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External microstructure of eggs from major owlet moth pests (Lepidoptera: Noctuoidea) associated with Brazilian soybean crops

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ABSTRACT

The owlet moth group of Noctuoidea (Lepidoptera) includes several significantly important pest species that cause severe defoliation or can act as borers of the reproductive structures of plants (pods, cotton bolls, corn ears). The descriptions of the microstructures and scanning electron photographs of the eggs of *Anticarsia gemmatalis* Hübner (Erebidae: Eulepidotinae), *Chrysodeixis includens* (Walker), *Rachiplusia nu* (Guenée) (Noctuidae: Plusiinae), *Spodoptera albula* (Walker), *Spodoptera cosmioides* (Walker), *Spodoptera eridania* (Stoll), *Spodoptera frugiperda* (J.E. Smith) (Noctuidae: Noctuinae), *Helicoverpa armigera* (Hübner), *Helicoverpa gelotopoeon* (Dyar), and *Chloridea virescens* (Fabricius) (Noctuidae: Heliothinae) are provided. The eggs of some of these species present peculiar morphological differences that can be useful for taxonomic purposes.

Introduction

The owlet moth group (Lepidoptera: Noctuoidea) comprises members of great economic impact such as certain species of the genera *Anticarsia* Hübner, 1818, *Chloridea* Duncan and [Westwood], 1841, *Chrysodeixis* Hübner, 1821, *Helicoverpa* Hardwick, 1965, and *Spodoptera* Guenée, 1852. These species are pests of important commodities such as soybean (*Glycine max* L.), corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.). There are at least 74 representative species of Lepidoptera, mainly within Noctuoidea (n= 35), whose larvae have already been reported feeding on soybeans in Argentina, Brazil, Chile, and Uruguay (Formentini et al., 2015; Montezano et al., 2016; Santos et al., 2016; Brito et al., 2019; San Blas and Balbi, 2021; San Blas et al., 2022).

In Brazil, the most prevalent lepidopteran species before the introduction of Bt-soybean were *Anticarsia gemmatalis* Hübner (velvetbean caterpillar) and *Chrysodeixis includens* (Walker) (soybean looper) (Luz et al., 2018). However, after the commercialization of soybeans expressing the Cry1Ac protein in 2013, a significant impact on the lepidopteran pest complex was observed, akin to the halo effect described by Wan et al. (2012). By the 2020/21 crop season, over 30 million hectares were cultivated with these genetically modified soybeans, representing 80% of the country's soybean acreage at that time (Horikoshi et al., 2021). As a result, since the 2018/2019 soybean growing season, secondary pests have emerged, such as the sunflower looper (*Rachiplusia nu* Guenée) and an undescribed species of a Neotropical budborer (Horikoshi et al., 2021; Fernandes et al., 2024).

Additionally, members of Noctuinae have also increased in prevalence, on soybean, particularly *Spodoptera frugiperda* (J.E. Smith) (Montezano et al., 2018). Congeneric species with polyphagous larvae, such as *Spodoptera eridania* (Stoll) and *S. cosmioides* (Walker) (Montezano et al., 2014; Specht and Roque-Specht 2016; Brito et al., 2024), which in the past rarely reached pest status, have also become more common. In contrast, *Spodoptera albula* (Walker) continues to occur with lower frequency (Machado et al., 2020).

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Among the species of Heliothinae, *Helicoverpa armigera* (Hübner) is widely distributed in Brazil throughout the year (Sosa-Gómez et al., 2016). Nevertheless, its population density usually does not reach threshold levels to initiate control measures, except in the west of the state of Bahia and the central region of Brazil (Specht et al., 2021). *Chloridea virescens* (Fabricius) very rarely reaches pest status in Brazilian soybean crops. Although the presence of *Helicoverpa gelotopoeon* (Dyar) has been observed in Brazil (Tood, 1955; Hardwick, 1965; Perini et al., 2019), it does not have an economic impact on soybean crops in the country. However, in Argentina, it is one of the most important species in soybean and chickpea, mainly in the Northwest region (Scalora et al., 2012; Murúa et al., 2016; Acosta-Parra et al., 2023).

Since members of this superfamily are prevalent and the morphology of their eggs is diverse, characterizing them is useful for distinguishing individuals among genera or even among species (Salkeld, 1984; Rolim et al., 2013). Proper identification is a key step in adopting control measures for each of them, mainly because different species may have different susceptibility thresholds to conventional insecticides (Smirle et al., 2013), bioinsecticides (Sosa-Gómez, 2017), toxins (Bernardi et al., 2014; Machado et al., 2020), or parasitoids (Goulart et al., 2011). In this study, we characterized the external morphology of lepidopteran eggs by examining specific structural details, including the micropylar area, the design and size of the rosettes, the number of petals, the number and distribution of aeropyles, the number of first-order ribs, and the overall morphology. The objective was to utilize scanning electron microscopy to analyze and differentiate the microstructures of eggs from various owlet moth species commonly associated with soybean crops in Brazil and neighboring countries.

Materials and methods

To describe the general side view of eggs, we followed the terminology defined by Peterson (1964), Salkeld (1984), and Korycinska (2012). Eggs were obtained from lepidopteran adults reared in the laboratory, and preserved for a short time in 75% ethanol or mercaptoethanol. All the eggs were collected from specimens occurring in the Londrina region (GPS -23.191151, -51,176451) in Paraná state except the eggs from *H. gelotopoeon* that were collected in San Agustin, Tucumán, Argentina (GPS -26.829113, -64.861971). The identification of the species was performed by mounting the male and female genitalia on microscope slides and comparing them with the descriptions provided by Hardwick (1965), Eichlin and Cunningham (1978), Gregory Junior et al. (1988), and Pogue (2002, 2004). For each species, we describe the pattern of oviposition which was observed under laboratory rearing conditions, the height and width of the eggs (n= 15-30), and their color immediately after oviposition.

All eggs of owlet moth soybean pests were characterized using Scanning Electron Microscopy - SEM (FEI Quanta 200 FEG FEI Co., Eindhoven, The Netherlands). Eggs of most species were mounted on the metal SEM-stub still attached to small pieces cut from the substrate. Because representatives of *Spodoptera* oviposit in masses and cover the eggs with modified abdominal scales (ex. Rolim et al., 2013; Brito et al., 2019), we used ovipositions from the end of adult development in which the females already laid few eggs and no longer had scales to cover them. After fixing and drying, the samples (SEM-stubs) were dried in the CO_2, Critical Point Drying equipment and coated with 25 nm of gold. The microstructure of the eggs was photographed from various aspects at relatively low magnifications to show their shape and general appearance. Photographs were taken at higher magnifications to illustrate several features of the chorion that can be used as taxonomic characters, including the position and form of cellular markings and reticulation, the morphological characteristics of the micropylar area, the position and size of the aeropyles, and the surface texture of the chorion.

Results

Anticarsia gemmatalis

The eggs of *A. gemmatalis* are laid individually by moths on their host plants. They are green with bluish tones, hemispherical, and flattened at the base (Fig. 1A). The micropylar rosette has cells demarcated by delicate ribs and is formed by seven to 11 primary cells, and the number of micropyles ranges from four to six (Figs. 1B and 1C). The eggs have conspicuous primary and secondary ribs visible under an optical microscope at 400x magnification that are much more elevated and prominent than those delimiting the cells of the rosette (Figs. 1A, 1B, and 1D). The primary ribs are distinctly more elevated as they extend from the rosette and subdivide towards the lower pole of the egg, reaching the cells surrounding the micropyle, whereas the secondary ribs do not reach the micropylar rosette and are less elevated. Between the primary and secondary ribs, there are less elevated transverse commissures that connect one to another resulting in small rectangular areas or rib cells (Figs. 1A, 1B, and 1D). Aeropyles (1-2 μm) are located at the junction points of the radial and transverse ribs, sometimes present in pairs (Fig. 1D). Additionally, they can have 29 to 33 primary and secondary radial ribs. We found that the average diameter of the egg ranges from 590 μm to 615 μm. The texture of the chorion surface appears undulated at lower magnification (Figs. 1C and 1D) and relatively smooth at 12,000x magnification (Fig. 1E).

Chrysodeixis includens

The eggs of *C. includens* are laid individually by moths on their host plants. They are light green, hemispherical, and flattened at the base. The average diameter of the egg ranges from 490 μm to 544 μm. The rosette is delimited in an area where its cells connect to the longitudinal ribs by a sequence of somewhat geometric cells, formed by slight elevations of the chorion. The cells forming the primary rosette are partially fused at the base, with a conspicuously depressed distal end. The inner micropylar rosette consists of six to 10 petals (Figs. 2B, 2C) surrounded by two concentric petal series, with petals that gradually increase in size toward the outer edge (Fig. 2C); the distal end of these petals can appear faint (Fig. 2B). The lateral regions of the eggs exhibit conspicuous radial ribs that range from 32-37 ribs, including primary ribs (9-11), secondary ribs, and transverse ribs, which create a rectangular pattern on the egg surface (Figs. 2A, 2D). Aeropyles (0.3-1.2 μm), often found in pairs and are located at the junction points of the radial and transverse ribs (Fig. 2D). The texture of the chorion surface, observed under 12,000x magnification, appears rough (Fig. 2E).

Rachiplusia nu

The eggs of *R. nu* also are laid individually by moths on their host plants. They are bright yellow-white, sub-spherical, with a flat and smooth base (Fig. 3A). The diameter of the egg ranges from 544 μm to 620 μm. The rosette is delineated in an area with inconspicuous sculpturing, losing connection with the radial ribs. The number of micropyles varies from three to four. The micropylar region is slightly depressed. The micropylar rosette consists of seven to eight petals that fuse at the base and cover an approximate diameter of 45 μm (Fig. 3B). This rosette could be surrounded by two, three, or four almost concentric rosettes (secondary, tertiary, and quaternary). The petals

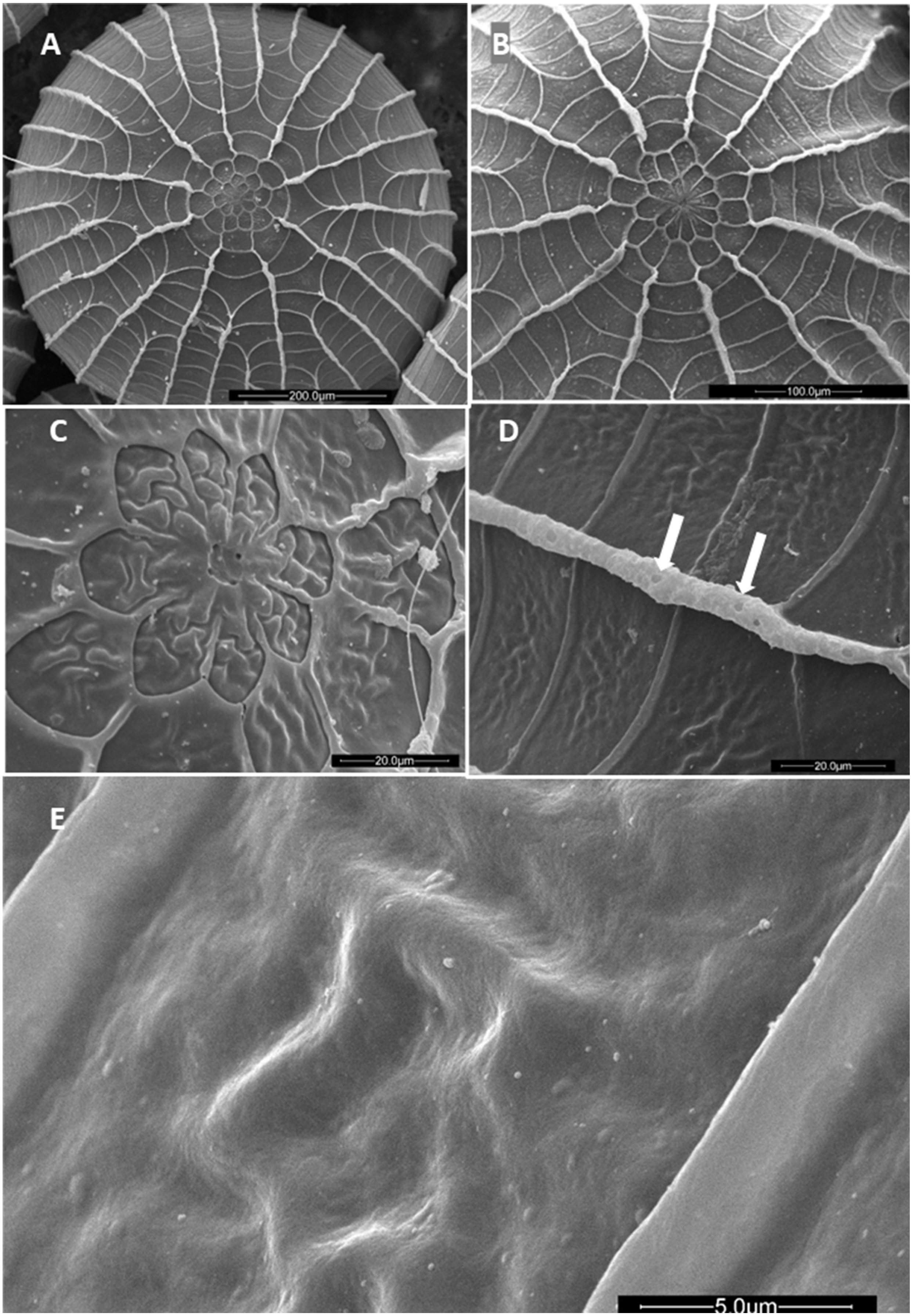

Figure 1 *Anticarsia gemmatalis* eggs. A) eggshell, B) micropylar region, C) petals of the micropylar area with 4 micropyles, D) aeropyles on the radial ribs (white arrows), E) texture of the eggshell surface.

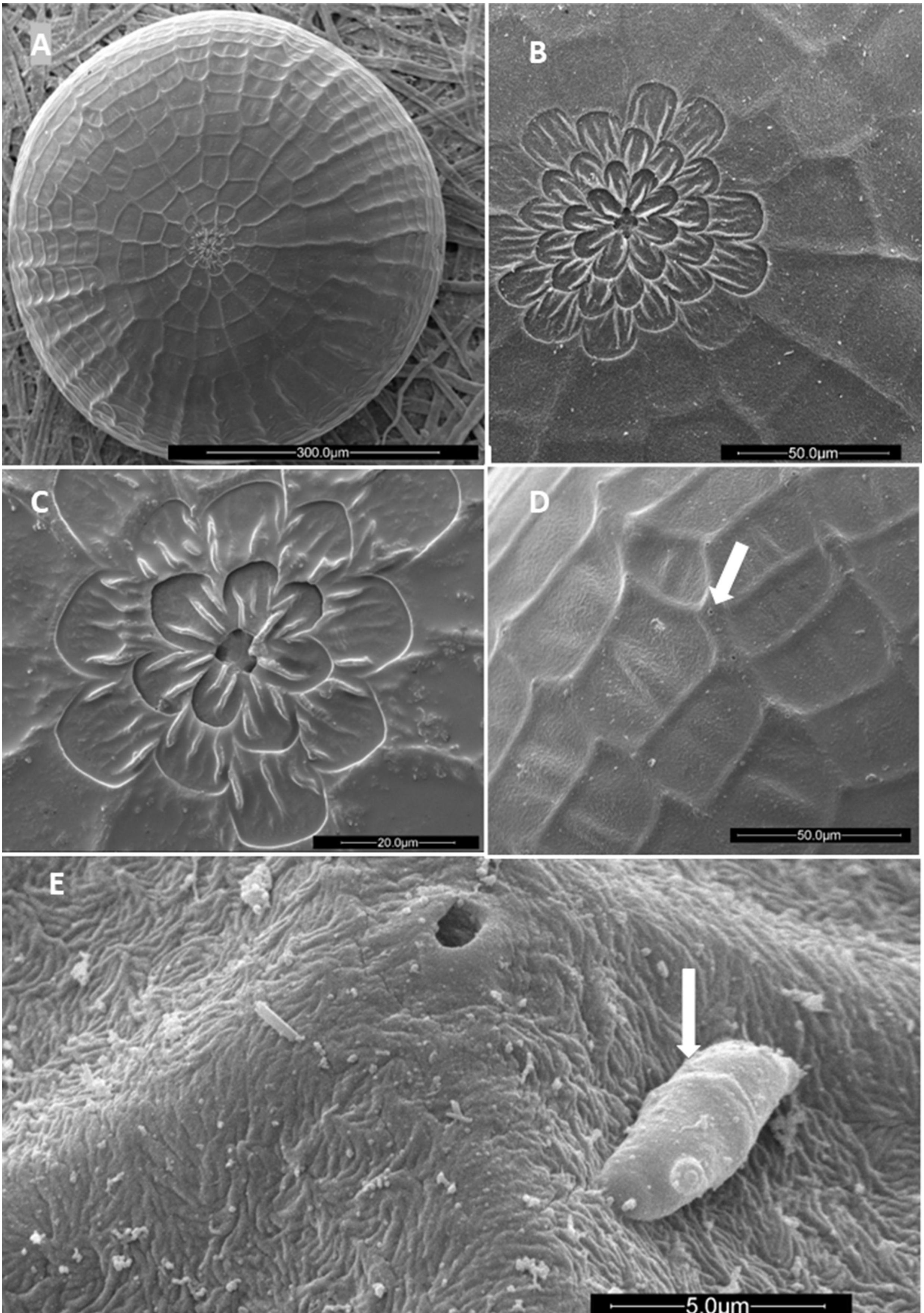

Figure 2 *Chrysodeixis includens* eggs. A) eggshell, B) micropylar region, C) petals of the micropylar area with micropyles, D) aeropyles at the junction of the radial and transverse ribs (white arrow), E) aeropyle and texture of the eggshell surface (presence of unidentified spore, white arrow).

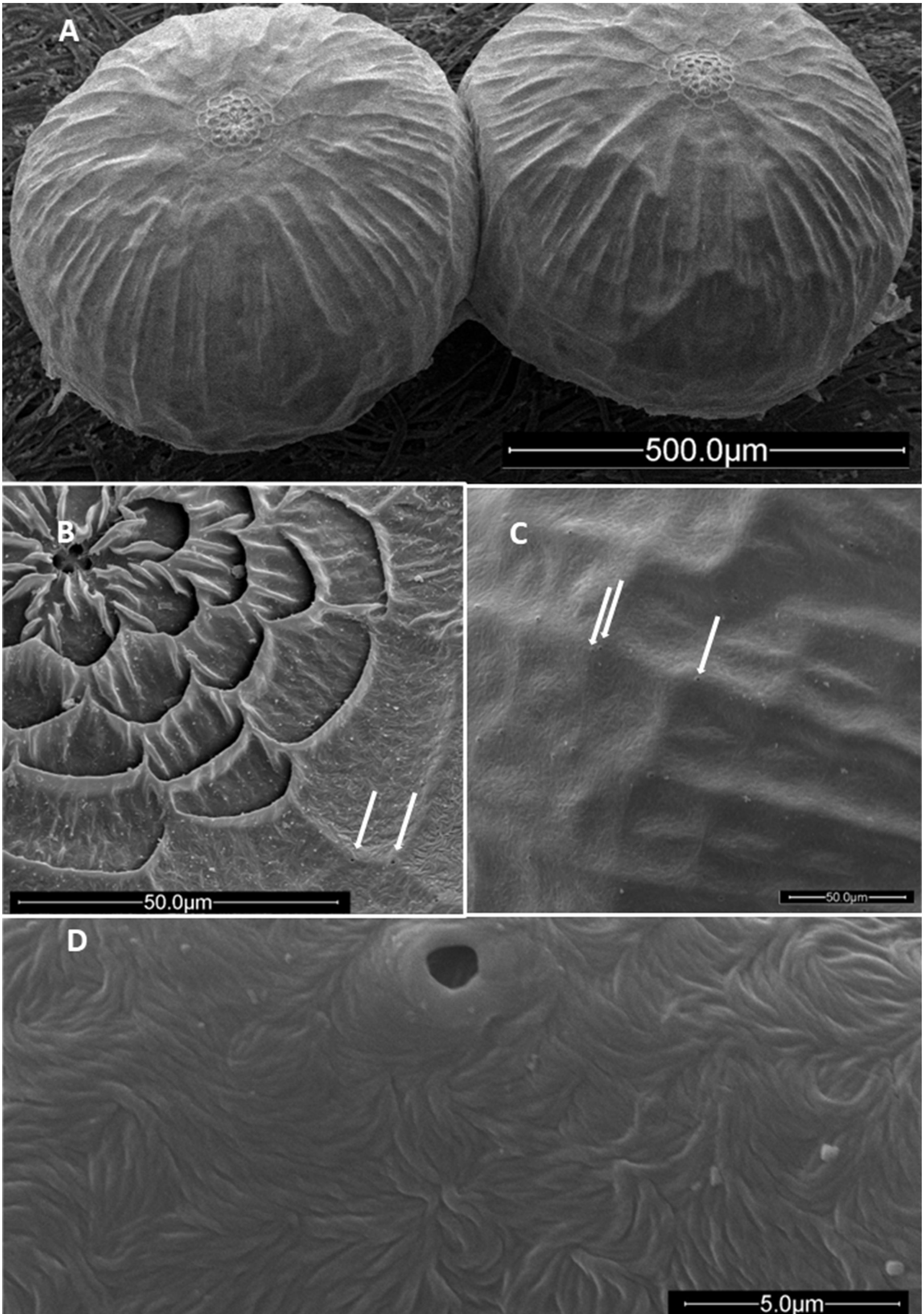

Figure 3 *Rachiplusia nu* eggs. A) eggshells, B) micropylar region and petals of the micropylar area with 3 micropyles, C) aeropyles at the junction of the radial and transverse ribs, sometimes with one pair of aeropyles (white arrows), D) texture of the eggshell surface.

of the rosette (composed of secondary, tertiary, and quaternary cells) are not evenly distributed in a concentric pattern; they may overlap, be unevenly arranged, or be absent. All petals have rounded distal margins gradually increasing in size towards the outer edge; the outer edge of the petals of the third rosette is not always evident. Tertiary or quaternary cells may have an incomplete distal edge and a rounded distal margin. The lateral regions of the eggs have fairly flat radial ribs, consisting of primary ribs, secondary ribs, and cross-ribs, the latter being less conspicuous. The number of ribs ranges from 36 to 38 and the primary ribs (n=12-13) reach the micropylar area (Figs. 3A and 3B). The aeropyles are present at the junction points of the ribs and cross-ribs and can often be found in pairs (Fig. 3C). Aeropyles (0.5-1.2 µm) can also be found on the primary ribs near the outer edge of the rosette petals, solitary or in pairs (Fig. 3B). The texture of the chorion surface, observed at a magnification of 12,000x, is rough and resembles a fabric texture, similar to a wool yarn (Fig. 3D).

Spodoptera albula

The eggs of *S. albula* are laid by the moths in clusters ranging from 100 to 450 eggs, covered by modified light beige abdominal scales (Fig. 4A). All the ribs are conspicuous and is not possible to classify them into primary and secondary ribs (Fig. 4A). The average number of micropyles is 4 (3–6). The micropylar area of *S. albula* eggs is surrounded by primary cells in the form of petals and secondary cells with somewhat angular distal ends, similar to the primary cells. Meanwhile, the tertiary and quaternary cells blend with the general reticulation of the chorion (Figs. 4A and 4B). The cross-ribs are less conspicuous than the ribs (Fig 4D). The aeropyles, which typically range in diameter from 0.5 to 1 µm, may or may not be present at the junction points of the ribs and cross-ribs, usually isolated but could be in pairs. They are predominantly located on the top third portion of the egg. (Figs. 4A and 4C). The texture of the chorion surface, observed at a magnification of 8,000x, is irregularly rugged (Fig. 4D).

Spodoptera cosmioides

The eggs of *S. cosmioides* display several colors, ranging from green, grey, and brown to non-glossy pinkish-brown, and they are laid in two to three layers. The egg masses are generally covered with filamentous modified scales (Fig. 5A) characteristic of many *Spodoptera* species. Aeropyles (0.5-1.5 µm) are distributed on the junction points where the ribs and cross-ribs converge, as well as, on the vertical ribs of the outer third series of cells from the micropylar region (Figs. 5C and 5D). The average numbers of ribs and micropyles are 48 (46–54) and 3 (3–4), respectively, and the micropyle is surrounded by a rosette composed of 10 (8–12) primary cells (Fig. 5B). The distal ends of the primary petals of the rosette are rounded (Fig. 5B). The texture of the chorion surface, observed at a magnification of 12,000x, is irregularly rugose (Fig. 5E).

Spodoptera eridania

The eggs of *S. eridania* are light green and somewhat shiny, and as in other *Spodoptera* species are deposited in one or occasionally two or three layers. Egg masses are generally covered with filamentous modified scales. They are hemispherical and flattened at the base. They may be covered with abundant filamentous scales (Fig. 6A). Their average diameter and height are 480.3 µm (430–521) and 370.2 µm (292–420), respectively (Fig. 6B). The average number of micropyles is 3.9 (2–5) (Fig. 6D) and they are surrounded by a rosette composed of 10.6 (8–14) primary cells (Figs. 6B and 6C). The distal ends of the rosette have rounded petals on their outer edge (Figs. 6B and 6C). The number of ribs in the eggs of this species ranges from 51 to 52, which falls within the range of *S. albula* and *S. cosmioides*. The aeropyles are arranged at the junction of the ribs and cross-ribs. The texture of the chorion surface, observed at a magnification of 12,000x is rugous.

Spodoptera frugiperda

Although this species was not commonly found in soybean crops, in recent years it has become more frequent as a defoliator and it can also exhibit behaviour similar to cutworms. The eggs are bluish-grey to green, laid in groups, and distributed in layers, making it difficult for some parasitoids to act (Beserra and Parra, 2005). The eggs can be covered with abundant filamentous scales. They are nearly spherical, ranging from 390 µm to 560 µm in diameter (Fig. 7A). The micropyle is surrounded by irregularly shaped 7–12 primary cells (petals) and the number of micropyles ranges from three to four (Figs. 7B and 7C). The aeropyles (0.6-0.9 µm) are located at the intersection of the ribs and cross-ribs (Figs. 7D and 7E). The number of ribs ranged from 50 to 63, with the cross-ribs slightly thinner than the ribs. The texture of the chorion surface, observed at a magnification of 12,000x, resembles a fabric texture, similar to intertwined wool yarn (Fig. 7F).

Helicoverpa armigera

The eggs of this species are laid individually. They are hemispherical, with a flattened base and initially light yellow. They measure between 500 µm and 540 µm in diameter (Fig. 8A). Micropylar area is convex, depressed at the center, surrounded by a rosette of 11 to 14 primary cells (Figs. 8B and 8C), narrower basally, distally elongated with margins broadly rounded appearance (Figs. 8A and 8B). The average number of micropyles is four (2–4). On the space between the micropylar area and the primary ribs, there are no chorion sculptures (Fig. 1A). At the top of the egg, the primary ribs are quite pronounced and elevated, reducing in height below the equatorial region where they are no longer visible (Figs. 8E and 8F). The primary and secondary ribs are somewhat blunt, particularly the transverse cross-ribs that along with the ribs, form a reticulate pattern of cells.

We found 23 to 27 ribs, being 11- 13 primary ribs, and 11 -14 secondary ribs (Figs. 8C and 8D). The eggs have one or two aeropyles (0.7µm – 1.4µm) located on the ribs, arranged infra-equatorially (Figs. 8E and 8F) in the transition zone between the keel region and the base of the egg, which has larger surface irregularities (Figs. 8D and 8F). The surface of the egg between the ribs has a fine rugosity, resembling fabric, which is observed more clearly at a magnification of 12,000x (Fig. 8G).

Helicoverpa gelotopoeon

Eggs are laid individually. They are hemispherical with a slightly flattened base, and do not have visible ribs at the base. Initially, they are light yellow and measure between 515 µm and 540 µm in diameter and 528 µm to 547 µm in height (Fig. 9A). The average number of micropyles is three (2–4) and they are surrounded by a rosette composed of 11 to 14 primary cells (Figs. 9B and 9C). The petals have outlines in low relief (Fig. 9B). The distal ends of the rosette petals have a rounded appearance (Figs. 9A and 9B). In specimens collected in northern Argentina, we found 27 to 32 radial ribs, with 12 to 16 primary radial ribs near the petaloid rosette and 15 to 16 secondary ribs. The surface of the petals of the primary rosettes is smoother than the surface outside the petals; additionally, the petals show a bubble appearance inside (Fig. 9B). The aeropyles (0.6 µm - 0.9 µm), from one to four on each radial keel,

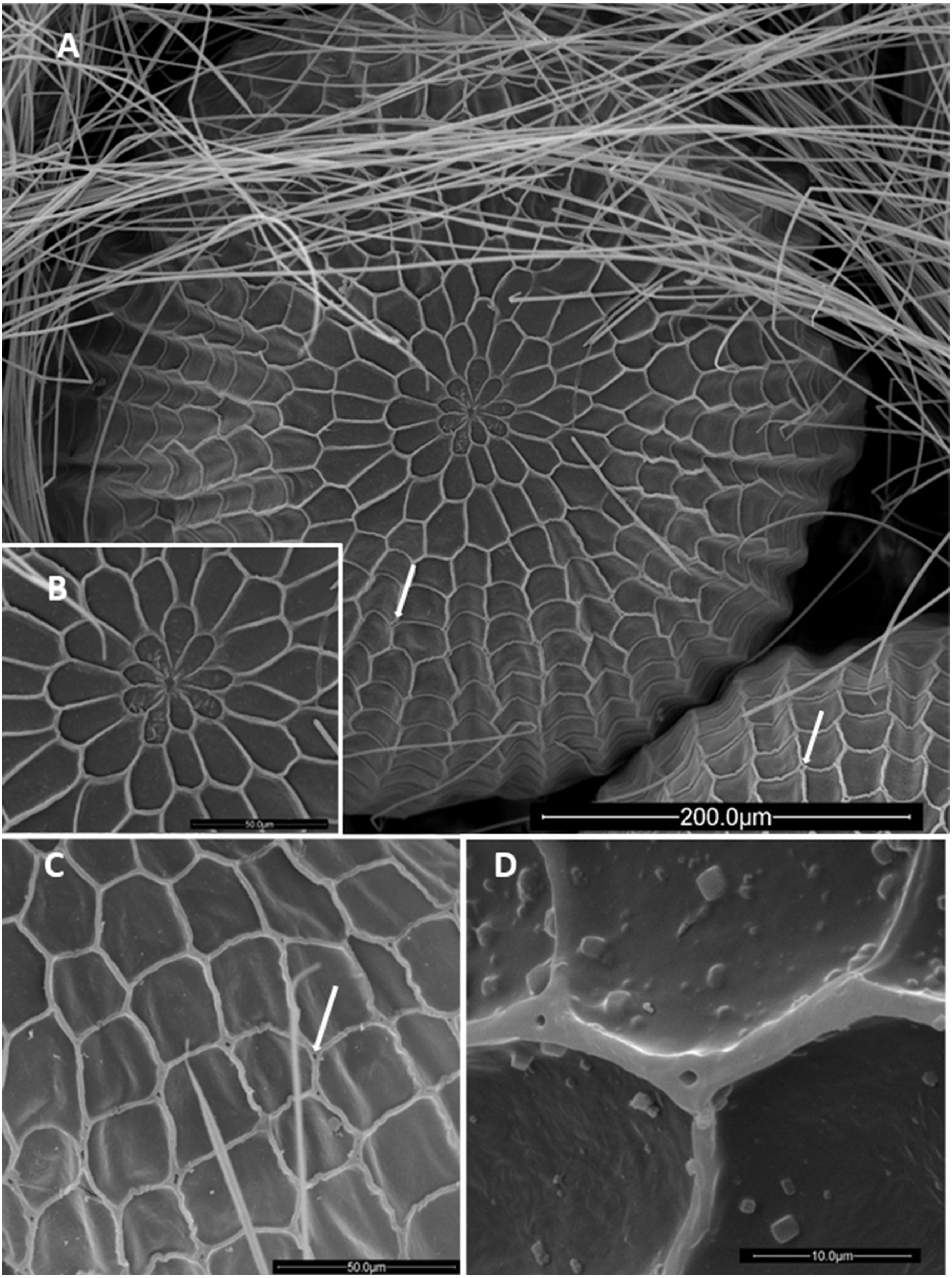

Figure 4 *Spodoptera albula* eggs. A) eggshell, B) micropylar region and petals of the micropylar area with 4 micropyles, C) aeropyles distributed along the radial ribs (white arrow), D) aeropyles and texture of the eggshell surface.

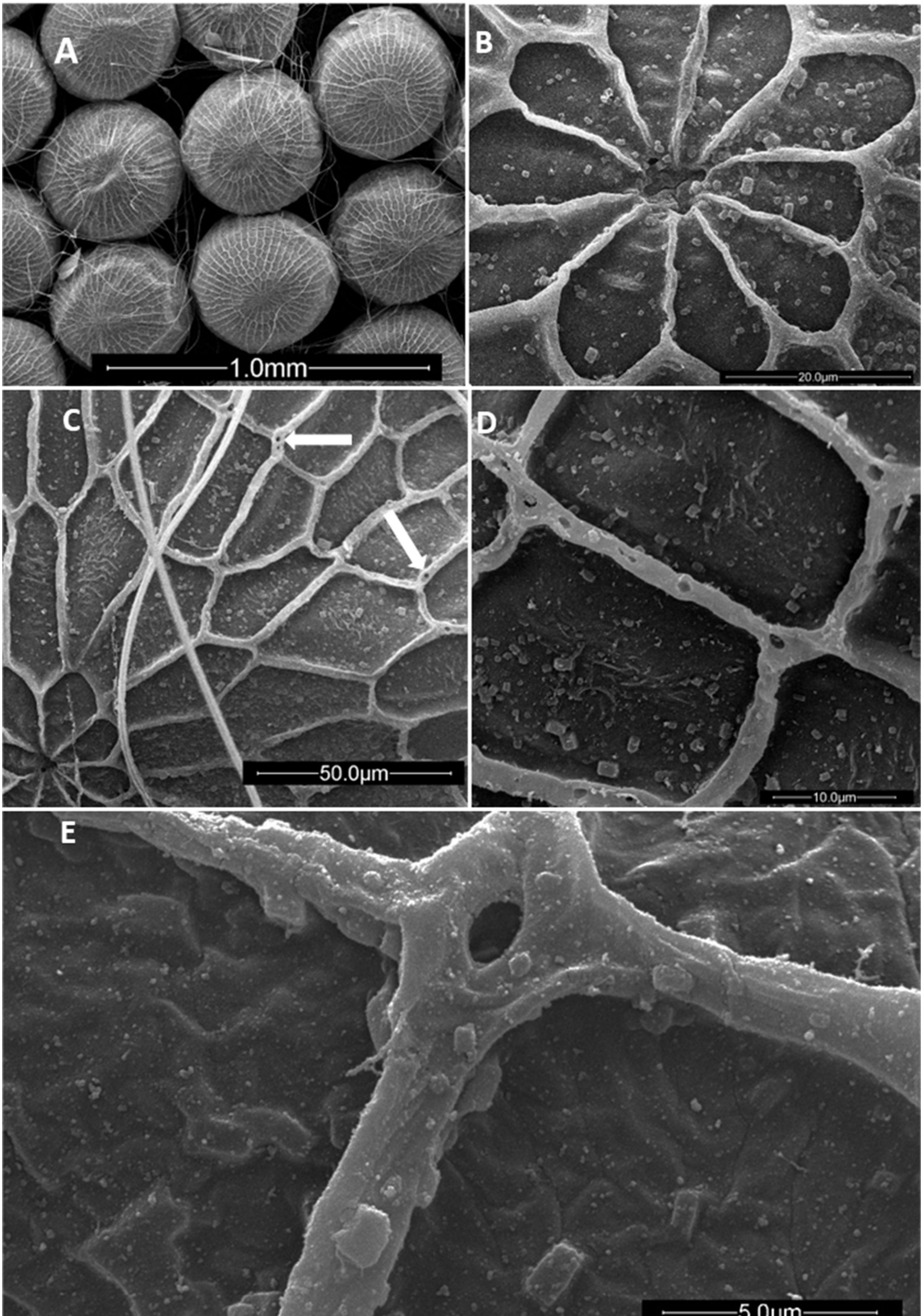

Figure 5 *Spodoptera cosmioides* eggs. A) eggshell, B) micropylar region and petals of the rosette, C) micropylar area with 3 or 4 micropyles, D) aeropyles distributed along the radial ribs, E) aeropyle and texture of the eggshell surface.

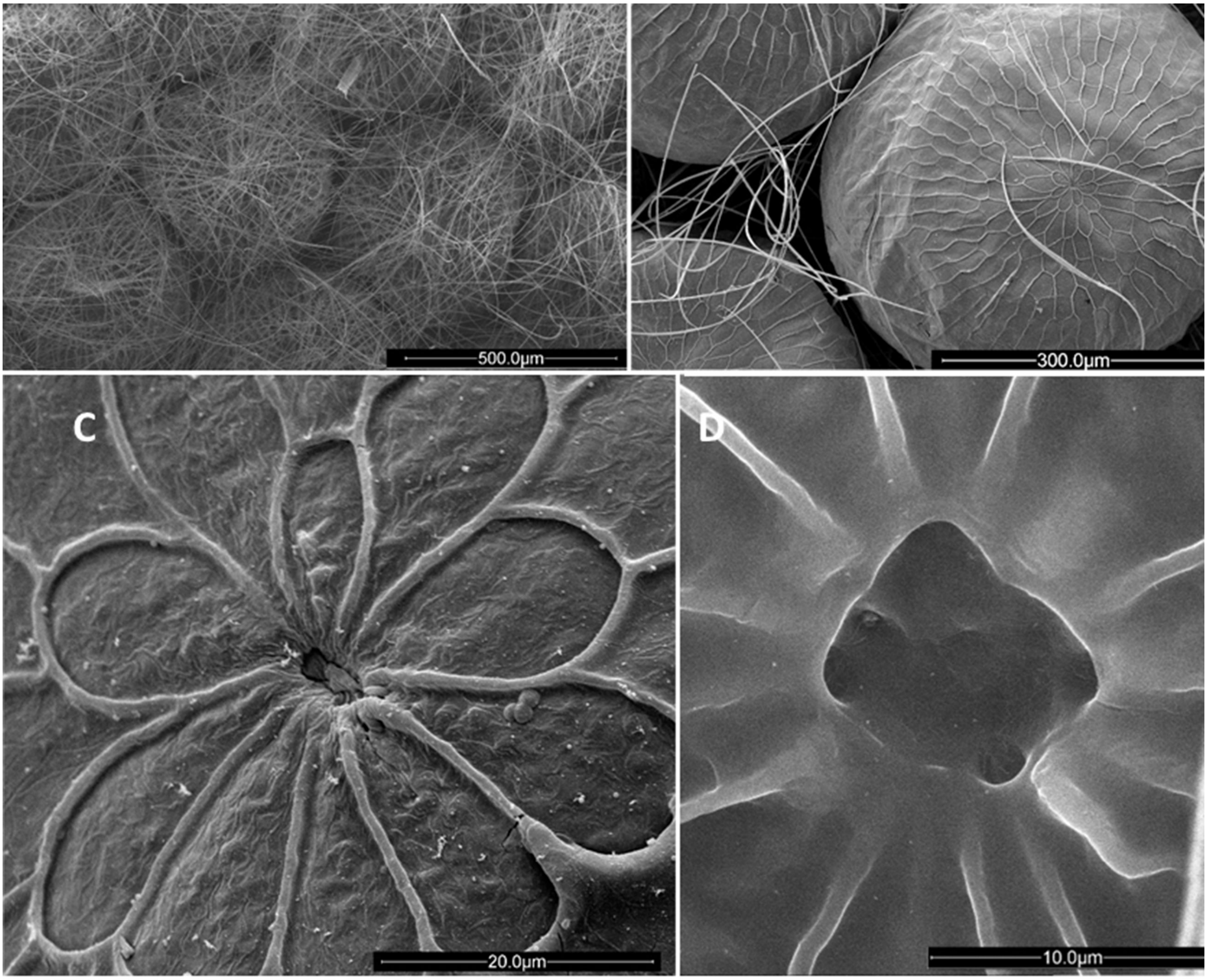

Figure 6 *Spodoptera eridania* eggs. A) eggs covered with filamentous scales, B) eggshell, C) texture of the petal surface, D), and E) micropylar area with 4 micropyles.

are distributed near the upper third of the egg, approximately at the height where the secondary ribs end, or lower than that (Figs. 9D and 9E). The texture of the egg surface is wrinkled and visible at magnifications between 12,000x and 15,000x (Fig. 9F).

Chloridea virescens

Eggs are hemispherical with a flattened base, laid individually, initially white or light yellow, and measuring 514 µm to 530 µm in diameter and 528 µm to 540 µm in height (Fig. 10A). The micropyles, from four to six, are surrounded by rosettes with a variable number of primary cells, ranging from 12 to 20 (Figs. 10A, 10B, and 10C). In the lateral region, there are 10 to 13 primary and 10-12 secondary ribs. The primary ribs terminate at the top of the egg, closer to the micropylar region than the secondary ribs (Fig. 10A). Aeropyles (0.6 µm – 2.5 µm), from two to five, are present on the primary ribs, mainly close to the top region of the egg (Figs. 10D and 10E), but sometimes they are present on the secondary ribs. The surface of the egg has a stippled texture (Fig. 10F).

Discussion

The surface ornamentation of the eggs exhibits variable morphological characteristics, including the number of radial ribs, micropyles, and aeropyles. Features such as egg size, number of primary cells of the rosette, and number of micropyles exhibit considerable variability, with overlapping values that make differentiation difficult. However, the number of radial ribs can distinguish between eggs laid individually (e.g., *A. gemmatalis*, *C. includens*, *R. nu*, *C. virescens*, *H. gelotopoeon*, and *H. armigera*) and those laid in groups (e.g., *S. albula*, *S. cosmioides*, *S. eridania*, and *S. frugiperda*) (see Table 1). Therefore, we aimed to characterize these variations and address previously undescribed aspects such as the surface texture and the distribution of aeropyles on the eggs of *S. cosmioides*, *C. virescens*, and *H. armigera*. Additionally, we incorporate more detailed descriptions of the eggs mentioned in the literature, such as in the case of *H. gelotopoeon* (Hardwick, 1965; Angulo et al., 2008).

Anticarsia gemmatalis. Cônsoli et al. (1999) determined a slightly larger diameter than what we observed, measuring 628 µm, with a height of 428 µm. They also noted the presence of numerous aeropyles along

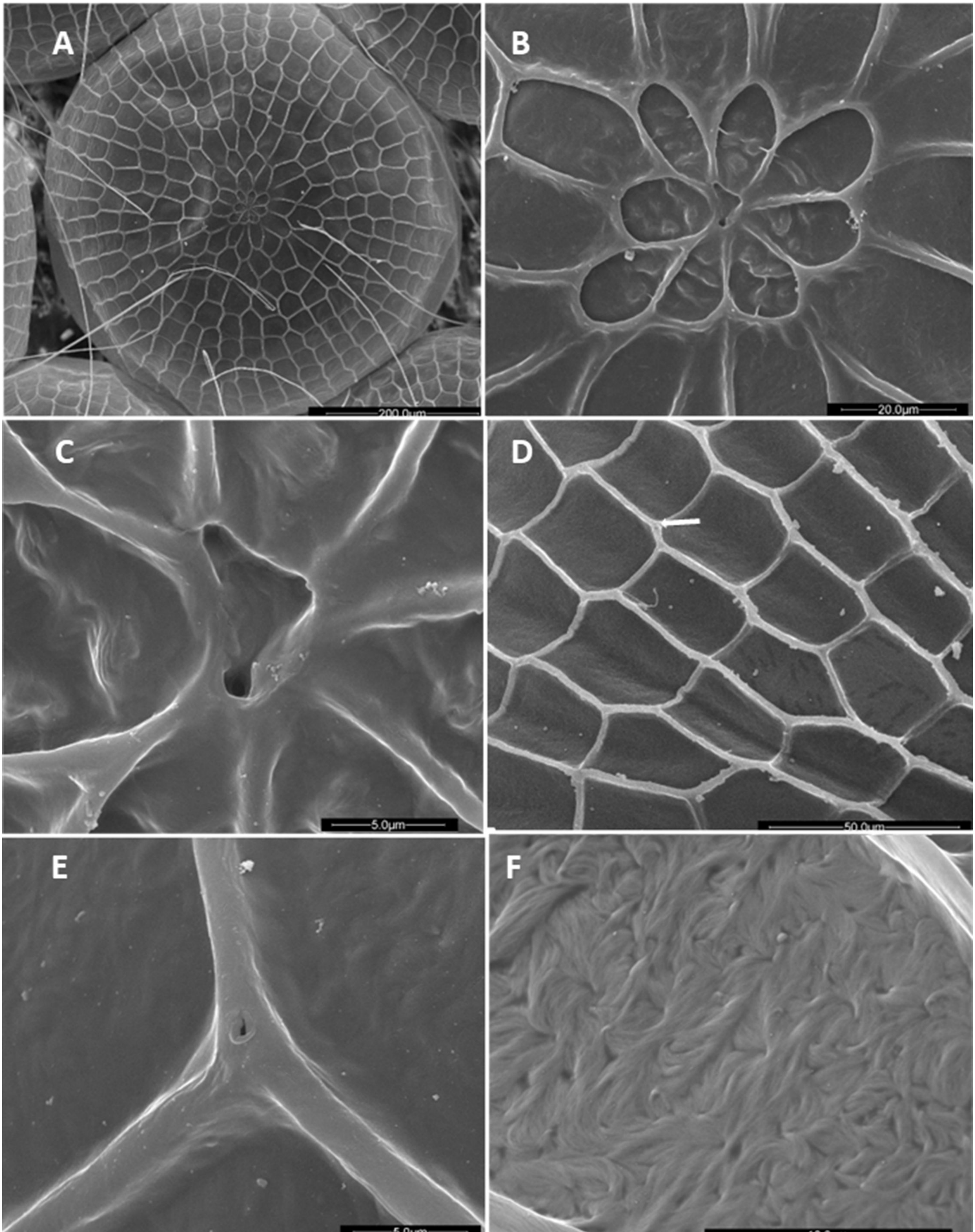

Figure 7 *Spodoptera frugiperda* eggs. A) eggshell, B) micropylar region and petals of the rosette, blistered surface inside the petals, C) micropyles, D) aeropyles distributed along the radial ribs (white arrow), E) aeropyle, F) texture of the eggshell surface.

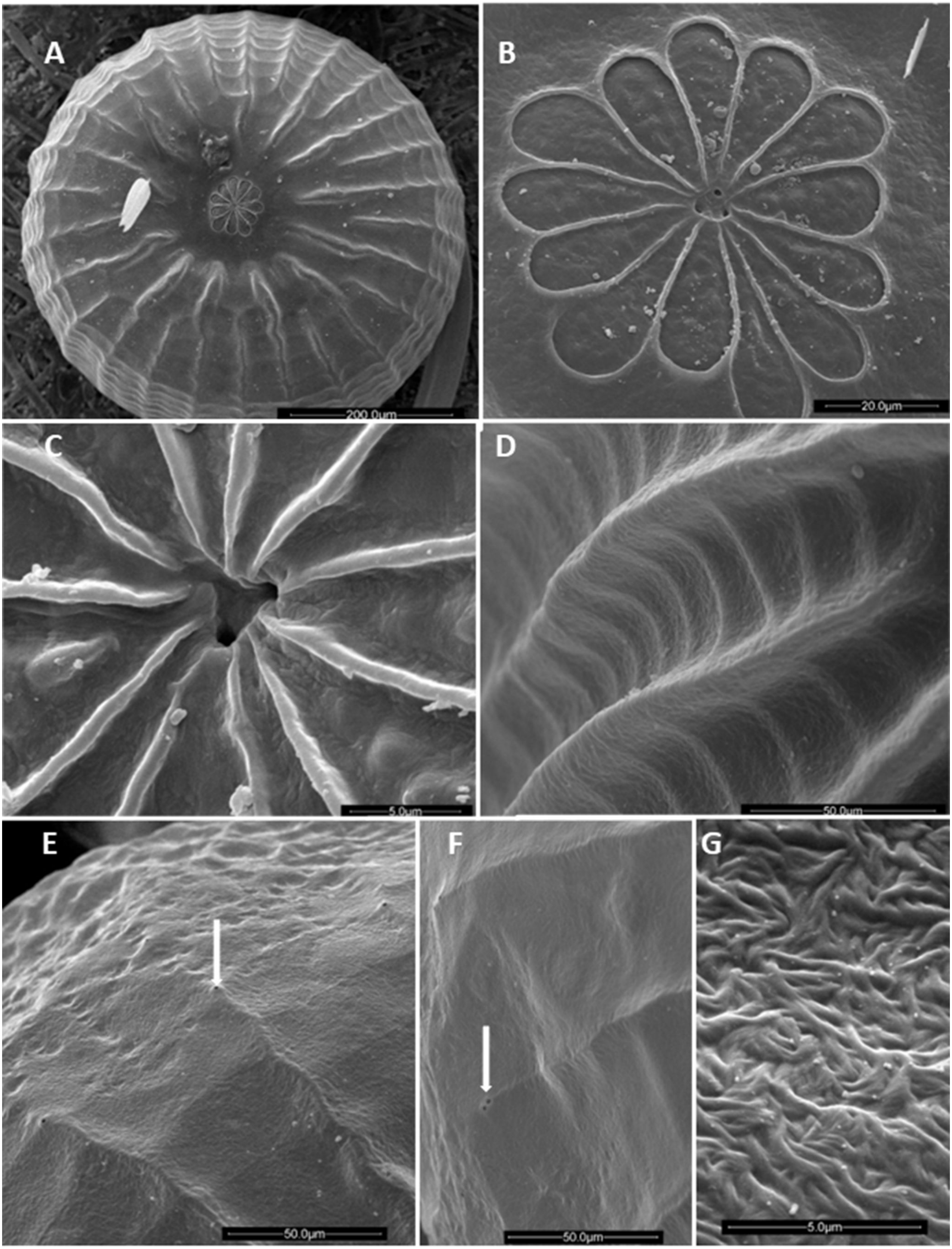

Figure 8 *Helicoverpa armigera* eggs. A) eggshell, micropylar region, and petals of the rosette, B) and C) micropyles, D) conspicuous radial ribs, E) aeropyles (arrow) distributed along the transition of the area with the ribs, F) lower rugose portion of the egg, lower than the equatorial region, G) texture of the eggshell surface.

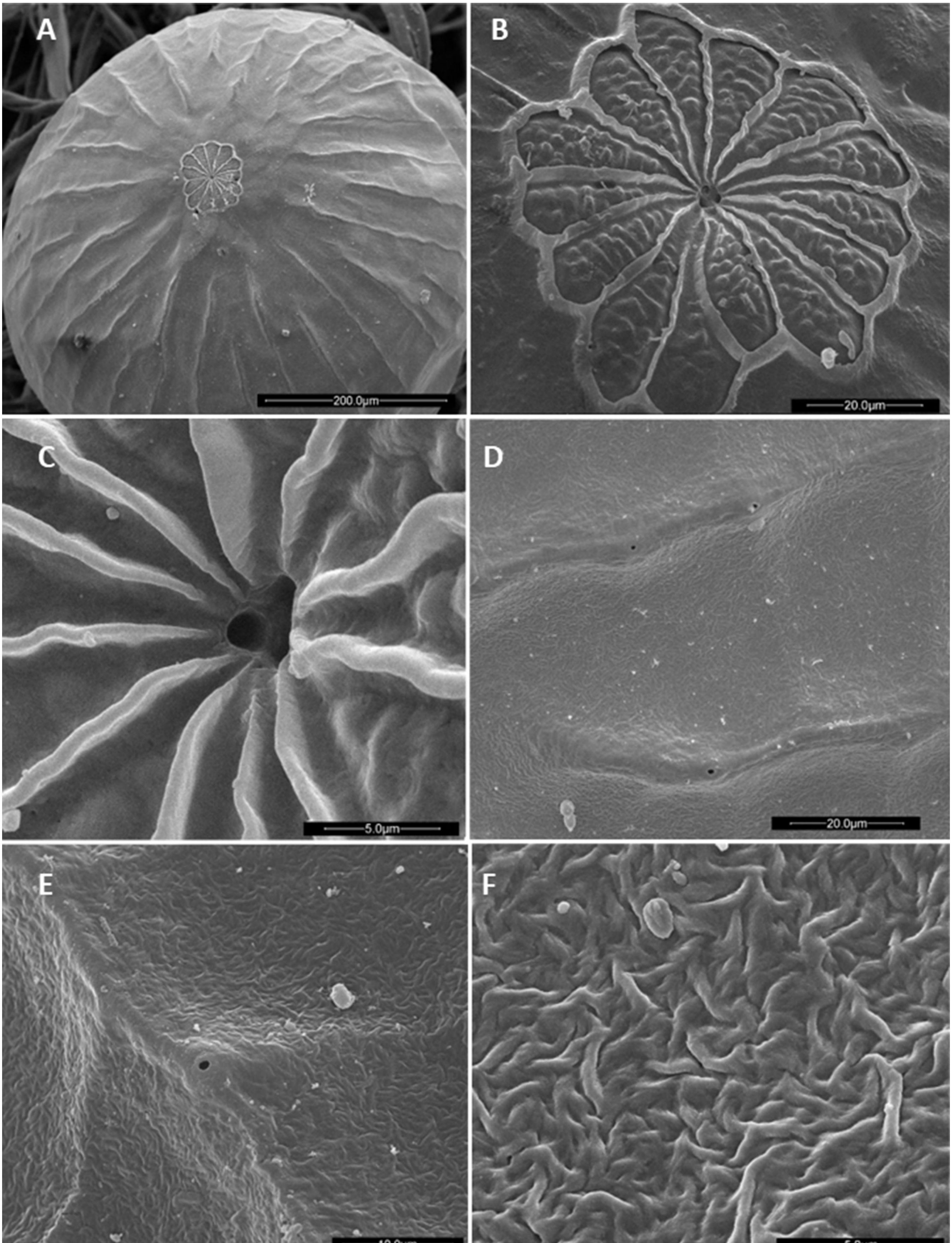

Figure 9 *Helicoverpa gelotopoeon* eggs. A) eggshell, B) micropylar region and petals of the rosette, C) micropyles, D) aeropyles distributed along the radial ribs near the top region of the egg, E) aeropyle, F) texture of the eggshell surface.

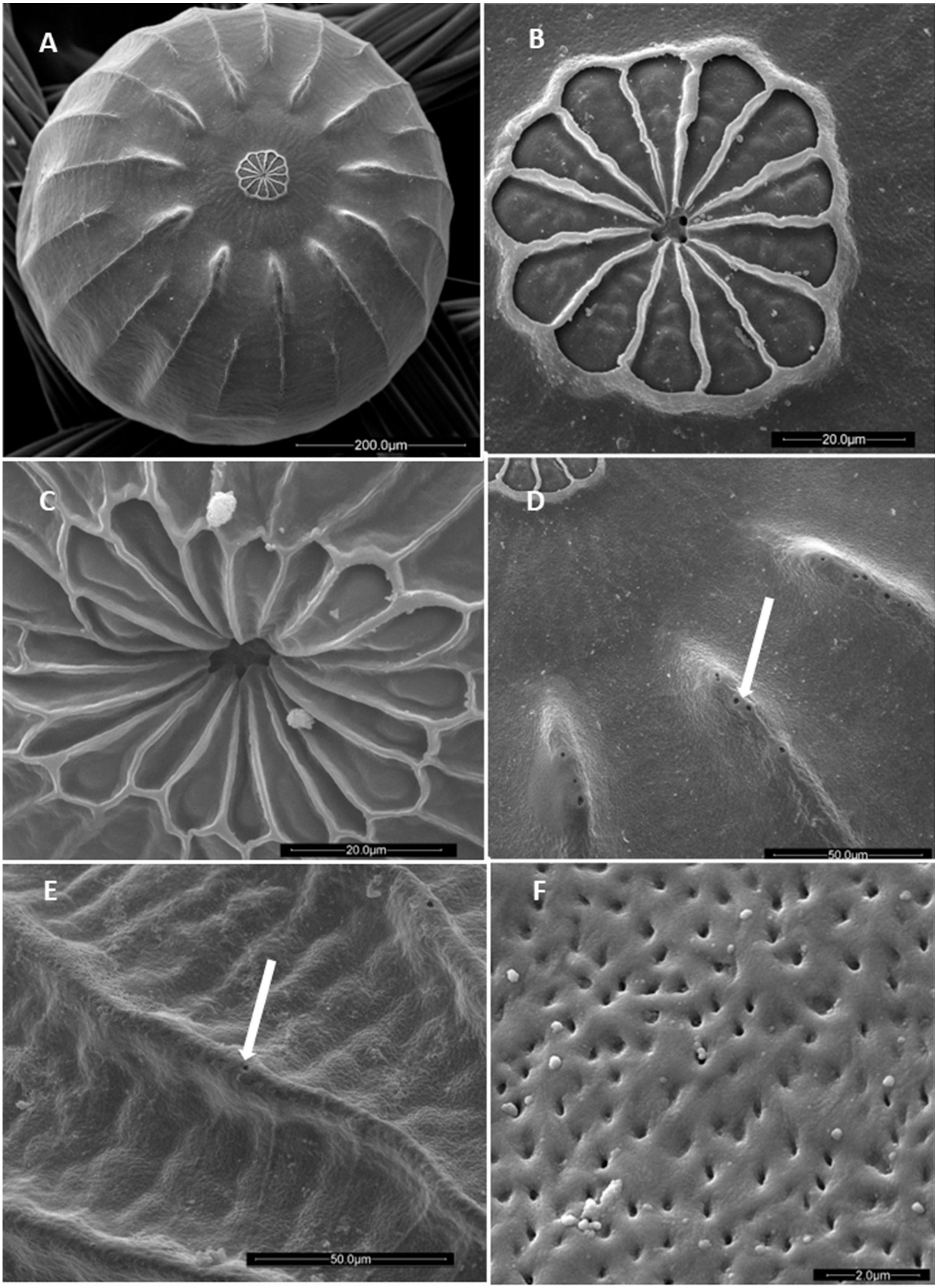

Figure 10 *Chloridea virescens* eggs. A) eggshell, B) and C) micropylar region and petals of the rosette with three micropyles, D) variable number of aeropyles distributed on the ribs (white arrow), E) variable number of aeropyles distributed along the radial ribs (white arrow), F) stippled texture of the eggshell surface.

Table 1
Characters related to eggs from major owlet moth pests (Lepidoptera: Noctuoidea) associated with Brazilian soybean crops.

Species	Egg laying	Initial color	Diameter µm	Micropyles	Number of primary cells	Number of ribs
A. gemmatalis	individually	light green	590 - 615	4 - 6	7 - 11	29 - 33
C. includens	individually	light cream	490 - 544	3 - 6	6 - 10	32 – 37
R. nu	individually	white yellow	544 - 620	3 - 4	6 - 9	33 - 41
S. albula	egg masses*	pale green with gray hues	475 - 540	3 - 6	6 - 11	45 - 48
S. cosmioides	egg masses*	green, gray, brown to dull pink brownish	396 - 517	3 - 4	8 - 12	46 - 54
S. eridania	egg masses*	light green	430 - 521	2 - 5	8 - 14	51- 52
S. frugiperda	egg masses*	light pink or pearl	390- 560	3 -4	7 - 12	50 - 63
C. virescens	individually	cream to white greenish	514 -530	4 - 6	12 - 20	18 - 25
H. armigera	individually	cream to light yellow	495 - 555	2 - 4	11 - 14	23 - 27
H. gelotopoeon	individually	cream to light yellow	515 - 540	2 - 4	11- 14	27 -32

*egg masses usually covered by modified scales detached from the female moth's abdomen.

the ribs and at the intersections between the vertical and transverse ribs. The number of primary ribs reaching the cells surrounding the micropylar region ranged from 9 to 11, which is slightly higher than the 9 to 10 reported by Cônsoli et al. (1999).

Chrysodeixis includens. Although the number of radial ribs often ranges from 30 to 37 (Fig. 2A), Peterson (1964) described a variation from 31 to 33, and in a photograph of *C. includens* egg published by Angulo et al. (2008) it is possible to count 37 ribs. Barrionuevo and San Blas (2016) also described the eggs of this species and mentioned the presence of 30-32 ribs and 8-9 primary cells.

Rachiplusia nu. The number of ribs ranges from 32 to 38 according to Barrionuevo (2011) or 39 according to Angulo et al. (2008), and a few of them extend from the base to the micropylar area. Many of the egg characteristics are similar or overlap with those of *C. includens* eggs. Consequently, even in dichotomous keys, differentiating between these species can be difficult (Barrionuevo and San Blas, 2016).

Spodoptera albula. The most distinctive characteristics that differentiate *S. albula* from other *Spodoptera* species are the fewer number of ribs compared to *S. eridania* and *S. frugiperda*, as well as the color of the eggs (Table 1). The other characteristics such as diameter of the egg, number of micropyles and number of primary cells from the rosette overlap.

Spodoptera cosmioides. Complementary to the features described by Rolim et al. (2013) is the distribution of the aeropyles, which differs from that of *S. albula* and *S. eridania*. These aeropyles are typically found at the distal end of the second series of concentric cells, which are distant from the rosette, and are usually solitary, although they can sometimes occur in pairs.

Spodoptera eridania. Valverde (2007) observed that *S. eridania* eggs collected in northern Argentina have 40–44 ribs and four micropyles, with the rosette composed of ten primary cells. This is a lower number than observed in the present study; however, a more extensive sampling is necessary. Aeropyles are visible at the distal end of the third series of concentric cells, which are distant from the rosette

Spodoptera frugiperda. As mentioned by Cônsoli et al., 1999, the aeropyles are located at the intersection of the ribs and cross-ribs as well as the other species of *Spodoptera* mentioned in this work. The number of ribs (50-63) were in a range wider than mentioned by Peterson (1964) of 47 to 50 ribs and by Kannan et al. (2021) of 58 to 60 ribs. The number of primary cells in the rosette ranged from 7 to 12, encompassing the value of 9 reported by Cônsoli et al. (1999).

Helicoverpa gelotopoeon. This species has limited occurrence in the southern region of Brazil and frequent occurrence in Central-Northern Argentina and can cause damage to soybean, chickpea, and corn crops (Murúa et al., 2016). The number of primary cells in the rosette, ranging from 11 to 14, aligns with the average number of cells (13.2) cited by Angulo et al. (2008).

Helicoverpa armigera. The introduction of *H. armigera* to the American continent and its subsequent hybridization and introgression into *H. zea* populations over successive generations (Valencia-Montoya et al., 2020; Rios et al., 2022) can complicate the morphological differentiation of these hybrids from the parent species. This species is occasionally found in the southern region of Brazil, in the beginning of the soybean season, but it occurs more frequently in the western part of Bahia and Mato Grosso, where it can cause damage to soybean crops. Given that its populations are typically controlled by natural enemies in the south region of Brazil, outbreaks of this pest do not occur in fields with proper pest management.

The morphological variations may be associated with the place of origin. For example, the rosette is composed of 11 to 14 primary cells (Figs. 8B and 8C). Nonetheless, Korycinska (2012) reported that the number of primary cells can vary from 15 to 19. In individuals collected in Brazil, we found 23 to 27 ribs (Figs. 8C and 8D). Coincidentally Queiroz-Santos et al. (2018) mentioned 23 ribs and 12 primary cells in the rosette, while Korycinska (2012) affirmed they can have 28 to 33 ribs. Therefore, a wide collection samples in different geographical locations should be performed to know the extent of this variability.

Chloridea virescens. This species is rarely found in soybean crops, but it can occasionally cause damage to it. In contrast, it can be important in chickpea and tobacco crops (Borella Júnior et al., 2022; Acosta-Parra et al., 2023). It is difficult to tell apart the eggs of *C. virescens*, *H. zea*, and *H. armigera*. Bernhardt and Phillips (1985) reported that the primary ribs of *C. virescens* eggs terminate before reaching the rosette of cells surrounding the micropyle, in contrast to *H. zea* eggs. This does not seem to be the case in comparisons between the eggs of *C. virescens* and *H. armigera* either (Figs 8A and 10A). The distance between the rosette perimeter and the primary ribs overlaps for both species: 54-65 µm for *C. virescens* and 40-56 µm for *H. armigera*. Peterson (1964) mentioned that the number of ribs ranges from 22-24, which is inside the range mentioned in this work. The cross-ribs in *C. virescens* may not be as evident as in *H. zea* or *H. armigera* (Neunzig, 1964, 1969) (Figs. 8A and 10A), but this feature does not appear suitable for species differentiation due to its significant intra-specific variation (Bernhardt and Phillips, 1985). Aeropyles are present in variable number (1-5) usually at the top of the cristae of the primary ribs (Fig. 10D) or less frequent at the top of secondary ribs (Fig. 10A). The higher number of primary cells observed (n=12-20) compared to the range reported by Cônsoli et al. (1999) (n=11-13) may be due to a broader sampling. The characteristic stippled surface texture of the egg (Fig. 10F) can be used to distinguish it from those of *H. armigera* and *H. gelotopoeon*.

Key to identify the Noctuoidea superfamily specimens associated with soybean in Brazil

1. Eggs deposited individually, without modified scales covering them (Figs. 1, 2, 3, 8, 9, 10) 2

1'. Eggs deposited in groups with two or three layers, covered by modified scales (Figs. 4A, 6A) 6

2. Eggs with a diameter approximately twice the height of the egg 3

2'. Eggs with a diameter approximately equal to the height of the egg 4

3. Eggs with vertical ribs less conspicuous than in *A. gemmatalis*, and a rosette with two to four concentric petals with radial striae inside each petal (Figs. 2B, 2C, 3B) .... *C. includens* or *R. nu*

3'. Eggs with conspicuous vertical ribs, principal ribs reaching the secondary or tertiary cells of the rosette, and secondary ribs in number of two or one between principal ribs. Instead of radial striae, the surface inside the petals features a smoothly rugose primary rosette (Fig. 1C) .... *A. gemmatalis*

4. Aeropyles distributed on the egg equator (Figs. 8E, 8F); surface of the egg appears like wool yarn (Fig. 8G) .... *H. armigera*

4'. Aeropyles at the top of the primary ribs, in numbers of two to five (Fig. 10D); egg texture with stippled surface (Fig. 10F) .... *C. virescens*

6. Eggs with petals of the secondary cells having somewhat angular distal ends, similar to the primary cells (Fig. 3A) .... *S. albula*

6'. Eggs with petals of the secondary cells having rounded distal ends, similar to the primary cells… 7

7. Eggs are light green and somewhat shiny .... *S. eridania*

7'. Eggs display several colors, ranging from green, grey, and brown to non-glossy pinkish-brown .... *S. cosmioides*

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Conflicts of interest

The authors declare no competing interests

Author contribution statement

DRS-G conceptualization, methodology, investigation, formal analysis, data curation, writing - original draft, review, and editing. AS investigation, writing - original draft, review, and editing. MGM methodology, investigation, formal analysis, writing - review and editing. CGTJA methodology, investigation.

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