Floral development of the divergent endemic Hawaiian genus *Schiedea* (Caryophyllaceae), with special emphasis on the floral nectaries

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Abstract   The endemic Hawaiian genus *Schiedea* has among the most architecturally complex flowers of any genus of Caryophyllaceae. To understand the developmental basis of the unusual floral features that characterize *Schiedea*, we studied the floral organogenesis of 6 of the 34 species of the genus using SEM, supplementing these observations with a focused investigation of nectary histology. The early ontogeny of *Schiedea* flowers is like that of many other Caryophyllaceae in that: (1) the five sepals are initiated in a 2/5 spiral, with the first, antesepalous whorl of stamens initiated in an imperfectly contrarotating sequence relative to the sepals; (2) the alternisepalous stamen whorl initiates slightly external to and at a lower level on the floral apex than the antesepalous whorl; and (3) petal primordia, if initiated (rare), arise on a primordium common with an alternisepalous stamen. Hence, most floral features that distinguish the genus, such as the (often) reflexed sepals and elongate stamen filaments, arise late in floral ontogeny. The only unique trait to *Schiedea* flowers are the elongate, tubular nectary extensions. They are antesepalous and arise from the abaxial surface of a ring-shaped proliferation of tissue that surrounds the bases of the developing stamens. The shape of each nectary in early ontogeny is highly similar to the mature "swallow's nest" nectaries present in many genera of a major crown clade of Caryophyllaceae (Pleurcaryophyllaceae, containing *Schiedea*), suggesting that the development of the needle- to scale-like nectary extensions of *Schiedea* might represent terminal additions onto a "swallow's nest" nectary ontogenetic program.

Keywords   Caryophyllaceae; floral development; floral dimorphism; floral nectary; Hawaii; *Schiedea*

■ INTRODUCTION

The endemic Hawaiian genus *Schiedea* Cham. & Schltdl. (Caryophyllaceae tribe Sclerantheae; Harbaugh & al., 2010; Greenberg & Donoghue, 2011), with 34 species (32 extant), comprises the fifth largest radiation of Hawaiian angiosperms (Wagner & al., 2005; Baldwin & Wagner, 2010). Recent molecular phylogenetic studies indicate that *Schiedea* is monophyletic and closely related to the northern boreal, perennial herbaceous genera *Honckenya* Ehrh. and *Wilhelmsia* Rchb. (both monotypic), suggesting the genus arose in situ, subsequent to a single dispersal event to the archipelago (Nepokroeff & al., 2005; Baldwin & Wagner, 2010; Harbaugh & al., 2010). Willyard & al. (2011) estimated the radiation of the crown clade of the genus to have commenced a maximum of 6.83 million years before present, during which time *Schiedea* underwent remarkable morphological diversification. Similar to other outstanding angiosperm examples of adaptive radiation in the Hawaiian Islands, several novel growth forms evolved within *Schiedea*, including scandent and secondarily woody species, facilitating niche diversification (Weller & al., 1990, 1995; Wagner & al., 1995, 2005; Sakai & al., 2006). Perhaps even more remarkable is the extent of reproductive trait diversification in *Schiedea*. *Schiedea* species exemplify the widest range of breeding systems known in the Hawaiian flora, which are associated with a diversity of pollination syndromes (Weller & al., 1998). Variation in these traits within *Schiedea* is largely mediated by corresponding differences in floral structure, which is considerably diverse in *bauplan* (body plan) and, especially, architecture (Fig. 1).

The flowers of most *Schiedea* species are individually small and inconspicuous—an impression accentuated by their characteristic lack of petals and often strongly reflexed sepals. Most *Schiedea* species have a floral bauplan of five free sepals, two whorls of five stamens (the outermost whorl alternisepalous), and a superior, eusyncarpous gynoecium of three to five (infrequently to 11) carpels with free styles (Wagner & al., 2005). Flowers so constructed are found occasionally in Caryophyllaceae (McNeil, 1962; Bittrich, 1993), but distinguishing the genus are the five, long-tapering tubular structures that radiate out from near the center of each flower, imparting to it a star-like appearance (Fig. 1A, B, D). These structures are a unique synapomorphy for *Schiedea*. In the original description of *Schiedea*, Chamisso & Schlechtendal (1826) interpreted them as petals—probably on account of their position between the calyx and androecium, notwithstanding their placement opposite, not alternate, with the sepals. Even so, most subsequent workers up to the late 20th century interpreted these tubular structures to be staminodia with basal nectaries (Fenzl, 1833; Gray, 1854; Pax & Hoffmann, 1934; Sherff, 1945; McNeill, 1962). Having the advantage of making observations from living material, St. John (1970) interpreted these structures to be extensions of the floral nectaries; his perspective has been sustained to present (Wagner & al., 1990, 1995, 2005;

Weller & al., 1995; Wagner & Harris, 2000). Nectar is delivered through these needle-like tubes and presented as drops at their apices (Fig. 1A, D). Thus, the function of these enigmatic floral organs is clear and justifies the terminology currently applied to them. However, their identity/homology remains poorly understood and structural information that could help clarify this problem is scarce (see Wagner & Harris, 2000). Structural and developmental differences between the tubular nectaries described above and the scale-like nectary extensions that characterize the four species contained in the clade inclusive of *Schiedea* sect. *Nothoschiedea* H. Mann and sect. *Alsinidendron* (H. Mann) Pax & K. Hoffm. (Fig. 1C; previously contained in the genus *Alsinidendron* H. Mann) are also obscure.

Schiedea flowers were probably ancestrally hermaphroditic and dependent on biotic pollen vectors to effect pollination (Weller & al., 1995; Nepokroeff & al., 2005; Sakai & al., 2006). Transitions to dimorphic flowers, associated with gynodioecous to dioecious breeding systems and a shift to wind pollination and dry habitats, occurred at least twice within the genus (Weller & al., 1995; Nepokroeff & al., 2005; Willyard & al., 2011). Although *Schiedea* serves as a model system for understanding the evolution of breeding systems in the Hawaiian flora (Sakai & Weller, 1991; Weller & al., 1995, 1998, 2001, 2006, 2007; Golonka & al., 2005), the developmental basis of floral dimorphism in the genus remains unknown.

Because of the biological significance of the unusual floral nectaries and presence of dimorphism in flowers of *Schiedea*, we analyzed the floral ontogeny of selected species to gain an understanding of these traits from a developmental morphological perspective.

■ MATERIALS AND METHODS

The complete floral ontogeny of four species of *Schiedea* was studied with the scanning electron microscope (SEM). Each of these species serves as an exemplar of a major infrageneric clade identified in phylogenetic studies of the genus
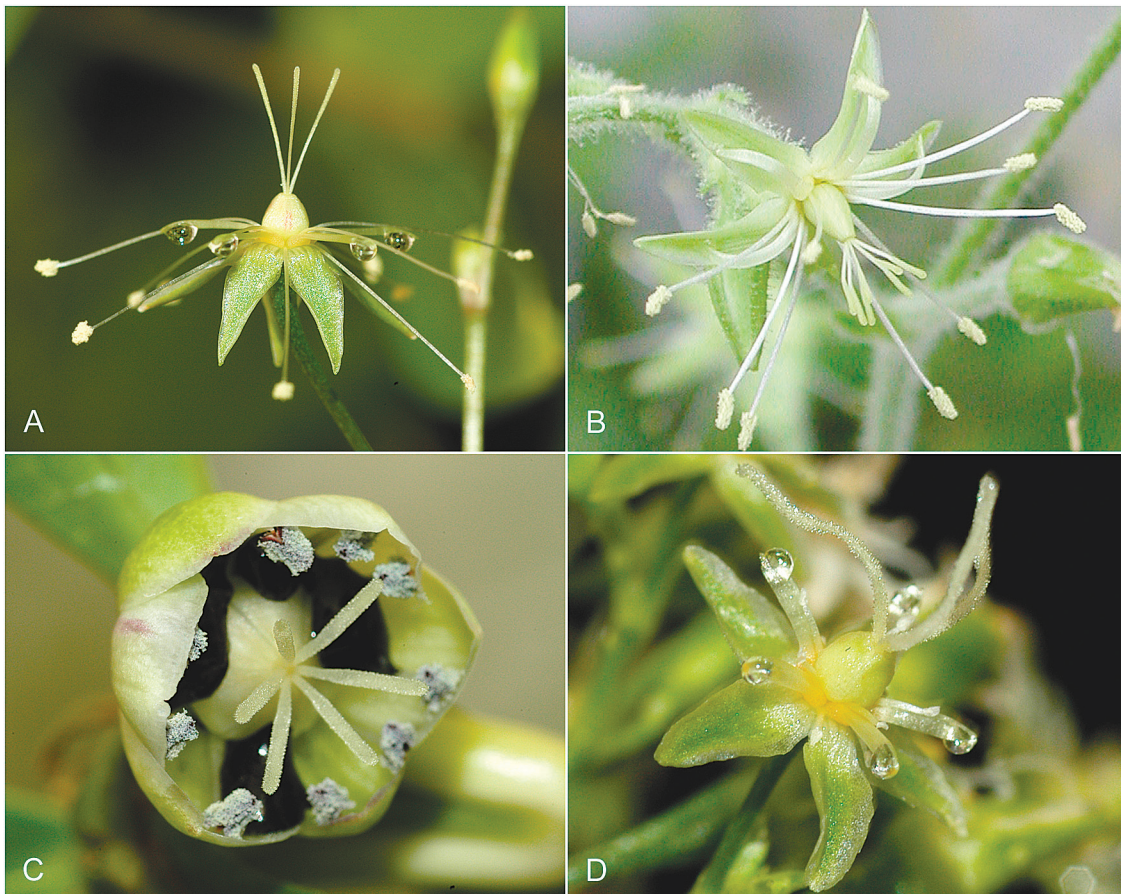

Fig. 1. Anthetic flowers of *Schiedea*, showing four types of nectar presentation resulting from modifications in breeding system and related changes in nectary extensions. **A,** *Schiedea lydgatei* Hillebr. (3 Mar. 1989, *Weller & Sakai s.n.* [cult. *Weller & Sakai 870*]), example of a typical hermaphroditic outcrossing species with straight or slightly arched nectary shafts that extrude drops of nectar for insect pollinators. **B,** *Schiedea verticillata* F. Br. (from U.S. Fish and Wildlife personnel & S. Conant, *Weller & Sakai 880* [cult. *Wagner & Shannon 6819*]), a hermaphroditic outcrossing species in which the nectary shaft arches to touch the sepal, where nectar pools. **C,** *Schiedea obovata* (*Perlman & Obata 5800*), example of hermaphroditic species with presumed bird pollination syndrome showing flap-like nectary extensions and black nectar pooling at their bases. Flowers are hexamerous. **D,** *Schiedea adamantis* H. St. John (*Weller & Sakai 847*), female flower of a gynodioecious species with reduced nectary shafts. — Photos: A, C, and D by Nobumitsu Kawakubo; B by W.L. Wagner.

based on morphological data (Wagner & al., 1995; Weller & al., 1995). Additionally, floral nectary ontogeny was investigated for *S. lychnoides* and *S. obovata*, representatives of sections *Nothoschiedea* and *Alsinidendron* (both included within the previously recognized genus *Alsinidendron*), because they have an unusual nectary type within *Schiedea*. Voucher information, provenance, and breeding system type are listed in Table 1. Hypotheses of *Schiedea* phylogeny based on molecular data (e.g., Nepokroeff & al., 2005; Willyard & al., 2011) do differ from those of the earlier, morphological studies of the genus; nevertheless, they suggest that our sampling scheme covers a diversity of lineages within the genus. *Schiedea ligustrina* and *S. kealiae*, both currently included in section *Schiedea* (Nepokroeff & al., 2005; Willyard & al., 2011), were chosen because they have dimorphic flowers.

Floral buds were obtained from plants cultivated at the University of California Irvine. Inflorescences were fixed in formalin/acetic acid/ethyl alcohol (FAA—5 : 5 : 90 of 50% EtOH) in the greenhouse and subsequently stored in 70% EtOH. For SEM study, the preserved material was dissected, dehydrated in an ethanol/acetone series, and processed in a Samdri PVT critical-point dryer (Tousimis, Rockville, Maryland, U.S.A.). Specimens were mounted, sputter-coated with gold-palladium and studied with a Cambridge Stereoscan 250 Mk2 (at 5 or 10 kV) or a Hitachi 570 (at 10 kV).

Histological data pertaining to the floral nectaries of *Schiedea* were obtained from floral buds of *S. ligustrinum*, *S. membranacea*, and *S. nuttallii*. Floral buds stored in 70% EtOH were dehydrated in a tertiary butyl alcohol series and embedded in Paraplast (McCormick Scientific, St. Louis, Missouri, U.S.A.). Sections of 7 µm thickness were cut with a rotary microtome and mounted on glass slides. Using a protocol modified from Joel (1983), we stained the sections sequentially with safranin and alcian blue 8GX (Sigma-Aldrich, St. Louis, Missouri, U.S.A.) for study with the light microscope.

■ RESULTS

Schiedea nuttallii. — The five sepal primordia are the first floral organs to form on the floral apex (Fig. 2A). They initiate in a regular 2/5 phyllotactic spiral and render the remaining floral apex pentagonal in outline. The two whorls of stamen primordia initiate next, forming at the periphery of the floral apex. The five antesepalous stamen primordia (which later become the inner whorl of stamens) initiate sequentially, with the first primordium forming opposite the fourth sepal. The sequence of initiation of this whorl of stamen primordia is imperfectly spiral, but opposite that of the calyx (the sequence, relative to the sepals, is: 4, 5, 3, 2, 1). The alternisepalous, or outer whorl, stamen primordia initiate shortly after the formation of the antesepalous stamen primordia. This whorl of primordia forms between the two established whorls of primordia, and at a lower level on the floral apex than the antesepalous stamen primordia. Petal primordia, or defined spaces for such, were not apparent on any of the floral apices of *S. nuttallii* that we observed. Subsequent to the initiation of the antesepalous stamen primordia, the remaining, uncommitted center of the floral apex enlarges to a height that exceeds the level of the stamen primordia and forms a ring meristem that will differentiate into the trimerous gynoecium. As the gynoecial primordium enlarges, septa form within that delimit three locules (Fig. 2B). A contrast in the tempo of differentiation and growth rate between the two whorls of stamen primordia now becomes manifest, with those of the antesepalous whorls beginning to differentiate into anthers and filaments (IA in Fig. 2B), while the alternisepalous stamen primordia remain uniformly shorter and peg-shaped. The carpels elongate apically while also enlarging basally, resulting in the formation of an ovary with three stylodia (Fig. 2C). By this point, all the stamen primordia have differentiated, but the contrast in size remains between the smaller alternisepalous and the larger antesepalous stamens. The process of stamen differentiation is also attended by the proliferation of receptacular tissue surrounding the filament bases, which becomes a low, ring-shaped outgrowth, into which the filaments appear embedded.

It is at this stage in floral development that the initiation of the nectary primordia becomes externally evident. Each of the five nectary primordia forms on the abaxial surface of the ring-shaped proliferation of receptacular tissue, opposite the base of an immature antesepalous stamen filament. At its inception, each nectary primordium arises as a broad, low mound of tissue that is horizontally furrowed (i.e., in the same plane as and perpendicular to the radii of the flower; Fig. 2C). As

Table 1. *Schiedea* collection information: species, voucher information, original island locality in the state of Hawaii and breeding system. For further voucher information, consult Wagner & al. (2005).

| Species	Voucher	Distribution	Breeding system
Schiedea kealiae Caum & Hosaka	*Weller & Sakai 791* (US)	Oʻahu	Subdioecious
S. ligustrina Cham. & Schltdl.	*Weller & Sakai 858* (BISH, PTBG, US) *Weller & Sakai 873* (BISH, PTBG, US)	Oʻahu Oʻahu	Dioecious
S. lychnoides Hillebr.	*Weller & Sakai 867* (US)	Kauaʻi	Facultatively autogamous
S. membranacea St. John	*Weller & Sakai 864* (BISH, PTBG, US)	Kauaʻi	Hermaphroditic
S. nuttallii Hook.	*Weller & Sakai 861* (BISH, PTBG, US)	Oʻahu, Kauaʻi	Hermaphroditic
S. obovata (Sherff) W.L. Wagner & Weller	*Weller & Sakai 868* (US)	Oʻahu	Facultatively autogamous
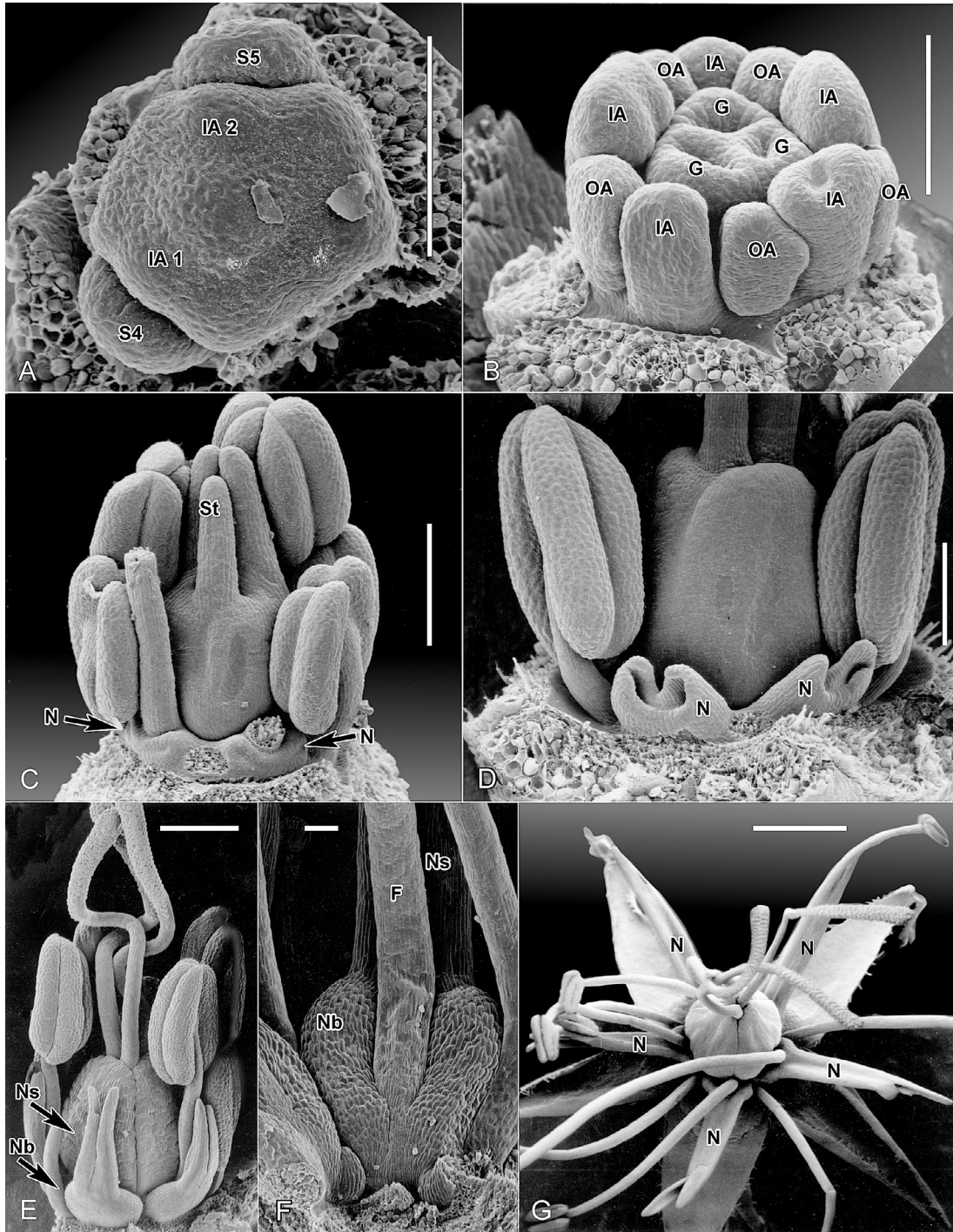
Fig. 2. Floral organogenesis and development of the hermaphroditic *Schiedea nuttallii* (*S.* sect. *Mononeura* W.L. Wagner & Weller). **A,** Polar view of floral apex with three sepals removed and two antesepalous stamen primordia present. Order of initiation of sepals and stamens is indicated numerically. Bar = 100 μm. **B,** Lateral/oblique view of developing flower with all sepals removed. Three locules have differentiated within the gynoecial primordium. The antesepalous whorl of stamens exceeds the alternisepalous whorl in size and begins to differentiate into anthers and filaments. Bar = 100 μm. **C,** Lateral view of developing flower at a later stage than B showing early stage of initiation of nectary primordium at the base of each filament of the antesepalous stamens. Bar = 200 μm. **D,** Lateral view of flower, with nectaries enlarged relative to C. Three stamens have been removed. Bar = 200 μm. **E,** Lateral view of developing flower. Nectaries continue to differentiate, each with a bulbous base and apical shaft. Bar = 400 μm. **F,** Adaxial view of the base of a nectary and filaments as seen from the interior of a flower near anthesis (ovary removed). Receptacular tissue adjacent to the nectary bulb (Nb) proliferates and differentiates to completely surround the base of the filament. Bar = 100 μm. **G,** Polar/oblique view of an anthetic flower. Sepals are partially reflexed, and nectaries are fully extended. Bar = 1 mm. — F, filament; G, carpel primordia; IA, stamen primordia of the antesepalous or inner whorl; N, nectary primordium; Nb, nectary bulb; Ns, nectary shaft; OA, stamen primordia of the alternisepalous or outer whorl; S, sepal primordia ; St, style; X, scar of removed stamen.

each primordium grows, the apical furrow deepens and the nectary primordium becomes broadly channeled distally on its adaxial side so that the resulting structure closely resembles a smiling human mouth ("N" in Fig. 2D). Each nectary enlarges basally, resulting in a flattened "bulb" (arrow labeled "Nb" in Fig. 2E). Concomitantly, the nectary apex elongates to form the nectary shaft (arrow labeled "Ns" in Fig. 2E). The apical meristem of the nectary initially produces the shaft as a result of localized cell proliferation from two, discrete "C"-shaped centers of meristematic activity (i.e., at the "corners" of the smiling mouth). The zone of meristematic activity broadens later in development to form a complete, annuliform, intercalary meristem. As the nectary shaft elongates, it curves toward the apex of the floral bud. Receptacular tissue surrounding the base of each antesepalous stamen also contributes to nectary formation, so that at anthesis the base of each nectary bulb encloses the base of the associated stamen filament (Fig. 2F). At maturity, the nectary is an apically cleft, hollow shaft atop a bulbous base that appears compressed in the ad-/abaxial plane of the flower (Fig. 2G).

Schiedea membranacea. — Early stages of the initiation of the sepal and stamen primordia in *S. membranacea* are like those of *S. nuttallii*. The sepal primordia initiate in a 2/5 phyllotactic spiral, and the whorl of antesepalous stamen primordia forms first, followed rapidly by the alternisepalous stamen primordia. Again, the antesepalous stamen primordia ("IA" in Fig. 3A) initiate higher on the floral apex and become the inner androecial whorl. The alternisepalous stamen primordia initiate centrifugally to those of the previous whorl. Petal primordia, or defined spaces for such, were not apparent on any of the floral apices of *S. membranacea* that we studied. The remaining floral apex forms a ring meristem and rapidly differentiates into a tetramerous gynoecium (Fig. 3B). After the stamen primordia differentiate, nectary primordia initiate on the abaxial surface of a ring-shaped proliferation of receptacular tissue, opposite the base of each of the immature antesepalous stamen filaments (arrows, Fig. 3C; accompanying stamen primordia removed). Because of this, the five developing nectaries appear to abut each other (Fig. 3D) and form a continuous ring on the receptacle of the flower. The nectaries enlarge apically to form the beginnings of the shaft (Fig. 3E). The bulb of each nectary differentiates at a slower rate (arrow, Fig. 3F), so that it becomes obvious only shortly before anthesis (arrows, Fig. 3G). At anthesis (Fig. 3H), the nectaries extend from the flower perpendicular to the floral axis, and the sepals reflex.

Schiedea ligustrina, female flowers. — This species has a dioecious breeding system, but the female flowers retain the ability to initiate stamen primordia (Fig. 4A). Their early ontogeny, through the formation of the antesepalous stamen whorl, is like that of the species described above. After the initiation of the antesepalous stamen primordia ("IA" in Fig. 4A), the alternisepalous common petal/stamen primordia initiate centrifugally. Subsequent to a limited phase of enlargement of each common primordium, a petal primordium initiates centrifugally. At its inception, each petal primordium appears as a small bulge on the abaxial surface of the common primordium (arrows in Fig. 4A), toward the base of the (now committed)

alternisepalous stamen primordium. As the stamen primordia enlarge, the remaining floral apex forms a ring meristem that differentiates into a trimerous (or tetramerous) gynoecium (arrow labeled G, Fig. 4B). During the early differentiation of the stamen primordia, the petal primordia abort and soon disappear (former positions indicated by unlabeled arrows in Fig. 4B). At a later stage of development, shown in Fig. 4C, the stamens continue to develop normally and the gynoecium forms three stylodia. Also at this stage, the nectary primordia initiate—each as a small bulge on the abaxial surface of a relatively impoverished, ring-shaped proliferation of receptacular tissue, opposite the base of each of the antesepalous stamen primordia (arrows, Fig. 4C). The characteristic "smiling mouth" form of each nectary primordium soon becomes apparent (arrows, Fig. 4D) as the stamens and gynoecium continue to differentiate. Note that at this stage the anthers remain turgid and the individual thecae are rounded. As the nectary primordia begin to elongate and differentiate (Fig. 4E), the anther thecae begin to flatten and collapse. The apices of the nectary primordia elongate erratically (Fig. 4E, F), such that the mature nectary shafts have a very irregular apical margin. As the nectary shafts elongate (Fig. 4F), the thecae of both whorls of stamens collapse and so appear shriveled (arrows, Fig. 4F). Even so, both whorls of stamens are present at anthesis (Fig. 4G), but their filaments do not elongate and their anthers fail to produce any viable pollen. The sepals are only partially reflexed in this species, and are longer than the nectaries they subtend. The mature nectaries are moderately differentiated into bulb and shaft zones, and the shafts are strongly ad-/abaxially compressed (Fig. 4G).

Schiedea ligustrina, male flowers. — The early stages of floral organogenesis of the male flowers of *S. ligustrina* are essentially identical to those of the female flowers (Fig. 5A–C). After the formation of the sepal primordia and both whorls of androecial primordia, a petal primordium initiates on the abaxial surface of each common petal/alternisepalous stamen primordium at its base (arrows, Fig. 5A). The petal primordia abort and disappear from the floral receptacle concomitant with the differentiation of the stamen primordia into anthers and filaments (arrows, Fig. 5B). The remaining uncommitted floral apex forms a ring meristem that differentiates into the trimerous (or tetramerous) gynoecial primordium contemporaneous with these events (Fig. 5B). After the stamen primordia fully differentiate and begin to enlarge, the nectary primordia initiate on the relatively depauperate, ring-shaped proliferation of receptacular tissue, opposite the base of each of the antesepalous stamens (arrows, Fig. 5C). In contrast with the ontogeny of the female flowers, the stamen primordia continue to develop normally. The disparity in size between the two whorls of stamen primordia is sustained throughout most of their development (Fig. 5B–F), but the immature stamens become about equivalent in size as the filaments elongate during anthesis (Fig. 5G). The gynoecium develops three stylodia (Fig. 5C–E), but the ovary does not enlarge greatly and becomes a pistillode (Fig. 5D–G). The nectaries of the male flowers of *S. ligustrina* are similar in development and mature structure to those of the female flowers (Fig. 5D, E, G).
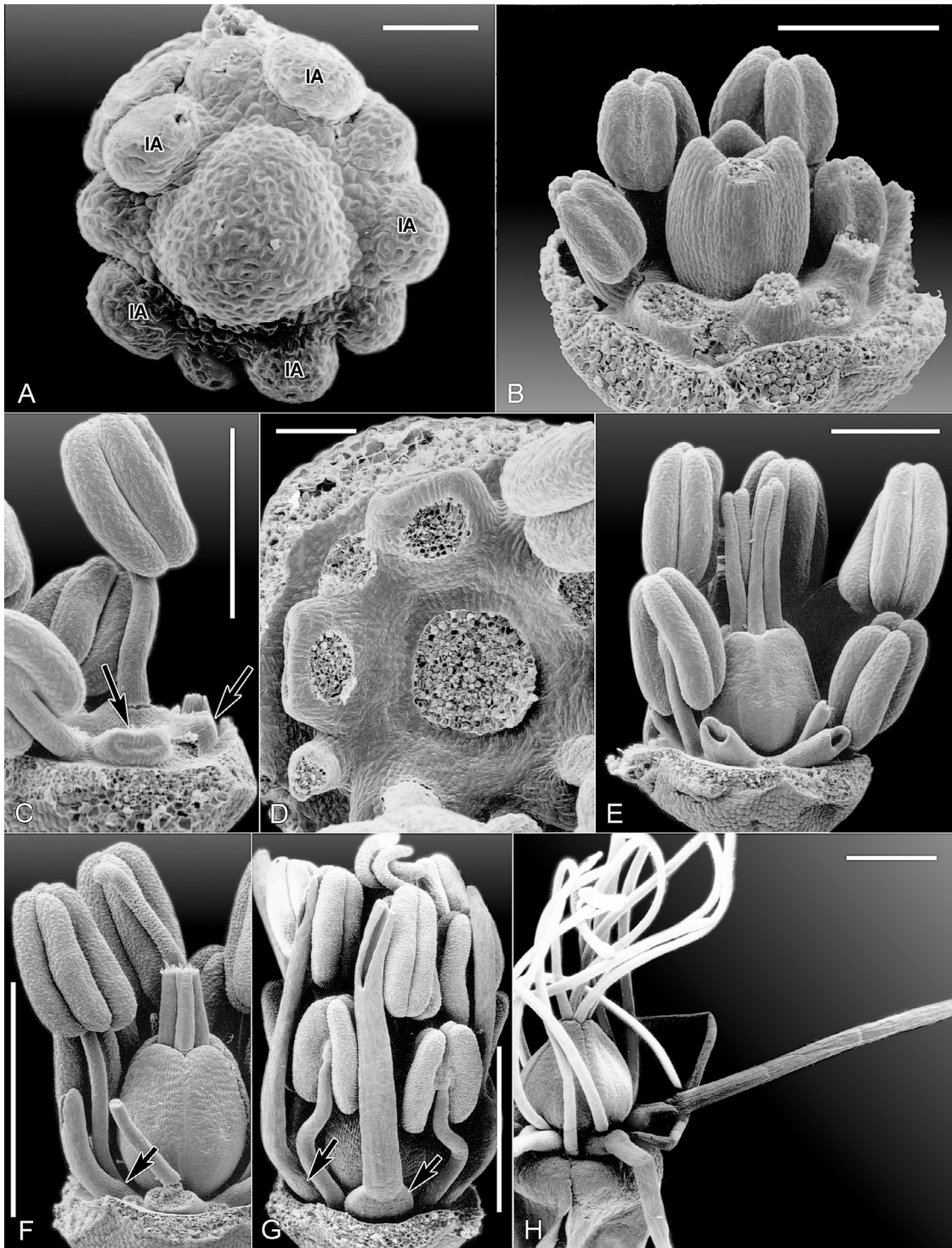
Fig. 3. Floral organogenesis and development of the hermaphroditic *Schiedea membranacea* (*S.* sect. *Alphaschiedea* W.L. Wagner & Weller). **A,** Polar view of floral apex after sepal and stamen initiation. The antesepalous stamen primordia are larger and are located higher on the floral apex than the alternisepalous stamen primordia. Bar = 40 μm. **B,** Lateral view of the developing flower with the sepals and several stamens removed. Bar = 200 μm. **C,** Lateral view of developing flower with sepals and gynoecium removed. Arrows indicate nectary primordia at early stage of initiation. Bar = 400 μm. **D,** Polar view of developing flower with sepals, stamens and gynoecium removed to expose the essentially confluent nectary primordia. Bar = 100 μm. **E,** Lateral view of developing flower with sepals and two stamens removed. Nectary primordia have begun to elongate apically. Bar = 400 μm. **F,** Lateral view of developing flower with sepals and some stamens removed. The base of the nectary is beginning to differentiate (arrow). Bar = 1 mm. **G,** Lateral view of a flower prior to anthesis with the sepals removed. Differentiated nectary base is apparent (arrows). Bar = 1 mm. **H,** Lateral view of an anthetic flower, with extended nectaries and completely reflexed sepals. Bar = 1 mm. — IA, stamen primordia of the antesepalous or inner whorl.
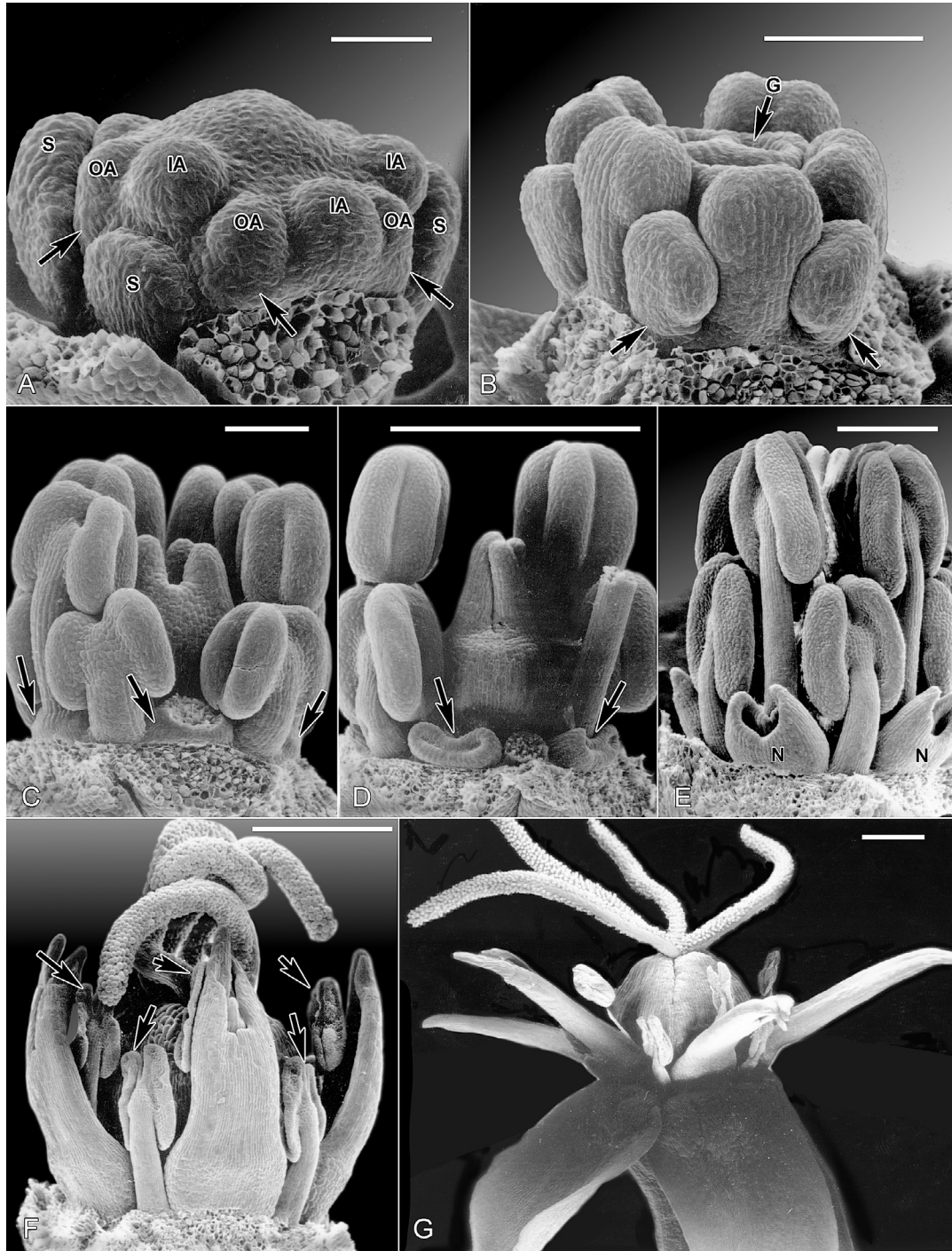
Fig. 4. Floral organogenesis and development of the dimorphic *Schiedea ligustrina*, female flowers (*S.* sect. *Schiedea*). **A,** Lateral view of the floral apex. One sepal in this view has been removed. Arrows indicate nascent petal primordia. Bar = 40 µm. **B,** Lateral view of a floral apex at a later ontogenetic stage than shown in A, with all sepals removed. Developing gynoecial primordium, with three locules differentiated, indicated with an arrow labeled "G". At the base of the alternisepalous stamen primordia are the spaces formally occupied by the petal primordia (arrows). The bulges of the petal primordia are no longer present, although the small space remains at this stage. Bar = 100 µm. **C,** Lateral view of a developing flower with all sepals and one of the antesepalous stamen primordia removed. Nectary primordia each initiate at the base of an antesepalous stamen primordium (arrows). Bar = 100 µm. **D,** Lateral view of a developing flower with all sepals and three stamen primordia removed. The nectary primordia have expanded to the "smiling mouth" form (arrows). Bar = 400 µm. **E,** Lateral view of a developing flower with all sepals removed. Nectary primordia are differentiating into a bulbous base and apical shaft. The thecae of the anthers have flattened. Bar = 200 µm. **F,** Lateral view of a developing flower primordium with all sepals removed. Anther thecae (arrows) have collapsed. Bar = 400 µm. **G,** Lateral view of an anthetic flower. Bar = 400 µm. — G, carpel primordia; IA, stamen primordia of the antesepalous or inner whorl; N, nectary primordium; OA, stamen primordia of the alternisepalous or outer whorl; S, sepal primordia.
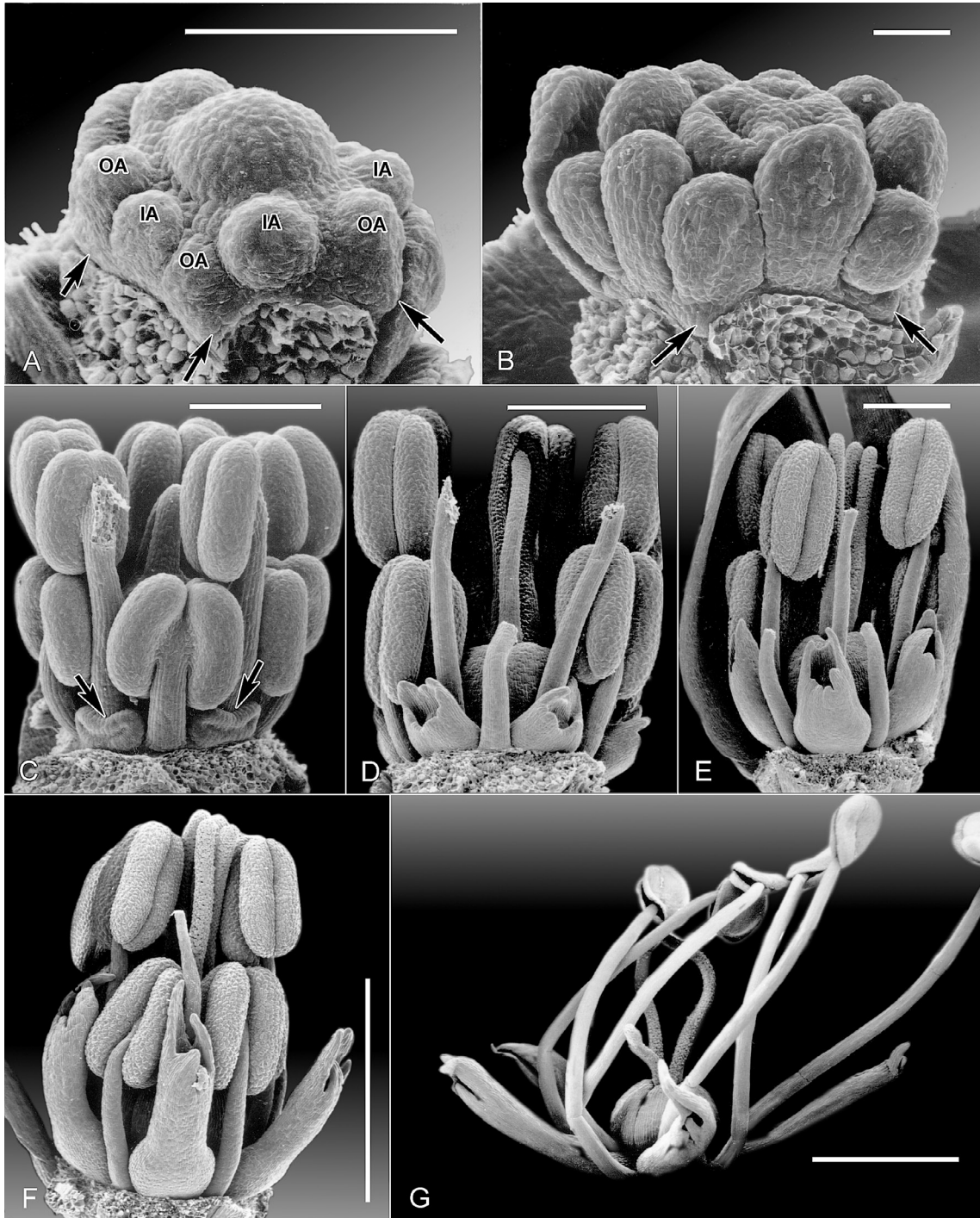
Fig. 5. Floral organogenesis and development of the dimorphic *Schiedea ligustrina*, male flowers (*S.* sect. *Schiedea*). **A,** Lateral view of a floral apex in which all sepal primordia have been removed. Both whorls of stamen primordia have been initiated. Petal primordia are each evident as a small bulge at the base of an alternisepalous stamen primordium (arrows). Bar = 100 μm. **B,** Lateral view of a floral apex in which all sepal primordia have been removed, at a later ontogenetic stage than shown in A. A trimerous gynoecium has formed with three distinct locules. Petal primordia have aborted and have mostly disappeared (arrows). Bar = 40 μm. **C,** Lateral view of a developing flower in which four of the sepal primordia have been removed. The stamens are fully differentiated and each nectary primordium has initiated (arrows) in the "smiling face" form at the base of an antesepalous stamen. Bar = 200 μm. **D,** Lateral view of a developing flower in which all sepals and three anthers have been removed. The nectary primordia are beginning to elongate apically. Bar = 400 μm. **E,** Lateral view of a developing flower in which three sepals and three anthers have been removed. Bar = 400 μm. **F,** Lateral view of a flower near anthesis, with four sepals and one anther removed. Bar = 1 mm. **G,** Lateral view of a flower at anthesis. Bar = 1 mm. — IA, stamen primordia of the antesepalous or inner whorl; OA, stamen primordia of the alternisepalous or outer whorl.

***Schiedea kealiae*, female flowers. —** The early stages of floral organogenesis are like those of the female flowers of *S. ligustrina*, except that petal primordia are not produced (Fig. 6A–E). The trimerous or tetramerous gynoecium differentiates from a ring meristem and rapidly enlarges to over-top the antesepalous stamen primordia in height (Fig. 6B, C). Subsequent to the differentiation of the stamen primordia into anthers and filaments, the nectary primordia initiate, each as a slight bulge on the abaxial surface of the relatively impover-ished, annuliform proliferation of receptacular tissue, opposite an antesepalous stamen (arrow, Fig. 6C). As the nectary pri-mordia mature to the "smiling face" phase of development, the anthers of all of the stamens begin to flatten (unlabeled arrows, Fig. 6D). Just prior to anthesis, the anthers appear distorted and lack turgidity (arrows, Fig. 6E). The nectary apices elongate to a relatively limited extent, with the apical margins show-ing irregular growth late in development (Fig. 6E, F). Mature nectaries are comparatively diminutive in size at anthesis, not or weakly differentiated into bulb and shaft zones, and have an irregular apical margin (Fig. 6F).

***Schiedea kealiae*, male/hermaphroditic flowers. —** The early stages of floral organogenesis are essentially identical to those of the female flowers (Fig. 7A, B). Each nectary primor-dium initiates on the abaxial surface of the relatively impover-ished, annuliform proliferation of receptacular tissue, opposite the base of an antesepalous stamen, and proceeds to enlarge as the stamens differentiate (arrows, Fig. 7C). Eventually, the tissue of each nectary base fully envelops the base of its associ-ated filament (Fig. 7D). Prior to anthesis, the nectaries enlarge, although only a short nectary shaft is produced that is not or weakly differentiated from the bulb zone (Fig. 7E). At anthesis, the sepals are moderately reflexed (Fig. 7F).

***Schiedea lychnoides*, floral nectaries. —** Each nectary primordium initiates on the abaxial surface of an annuliform proliferation of receptacular tissue below the base of an ante-sepalous stamen filament (Fig. 8A). The nectary primordia each become evident as a horizontally elongate furrow. The furrow divides each primordium into a distal and proximal ridge. Subsequent growth of a primordium is largely confined to the elongation of the proximal (lower) ridge (Fig. 8B), which becomes a discrete, scale-like flap of tissue that attains a height of about half that of the ovary at maturity (Fig. 8C). Nectar is secreted within the furrow and pools on the adaxial surface of the scale-like extension.

***Schiedea obovata*, floral nectaries. —** Nectary primor-dia are initially evident as a series of horizontally elongate furrows on the abaxial surface of an annuliform mound of receptacular tissue that is confluent with the stamen bases in each flower. The nectary primoridia sustain their horizontal elongation and fuse to form a common nectary meristematic zone shortly after their inception (Fig. 8D). This meristematic zone is divided by the furrow into proximal and distal ridges. As in *S. lychnoides*, subsequent growth is most dramatically evident in the elongation of the proximal ridge. Growth of the nectary extension occurs at an unequal rate, with the sectors op-posite the antesepalous stamens elongating at a faster rate than the adjacent alternisepalous sectors (Fig. 8E). At maturity, the

nectary extension is cupuliform—surrounding the androecium and gynoecium—and is with a deeply incised apical margin (Fig. 8F).

***Schiedea* nectary histology. —** The floral nectaries have limited anatomical complexity, and there is little anatomical variation present between the species we observed, despite their appreciable diversity in nectary shape. Nectaries lack a direct vascular connection to the floral stele. A vascular connection is effected by the antesepalous stamen traces, which extend through the nectary bulb and adjacent nectariferous tissues (Fig. 8G, K). Although there are no branches from each trace to the surrounding nectariferous tissue, the vascular bundle lacks sheathing. Conducting cells at the periphery of the bundle are, therefore, in direct contact with nectary tissue. The bulb of each nectary and confluent tissues adjacent to the stamen bases have a thin, uniseriate epidermis. Internal to this limiting layer is a solid, compact mass of cells with densely cytoplasmic contents (Fig. 8 H). Figures 8G and 8K illustrate approximately median longitudinal sections through the nectaries, emphasizing that receptacular tissue fated to differentiate into nectariferous tis-sue proliferates to encircle the base of each stamen (cf. Figs. 2F, 7D). Nectar is secreted through modified stomata that oc-cur at the juncture between the bulb and shaft (Fig. 8I). The nectary shaft is internally comprised of longitudinally elongate parenchyma cells. The ring-shaped proliferation of tissue that surrounds the stamen filament bases remains the most obvi-ous zone of undifferentiated, meristematic tissue at the time of inception of the nectary primordia (Fig. 8J).

■ **DISCUSSION**

Floral patterning of *Schiedea* in relation to other Caryo-phyllaceae. — Although the *gestalt* of *Schiedea* flowers is dissimilar from that of most other Caryophyllaceae, floral pat-terning in *Schiedea* is like that of many other members of the family. Basic similarities include the initiation of sepal primor-dia in a 2/5 spiral, with the second sepal in a median adaxial position, and the formation of each petal primordium (when present) on a common primordium that also forms an alternise-palous stamen primordium (Hofmann, 1994; Ronse De Craene & al., 1998; Leins & al., 2001; Ronse De Craene, 2010). Pattern formation of the androecium and corolla is the most diverse aspect of the early ontogeny of Caryophyllaceae flowers (Ronse De Craene & al., 1998; Ronse De Craene, 2010). As in many other core Caryophyllales lineages, this organ zone appears developmentally independent of the calyx and gynoecium on account of the (frequent) tendency toward centrifugal inception of primordia. In *Schiedea* flowers, the ontogeny of the stamen/ petal zone conforms to the most frequently observed pattern for the family, in which the first-formed, antesepalous whorl of sta-mens is sequentially initiated, with the first stamen primordium forming opposite the fourth sepal, and subsequent primordia initiated in an imperfectly contrarotating sequence to that of the sepals. Petal primordia, when present, initiate subsequent to the androecial primordia. Floral patterning, therefore, does not ac-count for the unique aspect of *Schiedea* flowers, which largely
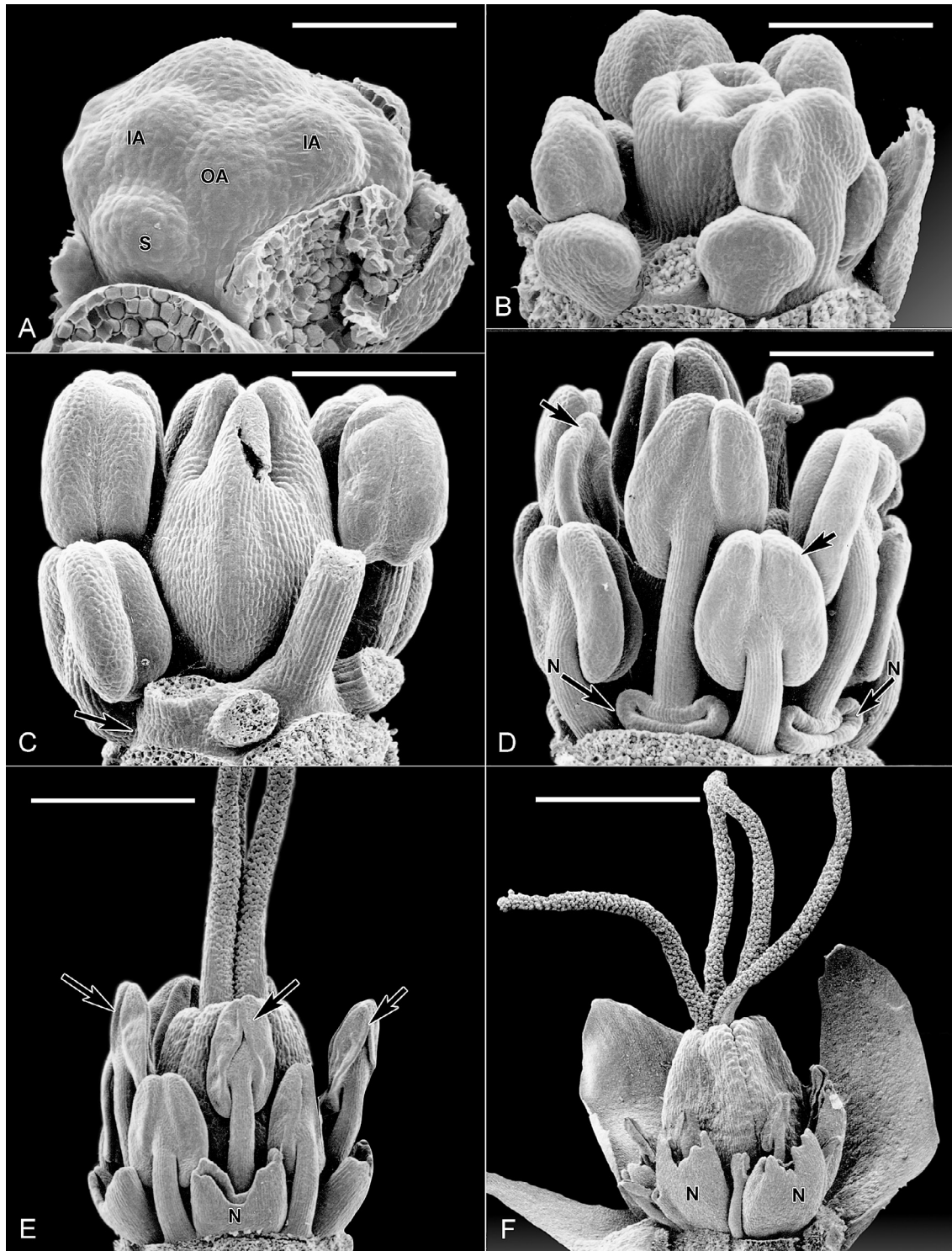
Fig. 6. Floral organogenesis and development of the dimorphic *Schiedea kealiae*, female flowers (*S.* sect. *Schiedea*). **A,** Lateral view of a floral apex with largest sepal primordia removed. The antesepalous stamen primordia initiate at a higher level than the alternisepalous stamen primordia. Bar = 75 µm. **B,** Lateral view of a floral apex, with four sepal primordia and one antesepalous stamen primordium removed. Bar = 150 µm. **C,** Lateral view of a developing flower with all sepals, two antesepalous stamens and two alternisepalous stamens removed. The nectary primordia are beginning to be initiated (arrow). Bar = 176 µm. **D,** Lateral view of a developing flower at a later ontogenetic stage than shown in C, with all sepals removed. Unlabeled arrows indicate the collapsing anthers. Bar = 300 µm. **E,** Lateral view of a developing flower near anthesis with all sepals removed. Anthers (arrows) are now fully collapsed. Bar = 500 µm. **F,** Lateral view of a flower at early anthesis with two sepals removed. Bar = 1.2 mm. — IA, stamen primordia of the antesepalous or inner whorl; N, nectary primordium; OA, stamen primordia of the alternisepalous or outer whorl; S, sepal primordia.
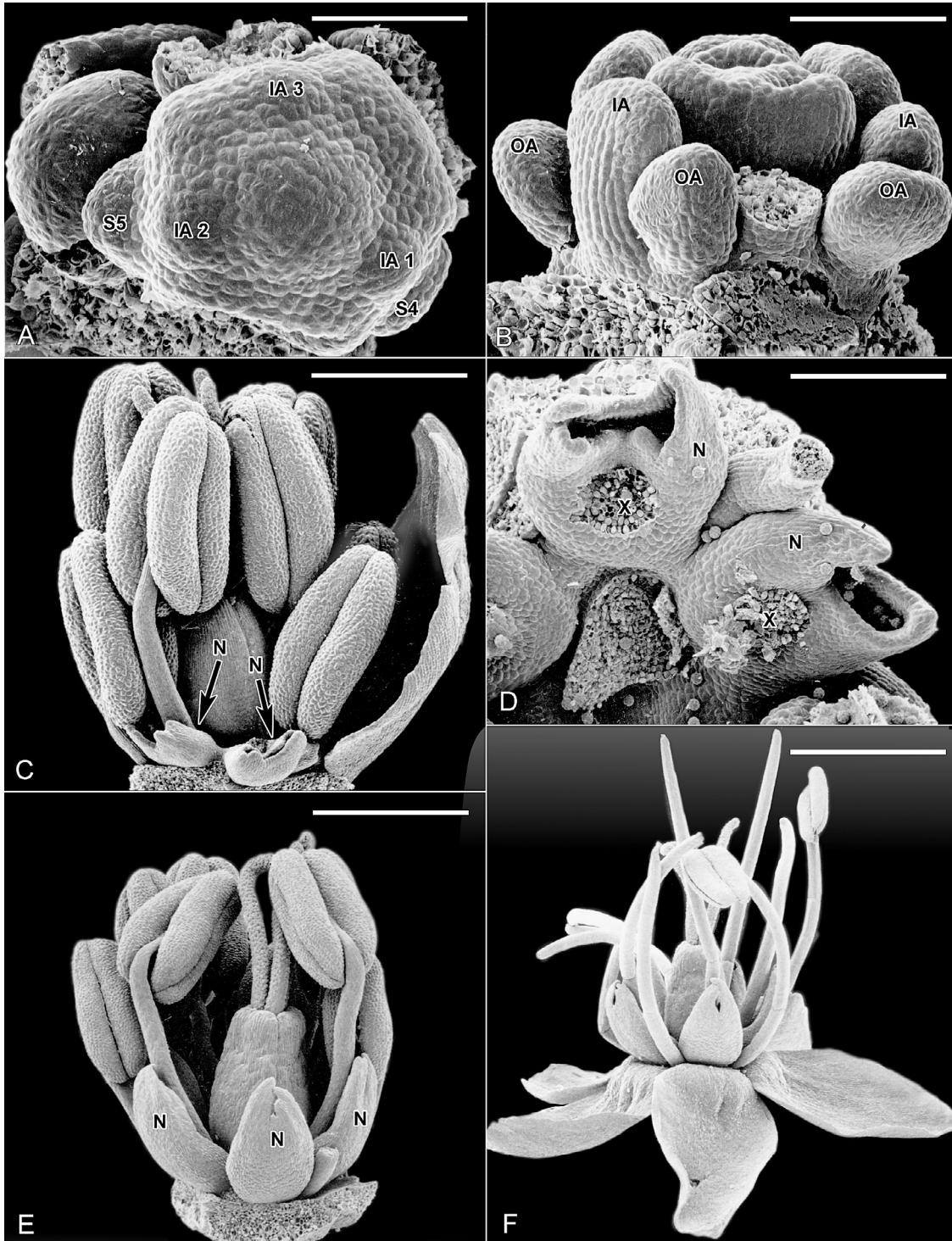
Fig. 7. Floral organogenesis and development of the dimorphic *Schiedea kealiae*, hermaphroditic flowers (*S.* sect. *Schiedea*). **A,** Polar view of a floral apex with three sepal primordia removed. Three antesepalous stamen primordia have initiated opposite the youngest three sepal primordia. Order of initiation of sepals and stamens as indicated numerically. Bar = 75 µm. **B,** Lateral view of a floral apex at a later ontogenetic stage than shown in A, with all sepal primordia and one antesepalous stamen primordium removed. Bar = 100 µm. **C,** Lateral view of a developing flower with all sepal primordia and one antesepalous stamen primordium removed. The nectary primordia have formed and are enlarging. Bar = 500 µm. **D,** Polar view of the developing flower with all stamens and the gynoecium removed. Nectaries are essentially contiguous at their bases to form a complete ring. Each "X" indicates a scar-like artifact resulting from the removal of a filament at these positions. Bar = 231 µm. **E,** Lateral view of a flower just prior to anthesis with all sepals, one antesepalous stamen and two alternisepalous stamens removed. Bar = 860 µm. **F,** Lateral view of an anthetic flower in which most of the anthers have abscised. Bar = 1.36 mm. — IA, stamen primordia of the antesepalous or inner whorl; N, nectary primordium; OA, stamen primordia of the alternisepalous or outer whorl; S, sepal primordia.

attain their characteristic appearance through developmental differences that occur late in floral ontogeny. This includes the architecture of the calyx, which becomes reflexed at anthesis in many _Schiedea_ species, and the extended stamen filaments that characteristically elongate to a length exceeding the sepals.

An important exception to the above generalization is the absence of petals in the large majority of _Schiedea_ flowers. The results of an ancestral state reconstruction of petal presence in Caryophyllaceae (Greenberg & Donoghue, 2011; shown in their fig. 5C) demonstrates that apetaly in _Schiedea_ represents a synapomorphic loss of petals. From a floral developmental perspective, Rohweder (1970) thought apetaly in Caryophyllaceae resulted from the complete loss of these organs. Our observations indicate that although his assertion might generally be true within the genus, _Schiedea_ flowers are, nevertheless, capable of forming (probable) petal primordia and even mature petals in rare instances (Wagner & al., 2005). Mature petals are uncommonly present in the anthetic flowers of some populations of _S. membranacea_ and _S. verticillata_, though corolla merosity is highly unstable when these organs are formed, suggesting petal development is probably a teratological phenomenon in these species (Harris & Wagner, pers. obs.). Moreover, our observations indicate that _S. ligustrina_ regularly forms an additional, alternisepalous whorl of organ primordia that, based on their position and common origin with an alternisepalous stamen primordium, can be interpreted as petal primordia. The petal primordia of _S. ligustrina_ abort and disappear shortly after their inception, indicating that apetaly in _Schiedea_ can be achieved by organ suppression, and not only organ loss like we observed in the other species. The genetic capability for petal formation is, therefore, likely retained to a degree in _Schiedea_—a suggestion bolstered by the fact that each of the three species mentioned above have nested phylogenetic positions within the genus (Willyard & al., 2011).

Petals are thought to be of staminodal origin within the core lineage of Caryophyllales (Ronse De Craene, 2010), and have several, independent origins within this clade (Brockington & al., 2009). Evolutionary lability in the development of a corolla is also manifest in Caryophyllaceae, on account of both the numerous, independent losses of petals within the family and the variability in robustness to which petals are expressed when they do occur (Bittrich, 1993; Harbaugh & al., 2010; Greenberg & Donoghue, 2011). Petal expression in Caryophyllaceae ranges from the development of large, showy structures that are differentiated into claw and limb zones and provided with a coronal appendage in many species of tribe Sileneae, to the formation of minute, filamentous structures that have been described as staminodes in species of _Paronychia_ (Bittrich, 1993; Hofmann, 1994; Leins & al., 2001). In light of this complexity, the modest ontogenetic lability in petal primordium expression we observed in _Schiedea_ seems unsurprising.

Development and comparative structural biology of floral nectaries in _Schiedea_. — This study demonstrates that the distinctive floral nectaries of _Schiedea_ initiate opposite the base of an antesepalous stamen primordium on the abaxial surface of a ring-shaped proliferation of receptacular tissue that surrounds the filament bases. During the early stages of their ontogeny, the nectary primordia are each low mounds of tissue that apically have an arcuate, horizontal furrow, and so resemble a smiling human mouth. The shape _Schiedea_ nectary primordia assume and the position in which they originate are, interestingly, like that of the mature nectaries of many other Caryophyllaceae taxa. These distinctive nectaries have been described as "swallow's nest-like [gland] appendages" (_schwalbennestartige Drüsenanhänge_) on the ring of nectariferous tissue adjacent to the stamen filament bases in each flower (Mattfeld, 1938; Rohweder, 1970; Bittrich, 1993). In this nectary type, nectar is secreted through modified stomata at the base of each nectary furrow, where it accumulates to form a drop that is exposed at the nectary apex (Zandonella, 1967; Mayer, 2003).

Pocketed or furrowed nectaries positioned opposite the base of the antesepalous stamens have been reported from many genera contained in a crown clade of Caryophyllaceae inclusive of tribes Sperguleae and Sclerantheae, designated by Greenberg & Donoghue (2011) as Pleurcaryophyllaceae (see also fig. 2 of Harbaugh & al., 2010, but not present in tribes Sileneae and Caryophylleae; Mattfeld, 1938; Thomson, 1942; Zandonella, 1967; Rohweder, 1970; Bittrich, 1993; Harris & Wagner, 1995; Mayer, 2003), which includes _Schiedea_ (Sclerantheae). Nectary structure among the genera of Sclerantheae is, however, diverse (Bittrich, 1993; Smissen & Garnock-Jones, 2002), and the genera most closely related to _Schiedea_—_Honckenya_ and _Wilhelmsia_—have entire, unfurrowed nectaries that are paired, one to either side of each antesepalous stamen base (Wagner, 2005a, b). Be that as it may, the common and widespread occurrence of "swallow's nest" nectaries within a major crown clade of the family may suggest a shared genetic basis for this nectary type within this group. If so, then the ontogeny of _Schiedea_ nectaries is clearly interpretable in relation to the "swallow's nest" nectary type. From a heterochronic perspective, the elongate, hollow nectary shaft that characterizes _Schiedea_ nectaries can therefore be seen to result from the addition of developmental stages to the end of an ontogenetic program of a "swallow's nest" nectary, in which the tissues surrounding the furrow become greatly elongated. Likewise, the unusual scale-like or cupuliform nectary extensions of sections _Nothoschiedea_ and _Alsinidendron_ are interpretable as a modification of the basic _Schiedea_ nectary ontogeny in that putative hypermorphic extension is limited to only the ridge proximal to (beneath) the furrow.

Despite these hallmarks of novelty, there is little evidence to suggest that the nectary extensions in _Schiedea_ and the evolutionary developmental nature of their origin are related to a (partial) shift in organ identity. The relatively early elongation of the nectar shaft is unlike that of the stamen filaments, which rapidly elongate just prior to anthesis, and they have no striking petaloid features. Furthermore, both the histology and nectar exposure mechanism of _Schiedea_ nectaries are like those of the "swallow's nest" nectaries in all fundamental respects. However, the extent to which nectar exposure, particularly in species with long, needle-like nectary shafts, is due to capillary action versus pressure-driven mass flow remains to be investigated.

Nectary size and shape vary considerably within *Schiedea*. The four species of the nested clade including sections *Alsinidendron* and *Nothoschiedea* have nectaries relatively different in kind from the other species of the genus. Within each flower, these nectaries manifest as a series of broad flaps that collectively form a cup, or else are congenitally fused to form a single cupuliform structure, in which accumulates copious amount of black nectar (Fig. 1C; Wagner & al., 2005). Compared with nectaries of the bulb-and-shaft type, structural differences are evident even at the earliest stages of nectary development, as the furrows of the nectary primordia are relatively much more horizontally elongate. Weller & al. (1998) suggested that the origin of the distinguishing floral features for the clade are correlated with a possible shift to bird pollination in the ancestors of these species (contemporary members of this clade are largely autogamous).
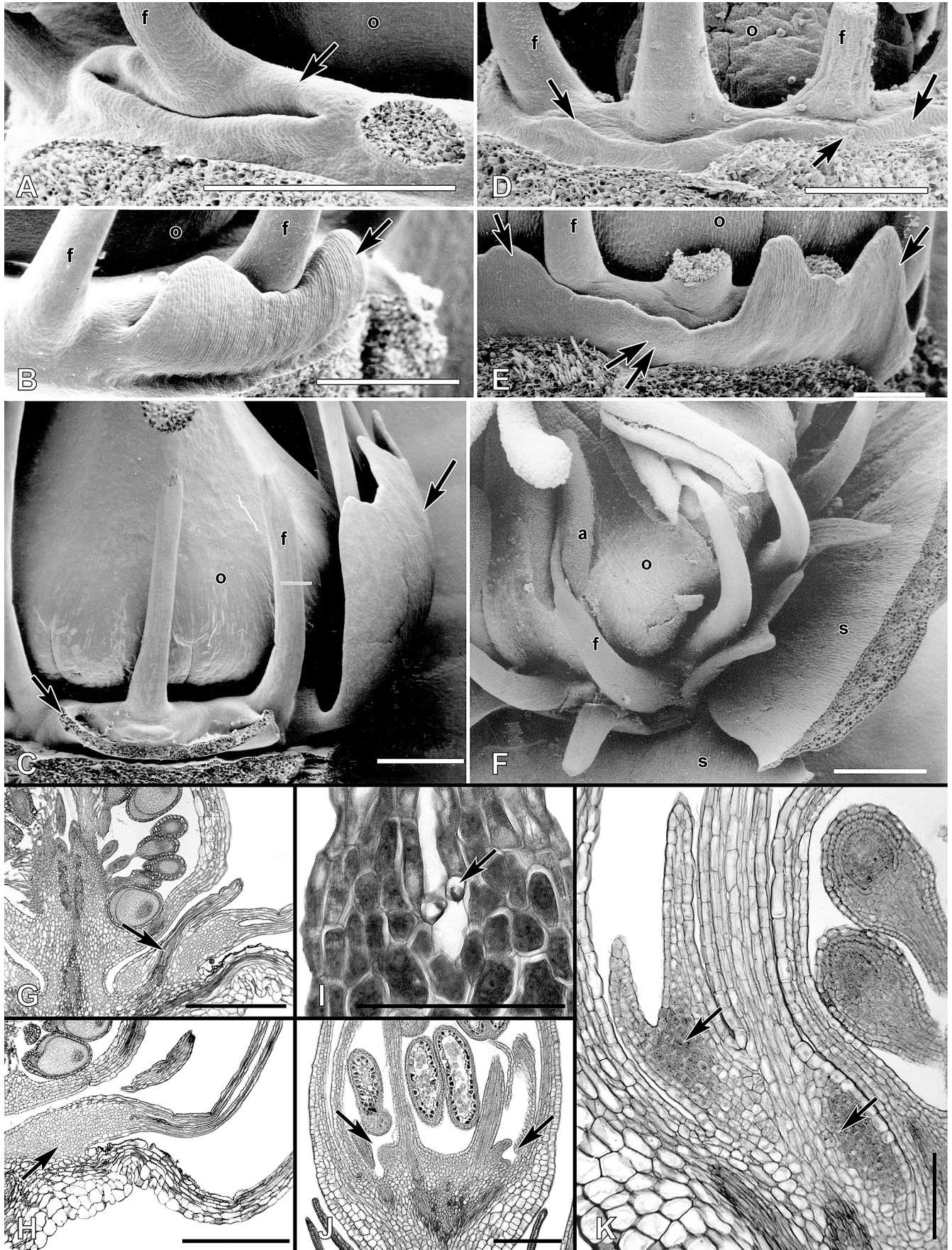
All other *Schiedea* species have floral nectaries broadly interpretable as having a bulb and shaft. In outcrossing species with consistently hermaphroditic flowers, the nectary shaft is typically elongate and well differentiated from the bulb zone, as in *S. nuttallii*. These species are presumed to be insect-pollinated, although this has been documented only in a single species—the Moloka'i endemic *S. lydgatei*—which is likely pollinated by native Pyralid moths (Norman & al., 1997; Wagner & al., 2005). *Schiedea* species with dimorphic flowers, which are all to a degree wind-pollinated, have nectaries that are functional at maturity, but with much shorter nectary shafts relative to those of hermaphroditic, outcrossing species (Wagner & al., 2005). Weller & al. (1998) indicated that while there are no obvious differences in the volume of nectar produced by nectaries of *Schiedea* species with dimorphic flowers relative to those with uniformly hermaphroditic flowers, the two groups differed in the relative concentrations of sugar types in the nectar they produced. Nonetheless, the nectar of all *Schiedea* species investigated, which includes representatives of all breeding system types, is strongly hexose-dominant (Weller & al., 1998). With limited or no visitation by biotic pollinators, selective pressures on nectary size and shape in dimorphic-flowered *Schiedea* species are likely relaxed. The nectaries of these species, exemplified in our study by *S. ligustrina* and *S. kealiae*, are relatively variable in shape, reduced in size, and with a very irregular apex. Relative to the bulb-and-shaft nectaries of the hermaphroditic, outcrossing species, our observations indicate that the above differences are attributable to developmental events occurring at all but the earliest stages of nectary ontogeny. Autogamous species of *Schiedea* (e.g., *S. diffusa* A. Gray) show pronounced plasticity in nectary form and development. Perhaps because these structures are essentially released from selective pressures, they are highly reduced in size and largely non-functional (Wagner & al., 2005). The early development of these nectaries reflects their bizarre mature structure. For example, in *S. diffusa*, individual primordia can differentiate into (even) more than one, very irregularly shaped nectary (E. Harris, unpub. data).

Developmental basis of floral dimorphism in *Schiedea*. — Male sterility in *Schiedea* is determined by a single nuclear gene that has a simple Mendelian inheritance pattern (Weller & Sakai, 1991). In the two species with functionally female flowers that we investigated, the anthers begin to abort relatively late in their development, well after the microsporangia and thecae have structurally differentiated. Thus, stamen initiation and early development appears identical in all *Schiedea* species that have been ontogenetically investigated (Wagner & Harris, 2000). Likewise, the cessation of normal anther development appears to occur at a similar stage in floral ontogeny among all species in which this phenomenon is characteristic, upon which the anthers begin to collapse inward, losing their plumpness. Despite the consistent and obvious presence of 10 stamens at anthesis, aborted anthers never form viable pollen. Although organ expression is variable among Caryophyllaceae species with dimorphic flowers (cf. *Silene latifolia* Poir., Grant & al., 1994), Hill (1996) documented a developmental program of male-sterilized flowers in *Arenaria* L. that is similar to what we observed in *Schiedea*. A cytological investigation of the developing stamens of *A. uniflora* (Walt.) Muhl. (Hill & al., 1992; = *Minuartia uniflora* (Walt.) Mattf.) demonstrated that the anther collapsing stage occurs just prior to what normally would have been the onset of microsporogenesis.

Fig. 8. **A–C,** Nectary development in flowers of *Schiedea lychnoides* (*S*. sect. *Nothoschiedea*): **A,** Nectary primordium initiation (at arrow) on abaxial surface of ring-shaped outgrowth of receptacular tissue, below the base of an antesepalous stamen filament. Note that a horizontal furrow divides the primordium into distal and proximal ridges. Bar = 400 µm. **B,** Proximal ridge of nectary primodium elongates to produce a discrete, scale-like extension (arrow). Stamen filaments and ovary are indicated. Bar = 400 µm. **C,** Anthetic flower showing mature form of nectaries (arrows). Nectary in foreground with the scale-like extension removed to show nectar-secreting furrow and unelongated distal ridge. Bar = 1.0 mm. **D–F,** Nectary development in flowers of *Schiedea obovata* (*S*. sect. *Alsinidendron*): **D,** Early stage of nectary initiation as horizontally elongate ridges (arrows). After initiation, meristematic zones of neighboring nectaries extend and fuse to form an essentially entire structure. Bar = 400 µm. **E,** Growth of nectary, with proximal scale-like extension differentially elongating at antesepalous (single arrows) and alternisepalous (double arrow) positions. Bar = 400 µm. **F,** Anthetic flower, with mature nectary extensions forming an essentially entire, cup-like structure surrounding the filament bases. Bar = 1.0 mm. **G–K,** Histology of *Schiedea* floral nectaries: **G,** Anthetic flower of *S. membranacea* in longitudinal section (LS) showing indirect vascular connection via associated antesepalous stamen (arrow). Bar = 500 µm. **H,** Anthetic flower of *S. membranacea* in LS showing nectary histology. Note densely cellular region of nectariferous tissue in nectary bulb (arrow). Bar = 500 µm. **I,** Nectary of *S. nuttalii* in LS, showing modified guard cells (arrow) at juncture between bulb and shaft. Nectar is secreted through the pore formed by the guard cells. Bar = 100 µm. **J,** Young, preanthetic flower of *S. ligustrina* in LS, showing inception of nectary primordia (arrows). Bar = 100 µm. **K,** Anthetic flower of *S. ligustrina* in LS showing nectary and associated antesepalous stamen base. Note densely cytoplasmic nectariferous tissue (arrows). Nectariferous tissue region located adaxial to filament base represents a proliferation of receptacular tissue differentiated around the filament, encircling it (cf. Figs. 2F, 7D). Bar = 100 µm. — a, anther; f, stamen filament; o, ovary; s, sepal.

■ ACKNOWLEDGMENTS

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