. immigrans*, distinctly modified ventral rim, vr, × 1104. (E) *D. virilis*, distinct but modified ventral rim, vr, with some follicle imprints, i, present, × 630. (F) Oviposited egg of an unidentified species collected from a decaying fern in Kipuka 9, Hawaii. Ventral rim, vr, is present but modified. It does not resemble that in any Hawaiian endemic and is probably from an introduced *Drosophila* species.

posteriorly along the lateral sides of the egg, by the edge of operculum area, up to the level of the bases of the posterior respiratory filaments, covering about two-thirds of the hatching region (Fig. 5H). Although always present in the species groups mentioned, the extent of the ventral rim varies among species, as illustrated in Fig. 15A, B. Members of the white-tip scutellum group have no ventral rim and the anterior ventral tip is covered with the typical follicle cell imprints found in the remainder of the chorion (Fig. 16). Among the continental *Drosophila*, the ventral rim is not present in the subgenera *Sophophora* or *Hirtodrosophila* (Fig. 15C), but it is found, although somewhat modified, in the subgenus *Drosophila* (Figs 15D, E). Species of the genus *Scaptomyza* also lack this structure. Figure 16 shows a diagrammatic representation of the species groups possessing a clear ventral rim. It is interesting to note that once again the white-tip scutellum species diverge from the remainder of the Hawaiian *Drosophila*.

The ventral rim may be present in other *Drosophila* groups as well. The detailed drawings of eggs in Okada's monograph (1968) suggest that some Asiatic species, like *D. alboralis* and *D. quadrivittata* of the subgenus *Hirtodrosophila*, may also possess this structure, but this needs to be verified from scanning electron micrographs.

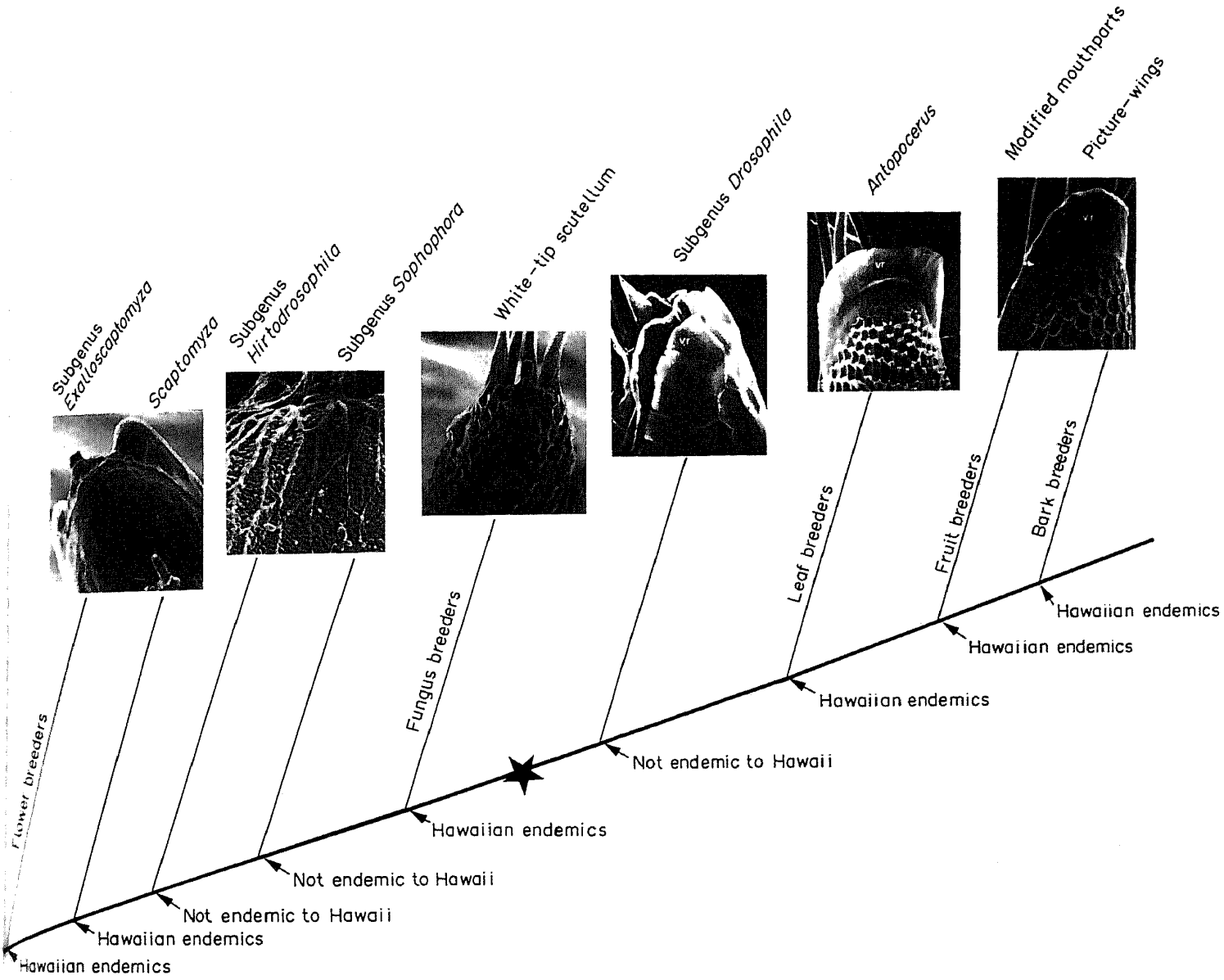

FIG. 16. Summary of phylogenetic variation in form of ventral rim, vr, among members of genera *Drosophila* and *Scaptomyza*. SEMs from representative species are as follows from left to right: *Scaptomyza* (*Tantalia*) *albovittata*, *D.* (*Hirtodrosophila*) *pictiventris*, *D.* (*Drosophila*) *dolichotarsus*, *D.* (*Drosophila*) *virilis*, *D.* (*Drosophila*) *diamphidiapoda*, and *D.* (*Drosophila*) *hanaulae*. Taxonomic grouping of Hawaiian endemics is indicated above micrographs, and their breeding substrates, where known, below. Taxonomic groups to right of asterisk have a ventral rim; those to left lack this structure.

Cross-section of the chorion

The structures typically seen in a scanning electron microscope cross-section of the chorion are the inner endochorion, the pillars and the outer endochorion (Margaritis *et al.* (1983) and Fig. 17). The thickness of the inner endochorion is comparable in all species. The height and numbers of the pillars appear to show some interspecific variability, but are difficult to quantify, because variability appears to exist even among different regions of the egg. The outer endochorion shows the most striking variability. The picture-winged species and the few modified-mouthpart species analyzed all have a

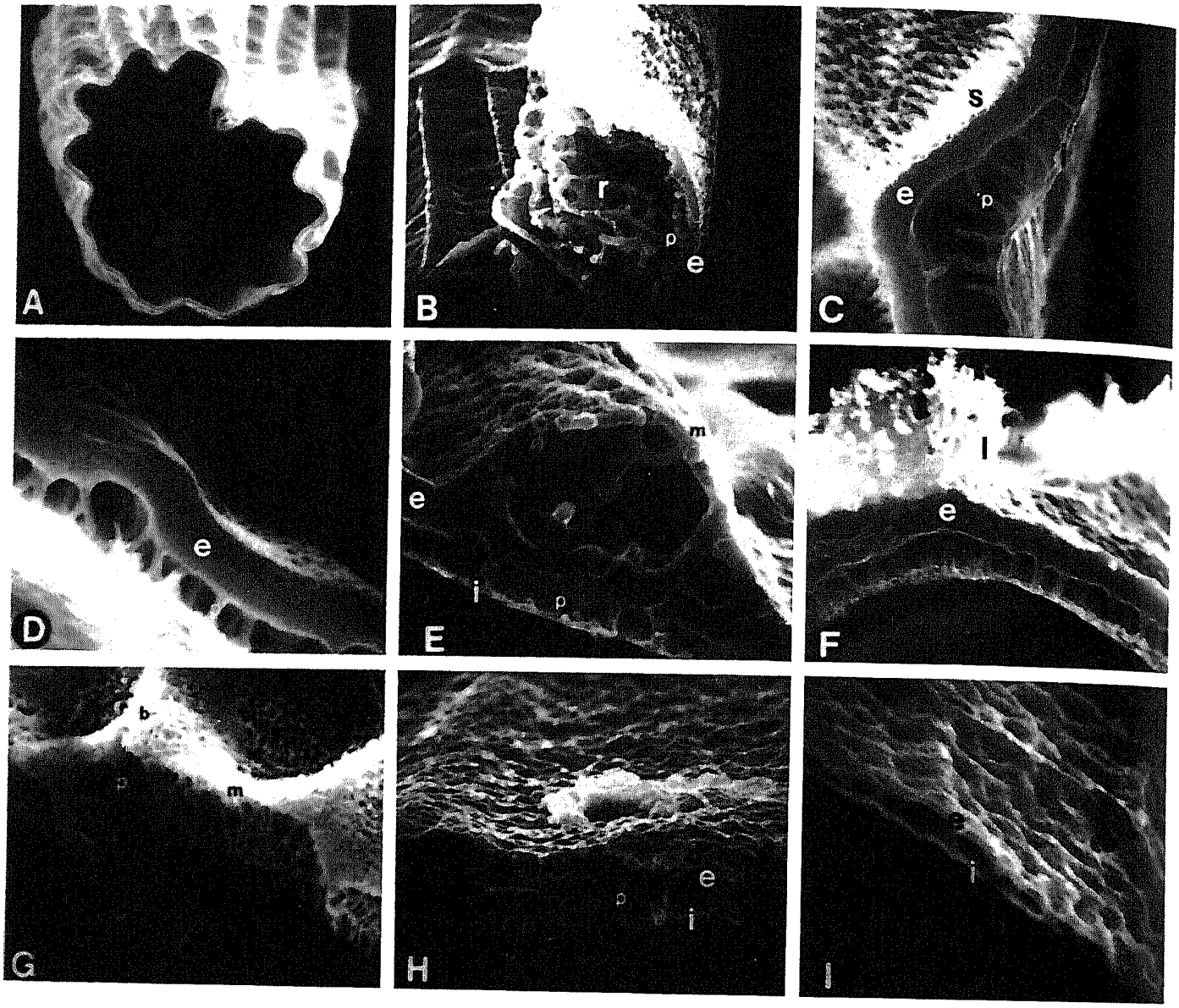

FIG. 17. SEMs of cross-section of various chorionic regions. (A) *D. grimshawi*, cross-section of whole egg showing dorsal ridge, r, and wavy surface of chorion, × 355. (B) *D. crucigera*, section of dorsal ridge, r. Note outer endochorion, e, and extensive respiratory channels formed by interconnected pillars, p, × 2698. (C) *D. fasciculisetae*, cross-section at border of follicle imprints. Note thick outer endochorion, e, covered with spikes, s, thin inner endochorion, i, and tall thin pillars, p, × 2840. (D) *D. clavisetae*, note thicker outer endochorion, e, and short pillars, p, × 7100. (E) *D. silvestris*, cross-section through dorsal ridge showing inner endochorion, i, pillars, p, and outer endochorion, e. Note that outer endochorion in dorsal ridge region is divided into outer, e, and outermost, m, endochorion layers. Pillars between these 2 layers are tall and branched, × 6390. (F) *D. silvarentis*, cross-section through follicle imprint border. Note that tall lacy structure, I, is an expansion of the outer endochorion, e, × 7100. (G) *D. dolichotarsus*, note multiple layers of pillars, p, replacing solid outer endochorion. Outermost endochorion, m, is clearly visible at follicle imprint borders, b, × 1349. (H) *D. adunca*, thin inner, i, and outer, e, endochorion with thick pillars, p, × 8520. (I) *D. willistoni*, note thin inner, i, and outer, e, endochorion typical of all continental species, × 7100.

rather thick outer endochorion (Fig. 17C, D), similar to that of *D. grimshawi* (Margaritis *et al.*, 1983). Species of the white-tip scutellum group have a highly modified outer endochorion. Unlike the solid one found in the picture-winged species, they have a very open, scaffold-like structure with several layers of interconnecting pillars, and the outside covered with a thin porous layer of endochorion we call the "outermost endochorion" (Fig. 17G). The number of layers of pillars and the thickness of the outermost endochorion vary among species (data not shown). The *antopocerus* group have a thin outer endochorion equivalent to the inner endochorion (Fig. 17H). The continental *Drosophila* and the *Scaptomyza* have a very thin outer endochorion as in *D. melanogaster* (Margaritis *et al.*, 1980) or *D. willistoni* (Fig. 17I). A diagram showing the thickness of the outer endochorion in various taxonomic groups is presented in Fig. 18.

Cross-sections at the follicle imprint borders show that they are formed either by elevation of the outer endochorion (Fig. 17C, D), or by addition of new proteinaceous material on top of the existing outer endochorion (Fig. 17F). Cross-sections through the dorsal ridge show that it is formed on top of the outer endochorion, and consists of a series of complex pillars (Fig. 17B), or pillars covered with an additional layer of outermost endochorion (Fig. 17E).

The thickness of the outer endochorion appears to correlate to both the oviposition substrate, and the duration of embryonic development. Species with a thick outer endochorion oviposit in decaying trees (Fig. 18), and have a prolonged period of embryogenesis. Species with a thin outer endochorion oviposit in decaying leaves or flowers, and have a brief embryogenesis. The flower-breeders, *Exalloscaptomyza*, are extreme cases, embryogenesis occurring while the egg is in the common oviduct; the larva hatches soon after oviposition or even before (i.e. larviposition). These observations suggest that the major function of the outer endochorion may be mechanical protection for the embryo.

The white-tip scutellum species constitute a special situation. They oviposit on fleshy fungi, some of which are epiphytic on endemic trees and release ammoniacal vapors. Apparently, as a response to this hostile environment, such species have evolved these elaborate structures of the outer and the outermost endochorion to facilitate respiration. Further studies are needed to understand their mode of respiration and to demonstrate the relationships between type of fungus substrate and chorion morphology.

Phylogenetic considerations

The value of specific chorionic characters, e.g. the dorsal ridge (Fig. 11), the ventral rim (Fig. 16), and the outer endochorion (Fig. 18), in assigning species to taxonomic groups has been suggested above. In addition, detailed analysis of all the chorionic structures provides useful information for phylogenetic reconstruction. In fact, towards that effort, in an analysis to be presented elsewhere, 26 characters were identified from the 8 chorionic regions analyzed (Fig. 3), resulting in a total of 122 possible character states. A computer analysis using parsimony (Swofford, 1990) produced 3 equally parsimonious phylogenetic trees. The consensus tree agrees well with phylogenies derived from polytene chromosome inversion differences (Carson, 1992). The species in the subgroups *grimshawi, planitibia,* and *adiastola*, for example, form distinct clades as do the *antopocerus* and white-tip scutellum species. There is a discrepancy in the *hawaiiensis* subgroup, in that the homosequential species *D. silvarentis* and *D. heedi* do not cluster together, possibly a reflection of their distinct ecological microhabitats (Table

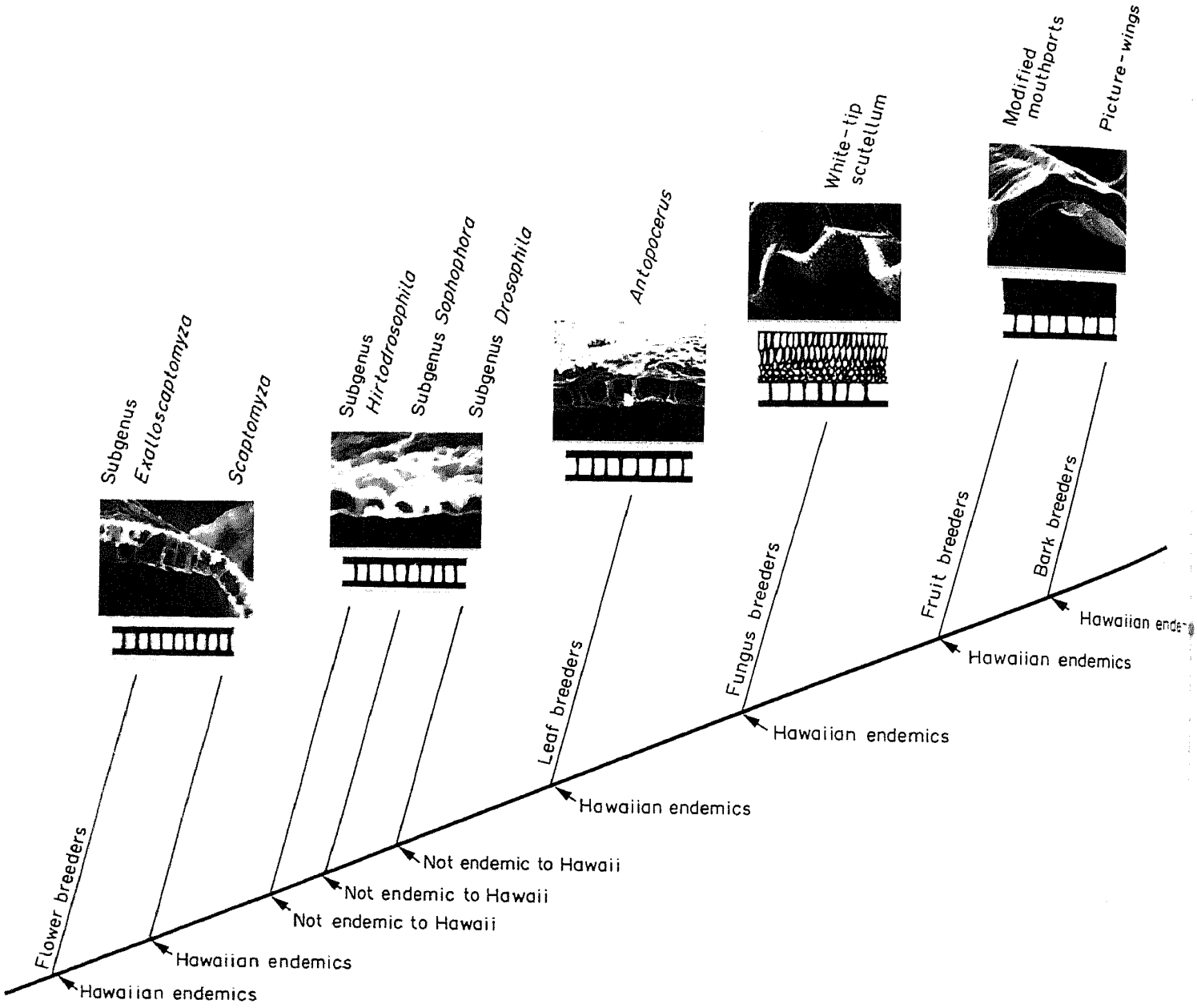

FIG. 18. Diagram of cross-section of chorion showing variation in relative thickness of outer endochorion among members of genera *Drosophila* and *Scaptomyza*. SEMs from representative species are as follows from left to right: *Scaptomyza* (*Tantalia*) *albovittata*, *D.* (*Hirtodrosophila*) *pictiventris*, *D.* (*Drosophila*) *adunca*, *D.* (*Drosophila*) *dolichotarsus*, and *D.* (*Drosophila*) *disjuncta*. Taxonomic grouping of Hawaiian endemics is indicated above micrographs, and drawings of cross-section below. Breeding substrates, where known, are indicated.

1 and Kaneshiro *et al.* (1973)). Despite this, chorionic ultrastructural characters are clearly important in studying evolutionary relationships among the Drosophilidae.

Acknowledgements – The author wishes to thank Dr Ken Kaneshiro for his generous support in providing facilities at the University of Hawaii, for coordinating the field trips and helping in some collections, and for verifying or identifying the Hawaiian species analyzed. Tina Dellas' superb S.E.M. expertise resulted in most of the photomicrographs. Fabio Piano contributed his computer expertise for the phylogenetic analysis and also accumulated some of the data. Dr Monroe Yoder of New York University was instrumental in training both Dellas and Piano in S.E.M. techniques and also took some of the micrographs. Dr Elysse Craddock of SUNY

Purchase who has contributed generously to many facets of this work, including the field collections, stimulating discussions of the results, and preparation of the manuscript, is especially acknowledged. Thanks also to Dr Lukas Margaritis of the University of Athens, Greece, for inviting the author to participate in the Symposium on Ultrastructural and Molecular Architecture of Egg Chorion at the XIX International Congress of Entomology in Beijing, at which this paper was presented. This research was supported in part by NSF grant PCM-7913074, and a New York University Research Challenge Fund award.

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