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POLLEN OF *PASSIFLORA SUBEROSA* LINNEUS IN EXCREMENTS OF THE CATERPILLAR OF *AGRAULIS VANILLAE MACULOSA* STICHEL

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Scope

This paper deals with the interaction of the *Agraulis vanillae maculosa* butterfly and the *Passiflora suberosa*, through pollen analysis and its state of conservation in the excrements of its caterpillar. The presence of dispersed pollen grains and those adhered on the external part of the leaf remains in excrements, confirms the ingestion of leaves and anthers of this passion flower. The organic residue obtained from the chemical treatment of excrements reveals numerous well-preserved pollen grains (whole) and trichomes (hairs), dispersed and immersed in the clumps with traces of leaves. The results of this interaction based on observations over 10 years, indicate that *P. suberosa* seems to resist the attack of caterpillars, being able to remain alive even without leaves for several days, especially in underdeveloped plants with thin and sparsely lignified green stems that can photosynthesize. The fluorescence color of pollen grains mounted directly from the flower and excrement after applying chemical methods are compared and discussed. The accumulation of excrements in the sediments where they cohabit can be a source of pollen supply, despite the preservation of organic matter depending on the type of substrate and the taphonomic and fossilization processes in their final depocenter. Therefore, this is a useful contribution to pollen-bearing coprolites and producers scarcely known in the fossil record.

Abstract

In this paper, we analyze the content and state of preservation of the passionflower *Passiflora suberosa* pollen after being digested and excreted by the larval stage of the *Agraulis vanillae maculosa* butterfly. This butterfly visits the plants in the gardens (Diamante, Entre Ríos province, Argentina), mainly between Spring and Autumn, and lays its eggs on the leaves of *Passiflora suberosa* and other *Passiflora* species. The caterpillar feeds on passionflower leaves exclusively while walking actively on the plant stems, completing its pupal phase attached to a nearby surface (wall, bars, and stems). In this study, a fragment of *Passiflora suberosa* (c. 50 cm in length), with four flowers (c. 1-2 cm in diameter) recently opened (anthesis) was placed on paper in a plastic tray kept inside a well-ventilated and illuminated environment. Dispersed pollen grains and pollen grains adhered on the external part of the leaves observed in the excrements of the caterpillar confirming that leaves and anthers were ingested. The organic residue obtained from the chemical treatment with boiling HCl 10% of 10 excrements reveals the presence of numerous well-preserved pollen grains under an optical microscope, associated with trichomes (hairs). These materials are found dispersed and into clumps bearing traces of leaves. In this insect-plant interaction, *P. suberosa* seems to resist the attack of caterpillars because it can remain alive even without leaves for several days, especially in underdeveloped plants with thin and sparsely lignified green stems that can photosynthesize. Concerning the fluorescence color of pollen grains mounted directly from the flower and those from excrements after applying chemical methods are compared and discussed. A soil sample of the garden is also analyzed and compared. The accumulation of excrements in the sediments where they cohabit can be a source of a well-preserved pollen supply. However, the organic matter will be preserved depending on the type of substrate and the taphonomic and fossilization processes in the final depocenter.

Keywords. Insect-plant interactions, excrements of caterpillar, butterfly conservation, Passionflower pollen, fluorescence, methodologies, urban vegetation, Argentina.

Resumen

En este trabajo analizamos el contenido y estado de conservación del polen de *Passiflora suberosa* luego de ser digerido y excretado por el estado larvario de la mariposa *Agraulis vanillae maculosa*. Esta mariposa visita las plantas de los jardines (Diamante, provincia de Entre Ríos, Argentina), principalmente entre primavera y otoño, y deposita sus huevos en las hojas de *Passiflora suberosa* y otras especies de *Passiflora*. La oruga se alimenta de hojas de pasiflora de forma exclusiva mientras camina activamente sobre los tallos de la planta, completando su fase de pupa adherida a una superficie cercana (pared, barras y tallos). En este estudio, un fragmento de *Passiflora suberosa* (c. 50 cm de longitud), con cuatro flores (c. 1-2 cm de diámetro) recientemente abiertas (antesis) se colocó sobre papel en una bandeja de plástico mantenida dentro de un lugar bien ventilado e iluminado. Granos de polen dispersos y granos de polen

adheridos en la parte externa de las hojas observados en los excrementos de la oruga confirman que hojas y anteras fueron ingeridas. El residuo orgánico obtenido del tratamiento químico de 10 excrementos con HCl hirviéndolos al 10% revela al microscopio óptico, la presencia de numerosos granos de polen bien conservados, asociados a tricomas (pelos). Estos materiales se encuentran dispersos y en grupos con restos de hojas. En esta interacción insecto-planta, *P. suberosa* parece resistir el ataque de las orugas porque puede permanecer viva incluso sin hojas durante varios días, especialmente en plantas subdesarrolladas con tallos verdes delgados y escasamente lignificados que pueden realizar la fotosíntesis. Se compara y discute el color de fluorescencia de los granos de polen montados directamente de la flor y los de los excrementos después de aplicar métodos químicos. También se analiza y compara el contenido palinológico de una muestra de suelo del jardín. La acumulación de excrementos en los sedimentos donde conviven puede ser una fuente de suministro de polen bien conservado. Sin embargo, la materia orgánica se conservará dependiendo del tipo de sustrato y de los procesos tafonómicos y de fosilización en el depocentro final. **Palabras clave**. Interacciones insecto-planta, excrementos de oruga, conservación de mariposas, polen de pasiflora, fluorescencia, metodologías, vegetación urbana, Argentina.

1 Introduction

Modern excrements (fecal pellets, feces) of specialized pollinator insects or their larvae stages that possibly accumulate in sediments, where they interact with plants for feeding or nesting, can contribute to a better understanding of palynofacies analysis (Tyson, 1995). Different groups of arthropods can release fecal pellets or excrements preserved as fossil coprolites bearing organic particles into the sediments (De Benedetti et al., 2023), or in a lesser frequency, into fertile parts (e.g. cones of gymnosperms, angiosperm flowers) of fossil plants (see Klavins et al., 2005). De Benedetti et al. (2023) documented 18 clumps of angiosperm species and three pteridophytes in Maastrichtian to Danian deposits of the La Colonia Formation in Patagonia Argentina. They concluded that there is a huge bias in paleopalynological studies linked to the processing methods applied to samples to recover this type of clumps. The scarcity of empirical studies based on extant groups producing clumps of pollen was also highlighted. Klavins et al. (2005) found pollen-laden coprolites in pollen sacs of a Middle Triassic permineralized cycad in the Queen Alexandra Range of the central Transantarctic Mountains. They concluded that the coprolites composed solely of relatively unaltered monocolpate pollen from *Delemaya spinulosa* Klavins, Taylor, Krings et Taylor, were similar to fecal pellets of modern arthropods like those produced by beetles (Coleoptera), supporting a cycad–insect interaction, which could have represented a precursory stage in the establishment of a more complex gymnospermic (cycad)–pollinator relationship.

The interaction of the "Espejitos" butterfly *Agraulis vanillae maculosa* Stichel (Nymphalidae, Canals, 2000; Soares et al., 2012; Moré et al., 2022) with *Passiflora suberosa* Linneus analyzed in this contribution may help the understanding of pollen-

bearing coprolites in ancient deposits. The finding of clumps of pollen-bearing plant fragments in excrements produced by the caterpillar of this lepidopteran arthropod allowed the microscopic evaluation of their fluorescence color and preservation state after being digested and excreted by this larval stage. This study was achieved after the direct observation of the behavior of this butterfly visiting passionflowers cultivated in a garden between 2014 and 2023, in Diamante, Entre Ríos province, Argentina (di Pasquo et al., 2022a). This species of butterfly is the most frequent of a few species of lepidopterans coming between September and April to this garden looking for the nectar of the large flowers of two passionflower species (Canals, 2000), the native *P. caerulea* Linneus (mburucuja), and the exotic P*. edulis* Sims. (maracuja) (Deginani, 2001). It was observed that this butterfly deposits its eggs exclusively on the leaves of the three passionflowers (Fig. 1). The organic content of excrements of one isolated caterpillar after being fed with *Passiflora suberosa* is evaluated in terms of the preservation state of pollen grains as well as their autofluorescence color and intensity. This feature in pollen grains and other palynomorphs depends on the original composition of the exine, and thus, on their biological nature. Moreover, it is affected by taphonomic (e.g. pedogenesis, oxidation, and corrosion by wetting/drying cycles, degradation by exposure to air and microbes during recycling) and diagenetic (e.g. thermal alteration) processes. Hunt et al. (2007) and Yang and Grote (2018) among others, demonstrated that the changes of the fluorescence spectrum (green, red, orange, and violet) up to its disappearance are correlated to the environment where palynomorphs are preserved. At the same time, Hunt et al. (2007) revealed the effect of chemical treatments on the autofluorescence color. Hence, other objectives of this work are to make a comparison of autofluorescence colors of pollen grains taken directly from the flowers also chemically untreated, with those from excrements, anthers, and a soil sample collected from the garden, after being chemically- treated. For this purpose, a great illustrative contribution is provided to show pollen grains after different acid treatments (acetolysis, HCl hot, HF, oxidation with nitric acid). The relevance of autofluorescence in modern pollen applied to interpretations of the fossil record is discussed.

2 Materials and methods

The behavior of *Agraulis vanillae maculosa* has been studied for ten years (from 2014 to 2023, di Pasquo et al., 2022a) through direct observation in a garden of a house in Diamante City, Entre Ríos (32° 3' 50" S, 60°38'37" W). A fragment of *Passiflora suberosa* c. 50 cm long bearing several leaves and four recently opened flowers (anthesis) of c. 1-2 cm in diameter, was placed on paper in a plastic tray kept inside an environment with good but not direct sunlight, at the first author´s home in Diamante. The effects of feeding the caterpillars on the leaves of *P. edulis* and *P. suberosa* are illustrated in Figure 1 for comparison. The material excreted (89 excrements) by one caterpillar of this butterfly was collected in April 2023 after seven days of being fed on this vine (Fig. 2). The rest of this fragment was herborized and cataloged into the

Herbarium collection together with its palynological pollen preparations, under the acronym LPPH that corresponds to the Palynostratigraphy and Paleobotany Laboratory (CICYTTP-CONICET-Entre Ríos-UADER) in replacement of CICYTTP-H used until 2021 (di Pasquo and Silvestri, 2014). This Herbarium is registered in databases such as Index Herbariorum, RCPol, and the SAB Herbaria network. Fresh pollen grains of *Passiflora* were directly extracted from flowers and from a soil sample collected in June 2023 from the garden where the passionflowers are cultivated (catalog under the acronym CICYTTP-Pl). The plant fragment was inspected and illustrated under a stereomicroscope Leica S6D bearing a video camera Leica EC3 (3 Mpixels). All the eighty-nine excrements collected from one caterpillar were placed in a Petri dish and pictured along with other small plant remains recognized such as anther-bearing pollen, fragments of leaves, and isolated trichomes. From these materials, slides were prepared manually with a pipette or a needle manually, mounted with glycerin.

On the other hand, ten excrements were selectively picked and disaggregated with HCl (10%) in a glass tube exposed to a direct flame, and the solution boiled for a few minutes into a hood. Afterward, the residue was rinsed with distilled water in a centrifuge and placed again into a Petri dish for microscopic analysis. Another two sets of c. ten excrements were treated. One with nitric acid and ammonium (2 minutes), was rinsed in a centrifuge, then boiled with HCl and rinsed again. The other set was acetolyzed following Traverse (2008). These same procedures were applied to the fresh pollen grains extracted from flowers and the soil sample. All these materials were mounted in slides using glycerin (a semi-permanent media), as recommended by di Pasquo et al. (2022b) to explore fluorescence using a Leica DM500 microscope, bearing a device with white and fluorescence LED lamps and a filter block for fluorescein of c. 450 nm. Pictures were taken with a video camera AmScope 14 MP, and with its software, the time of exposition up to 2000 milliseconds, and the gain between 1 and 3 are adjusted. These parameters were modified at the time of capturing the specimens. The highest time of exposition and gain was used to photograph the highest level of autofluorescence. Whether the morphology was precluded due to being very shiny, a lower period was applied. These parameters are indicated in the captions of the illustrations. A detailed morphologic analysis of excrements and anthers was performed in a scanning electron microscope (SEM) Phenom ProX at the CICYTTP (CONICET-Entre Ríos-UADER), applying low vacuum for which samples (stubs) do not require to be coated (di Pasquo and Vilá, 2019).

3 Results

3.1 Interaction *Agraulis vanillae maculosa* caterpillar-Passionflower

Observations of *Agraulis vanillae maculosa´*s behavior revealed that the caterpillars fed almost exclusively on *Passiflora suberosa* leaves. The caterpillars move along their stems very actively until they complete their cycle in the pupal phase, after adhering the chrysalid to some more or less rigid surface, such as walls, bars and stems (Fig. 1 C-D, J,

I). In this insect-plant interaction, *P. suberosa* seems to resist the attack of caterpillars and remains alive even without leaves for several days, especially in underdeveloped plants with thin and sparsely lignified green stems that can photosynthesize. According to the recent scheme by Labandeira and Wappler (2023), the main damage caused by caterpillars to the leaves was described as "Surface feeding". Following Coulson and Witter (1984), three types of external foliage feeding were observed in cultivated *Passiflora*: skeletonizing (Fig. 1A, 2A), whole feeding (Fig. 2A-B), and window feeding (Fig. 2E-G). Skeletonizing occurs when the insect feeds on the soft tissue, leaving only the veins as a "skeleton" of the leaves. Skeletonizing was intense in *P. suberosa* as well as in *P. edulis* despite the larger size of its leaves (Fig. 1 K-M). The whole feeding damage type in leaves of *Passiflora suberosa* is characterized by almost circular polylobate perforations of different sizes. Window feeding occurred mainly at the leaf base, where the caterpillar fed on only one surface of the leaves, making them thinner and allowing the light to penetrate. In addition, only a couple of oviposition-type remains were observed on leaves (Fig. 2I), although the birth of caterpillars was not observed (Fig. 2).

The interaction between the one isolated caterpillar and the *P. suberosa* fragment revealed that the caterpillar mainly caused damage to leaves and two flowers bearing anthers with pollen completely eaten up to their petiole. Individual coprolites are irregularly rectangular and measure up to 1 mm in length and c. 500 µm in diameter. Observation of excrements under the stereomicroscope allowed the identification of loose and adherent pollen grains on the external part of the leaf remains, small remains of leaves and trichomes (hairs), and two partially broken anthers with pollen grains (Figs. 2 and 3), confirming that the caterpillar also ingested anthers. Pollen grains in the coprolites appear to be relatively intact.

3.2 Autofluorescence color: comparison between fresh pollen, and those in excrements and soil

The autofluorescence color of pollen grains of passionflowers was compared considering different chemical treatments and three different sources: fresh pollen grains extracted directly from flowers, pollen grains from caterpillar excrements, and pollen grains from one soil sample.

A bright yellow with a light green ring of fluorescence color (Fig. 4) was observed in both the pollen found in untreated excrements and the anthers (Fig. 5). This demonstrates that pollen grains do not change after being excreted by the caterpillar. SEM images of anthers from untreated flowers (Fig. 6) showed excellent external and internal features of the anthers bearing pollen grains, which exhibit the hexa-colpi in different positions and the well-defined reticulum of their walls.

The microscopic organic material released from the excrements (Figs. 7-9) after applying chemical treatments (especially acetolysis), revealed numerous well-preserved (whole) pollen grains, not only dispersed but also immersed in the clumps with leaf remains, in which trichomes can predominate. This is also observed in SEM images from untreated excrements (Fig. 10). Pollen grains from excrements after being chemically treated showed a shift in

autofluorescence color from bright yellow with a green ring to a darker yellow/orange with a reddish ring. This change in autofluorescence color was also observed in the flower anthers after being treated with the same methods (Fig. 11-13) and in the palynomorphs obtained from the soil sample (Figs. 13-14).

Differences in palynomorph content and fluorescence are recognized when comparing acetolized and not acetolized soil samples. In the latter, pollen grains were identified with fluorescence as poorly preserved types of *P. caerulae* - *P. edulis* (Fig. 14. C, F) and *P. suberosa* (Fig. 14. D-E), and palm pollen of *Syagrus romanzoffiana* (Cham.) Glassman over a cuticle (Fig. 14. K-N). These pollen grains were not observable under light microscopy. Instead, in the acetolized residue, more palynomorphs were released from the clumps of amorphous organic matter such as a palm pollen grain of *Syagrus romanzoffiana* type well-seen under both white and fluorescent lights (Fig. 14. G-I), an arthropod egg (Fig. 14. O-P), and a fern spore of *Lophosoria* Presl type (Fig. 14. Q-R).

Among the methods applied herein, HCl boiled was not good for accurate identifications because the specimens still maintained their cell content precluding observation of morphologic features. It is a pity because it is an easier, faster, and less risky treatment than acetolysis. Concerning pollen grains mounted directly from anthers, the water medium enhances the expansion of the pollen wall allowing a better evaluation of morphologic features despite their cell content. Whereas glycerin prevents, or masks those features.

4 Discussion and conclusions

4.1 Insect–plant interactions

Animal–plant interactions in modern ecosystems can be classified into feeding, shelter, transport, reproduction, and coevolution. Siluro-Devonian ecosystems may have been based on detritivores and micro herbivores, herbivory evolving only much later (Devonian-Carboniferous?) when animals had developed gut microflora to aid in the breakdown of lignin, other recalcitrant materials, and toxins. Examples of these have been found in the fossil record (e.g. Rhinie Chert, Early Devonian), since older pieces of evidence of feeding like damage to sporangia and stem carried out by arthropods (Labandeira, 2007, 2013; Iannuzzi and Labandeira, 2008). Coincidental spore dispersal was documented in the older plant fossil deposits of the Silurian and Early Devonian (e.g. Habgood et al., 2003; Edwards et al., 2012). Instead, interactions on leaves or roots have been reported until the end of the Carboniferous (Shear, 1991; Labandeira et al., 2007; Labandeira, 2011). Despite early evidence of arthropods, even today, the cycling of nutrients through the decomposer niche has a greater impact, in most cases, than herbivory in terrestrial ecosystems.

Fossil evidence for animals feeding on plants comes from four sources: (1) plant morphology (anatomy and pathology), (2) animal morphology, (3) direct associations between animals and plants, and (4) coprolites (Gensel and Edwards, 2001). It must be highlighted that small arthropods are not easily preserved in rocks, so there is a black-out to establishing biological affinities and their methods of interactions.

Animals, particularly arthropods, exhibit a wide variety of methods of feeding on plants, including biting, sawing, cutting, chewing, rasping, mining, boring, mopping exudate, swallowing whole, and piercing and sucking juices. Some are particular to certain plant organs (e.g., boring wood and seeds, leaf mining, and eating whole seeds, pollen grains, and spores). Animal mouthparts can be a good clue to their feeding style, the lepidopteran "tongue,'' for example (Labandeira, 2010, 2011, 2013). However, piercing and sucking mouthparts occur in parasites of both plants and animals and chewing mouthparts may be considered generalized. So, morphology alone is often not good enough, and comparison with modern relatives of the systematic group is normally necessary to determine the probable style (Labandeira et al., 2007; Labandeira, 2013; Schachat et al., 2022; and their references).

On the other hand, external ovipositors currently are the most prominent in the orders Odonata, Orthoptera, Hemiptera, Coleoptera, Lepidoptera, and Hymenoptera. Worldwide, evidence for leaf damage produced by ovipositors is relatively abundant throughout the Phanerozoic (Labandeira, 2013). Complex patterns of ovipositional plant damage frequently allow reliable identification of the producers, chiefly when fossil ovipositor structure and egg-laying biology of modern counterparts are well-studied (Kawahara et al., 2019). *Lepidoptera* larval stages (caterpillars) develop into pupae (chrysalides) undergoing a complete metamorphosis. During growth, larvae store a lot of energy allowing the metamorphosis process.

Concerning *defense versus dispersal adaptations* in fossil and modern plants, many early terrestrial plants bore spines like in *Passiflora suberosa* leaves, which suggests they may have been defensive adaptations against herbivory. However, other reasons for the spines proposed that the short, upward-pointing, scalelike enations of early vascular plants, facilitated upward (but not downward) climbing of plant axes by arthropods. This would favor spore-eaters, which might jump or glide off the sporangium after feeding, thus aiding dispersal. Outgrowths from the axis in early vascular plants would undoubtedly increase surface area for photosynthesis, like in modern plants, in which chlorophyll is present, despite in fossil plants are difficult to prove. Nowadays, dense, silvery hairs are used by plants in exposed areas to prevent excess transpiration. Lateral branches and the separation of axes used to survive in dense stands, or for a scrambling habit. Different ways to store toxic substances (e.g. lignin) or distasteful to avoid herbivory is one of the main objectives of plants in the co-evolution with depredators. These allelochemicals can inhibit feeding, act as repellents, reduce digestibility, and even mimic animal hormones, speeding up or slowing developmental changes. Strategies available to herbivores (animals that feed on the living tissues of plants; instead, detritivores feed on dead or decayed plant material, Labandeira, 2007; Labandeira and Wappler, 2023) to circumvent allelochemical defenses include avoidance, detoxification by endogenous enzymes, detoxification by gut microorganisms, and sequestering. At least a few insects have co-opted plant toxins for their defense. There are special cases, those animals that feed primarily on seeds, spores, pollen, and flowers (ephemeral parts), which rarely

contain appreciable toxins. These plant parts, while living, are nutritionally very different from the vegetative parts of the plant. They can be high in calorific value and contain abundant lipids and protein. Seeds can be heavy-defended by recalcitrant shells and coats but usually contain little indigestible material, and the rewards for cracking the defenses are high. Spore and pollen coats are largely indigestible and must be cracked physically or chemically to be digested. Other functions of spines on fossil spores and pollen grains, which commonly have bent or bifid tips, may not be defensive but be dispersed and attached to the bodies of animals. Because of the low nutrient content of tissues of plants, herbivores have to consume enormous quantities to extract what they can. The gut becomes extremely large to handle the mass of food, and conversion values are low (Gensel and Edwards, 2001; Labandeira, 2013; Labandeira and Wappler, 2023; McElwain et al., 2024, and their references). This is a common strategy among orthopterans and lepidopteran larvae like the one studied in this work. Many caterpillar species eat their eggshell upon hatching before resting for a while up to a few days depending on the species, and then, start to eat the leaves (Bug.Guide.Net!).

In this study, the interaction between the *Agraulis vanillae maculosa* and the passionflower reveals that the caterpillar obtains a high subsistence benefit during the main caterpillar-butterfly season between September and April. *P. suberosa* seems to resist this attack being able to live without leaves after several days. This seems linked to thin, slightly lignified green stems that can do photosynthesis (Amela García, 2008). When the caterpillar enters the pupal/butterfly stage, the plants recover their leaves and can continue to flower and bring fruit. Due to this interaction, the damage caused by caterpillars to the passionflower seems to be slower and slightly lesser than the damage produced by black ants (*Acromyrmex lundii* Guérin-Méneville). The latter can eliminate all the foliage of a plant much faster as it was observed (not illustrated), and after this attack (c. one day or night, see Delabie et al., 2003; Folgarait et al., 2011) some plants die.

Although the isolated caterpillar fed mainly on the leaves of *P. suberosa,* it also ingested the flowers as evidenced by the presence of anthers with pollen in its excrements. The same type of damage was observed on the leaves of *P. caerulea* (Figure 15) and *P. edulis* carried out by the same caterpillars (di Pasquo et al., 2022a). However, no caterpillars were observed on the plants visiting and browsing their open flowers, possibly because of their larger size which can reach up to 9 cm in diameter (Deginani, 2001). *Passiflora suberosa* can survive the period of caterpillar activity, but the stems without leaves do not survive more than a month alive. Hence, for harvesting fruits, plants must be protected in some way because *Agraulis vanillae maculosa* exclusively uses passionflowers for reproduction (Canals, 2000; Soares et al., 2012). This thing was also confirmed by direct observation, as it does not interact with other plant species cultivated in the garden.

4.2 Relevance of the analysis of modern excrements (feces or fecal pellets) for ancient studies on coprolites

Coprolite-bearing plant remains provide direct evidence of plant-animal interaction since early

terrestrial ecosystems. Animals require calories and specific nutrients, such as certain amino acids and minerals. Thus, the value of their food depends not just on the available calorific content, but on the amounts of micronutrients and protein available. Gensel and Edwards (2001) state that coprolites are not uncommon in macerates of early terrestrial sediments. Another important point to elucidate is whether the coprolites are produced by herbivory or sediment-litter feeders. This evidence needs careful evaluation, and it must be said that micro-coprolites are underestimated in palynologic analyses, especially if their constituents are poorly aggregated, but also if they are composed of spores of the same type, in which case they show a superficial resemblance to sporangia. However, other possible explanations for the high concentration of spores or pollen grains seen in the coprolites of arthropods, were digestive processes, coprophagy, and selective feeding. Also, an important fact to consider is that standard processing methods can prevent the finding or recognition of coprolites in palynologic residues.

By Carboniferous times, coprolites had become relatively common (Scott and Taylor, 1983; Labandeira and Wappler, 2023). An impression of the size of the producer was deduced from the size of the coprolites and knowledge of the sizes of modern animals, so most collembolans, mites, and nematodes are generally small, whereas earthworms are large. Extant herbivores exploit gut fungi and bacteria to degrade cellulose, but even with their assistance, the nutrient value of living vegetative tissues would have been low in the mid-Paleozoic. Then, palynophagy could be an attractive possibility despite sporopollenin-impregnated spore walls being largely resistant to enzymes, a spore-eating animal would have to crack the spores to digest them. Litter feeders eat varied foods; studies of fecal contents of detritivores invertebrates showed a variety of matter passes through the gut, including spores and pollen grains, which remain undigested (Gensel and Edwards, 2001; Labandeira, 2010, 2011, 2013; McElwain et al., 2024, and their references).

The isolated caterpillar of this study fed mainly on the leaves of *P. suberosa* (i.e. herbivory) but also ingested the flowers remaining in the stem analyzed, confirmed by the presence of anthers and pollen in its excrements. Considering that abundant well-preserved pollen grains of this passionflower are incorporated into the coprolites, the caterpillar probably fed on the cell content of pollen without digesting the sporopollenin. The accumulation of coprolites bearing pollen grains in soil sediments should enhance their preservation. However, such organic matter will be preserved depending on the type of substrate into which it is incorporated, on the taphonomic and fossilization processes that occur at the accumulation site (intermediate and final depocenter), and weathering during exhumation (Tyson, 1995). The microscopic analysis carried out on the soil sample revealed the presence of poorly preserved types of *P. caerulae- P. edulis* (Fig. 14. C, F) and *P. suberosa* (Fig. 14. D-E) that confirm their accumulation in the sediments below they are growing. The palm pollen of *Syagrus romanzoffiana* type (Fig. 14. G-I, K-N) comes from the plants in the neighborhood house brought by bees or even by the wind,

and probably the same occurs with a fern spore of *Lophosoria* type (Fig. 14. Q-R). The arthropod egg (Fig. 14. O-P), which is very similar to the Permian acritarch *Lancettopsis harringtonii* Di Nardo et al. (2022), can be the egg of the butterfly or other arthropods visiting the plants of the garden (Fig. 13). Another thing to consider is the way samples are processed to recover this type of clumps into excrements. Generally, with successive acids and washings as standard methods, clumps of pollen grains can be disintegrated releasing single grains. Therefore, the step-by-step chemical procedures are highly recommended. This implies the analysis of the organic particles into residues under the stereomicroscope, and mounting slides after each acid attack, instead of following "standard methods" (di Pasquo et al., 2023, Fig. 16).

Furthermore, as part of the observed insect-passionflower interactions, di Pasquo et al. (2022a) carried out the analysis of pollen carried by bumblebees of the genus *Xylocopa* that visited *P. caerulea* and *P. edulis* in December 2019 when both species were in bloom. The pollen grains in clumps collected by one of these bumblebees revealed under the microscope that 99% corresponded to both passionflowers. Since the pollen of both species is so similar, their percentage cannot be estimated. These insects take pollen from flowers and bring them to their nests to feed their young (Silva et al., 2020 and references therein), so their contribution to the sediments is possibly low compared to that produced through the excrements of caterpillars. Hence, this contribution is useful to understand pollen-bearing coprolites and their producers in the fossil record.

4.3 Pollen analysis and autofluorescence comparison

Concerning the pollen morphology, due to the cell content of modern pollen grains, their morphological characterization generally is poor or precluded under white light microscopy in not treated and treated with HCl samples. Whereas, fluorescence helps to reveal their presence, or even features not observed with white light. Instead, acetolysis was an effective method to identify the pollen grains from anthers, excrements, and soil under both microscope lights. SEM images of pollen grains confirmed their hexa-colpi character (see discussion in di Pasquo et al., 2022a), as revealed in different positions along with well-defined reticulum of their walls (Fig. 6).

In the present study, no shift in autofluorescence color was observed in pollen grains of passionflowers after being digested by *Agraulis vanillae maculosa* caterpillar compared with pollen grains taken directly from in situ flowers. However, in both cases, the same shift in autofluorescence color of pollen grains from bright yellow with a green ring to a darker yellow/orange with a reddish ring was noticed after chemical treatments. This result confirms that the laboratory methods affect the autofluorescence color and its intensity of modern palynomorphs in agreement with Hunt et al. (2007). Therefore, the autofluorescence yellow (+green ring) color revealed by the *in situ- untreated* modern pollen and those from the excrements changed to a yellow-orange with a red ring in dispersed modern, sub-fossil, and fossil palynomorphs (i.e. deposited into sediments) not only due to the chemical treatment applied, but also because of the original

composition of the exine related with the biological nature of each fossil group. Obermeyer et al. (1999) explained that irreversible changes in the structure of sporopollenin associated with the loss of the hydroxyl group by dehydration and increasing aromaticity, produce a progressive red shift in fluorescence.

Moreover, taphonomic (e.g. oxidation, corrosion, degradation) and diagenetic (e.g. thermal alteration) history (see Yang and Grote, 2018), affect their color and intensity. Hunt et al. (2007) related bright red colors (a shift to brighter, redder colors) with pedogenesis in the past. A full range of autofluorescence colors of fossil palynomorphs (mainly spores, pollen, and algal remains), indigenous of Carboniferous age, associated with Devonian reworked (temporal *ex-situ*) one was observed by di Pasquo et al. (2022b, and references therein). After applying different chemical treatments (e.g. HF, HCl, nitric acid, acetolysis), these authors concluded that autofluorescence is not a good method to discriminate between reworked and indigenous palynomorphs, also due to their colors depended on the type of substrate and the taphonomic and fossilization processes at their final place of deposition, and weathering during exhumation.

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Despite being evaluated, this work has to be retired due to the lack of funding.

A suggestion to solve this situation is that the Open Access Editorials claim the corresponding countries of the authors to pay for the costs of the articles published each year.

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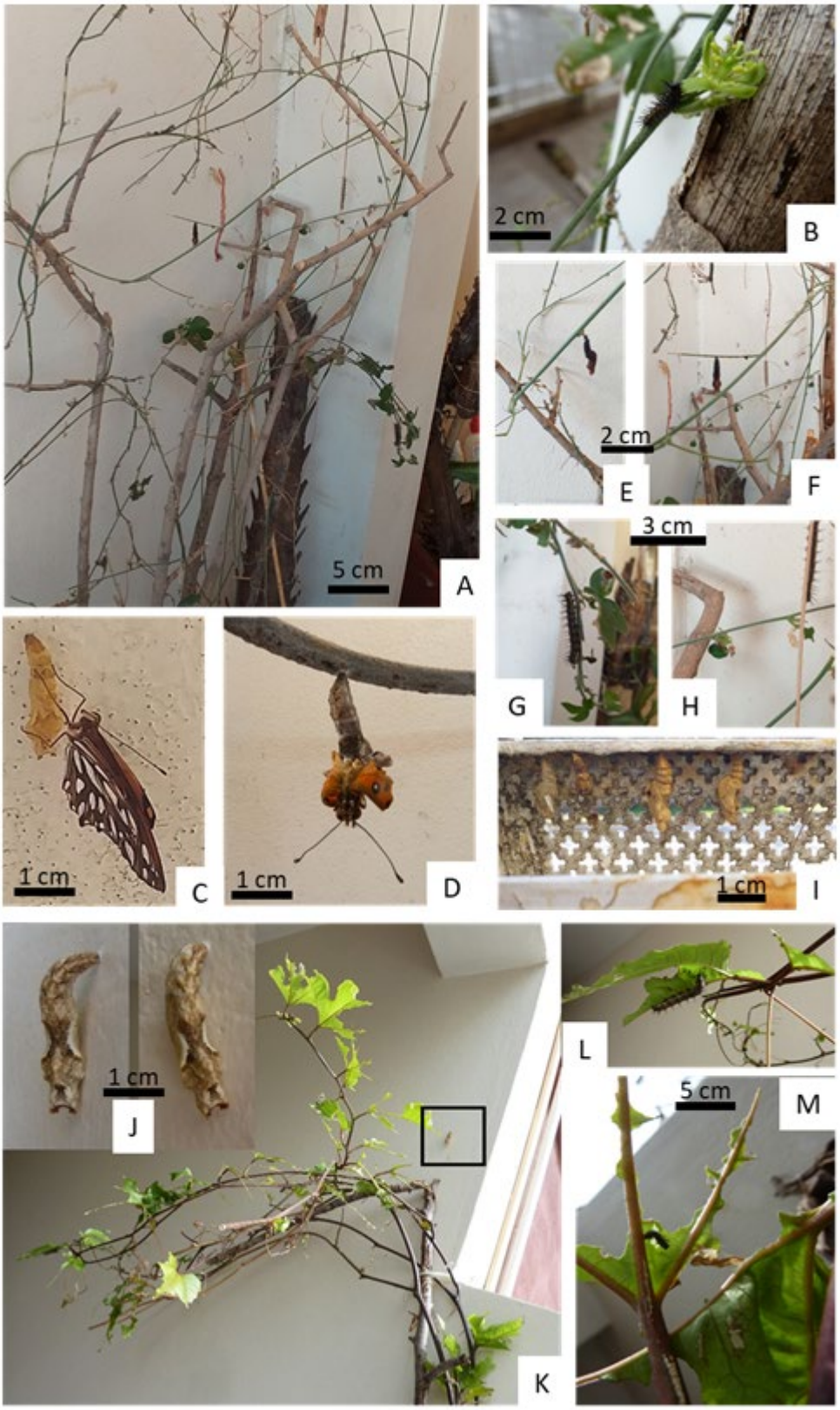

Figure 1. A-M. *Passiflora suberosa* (A-H) and *P. edulis* (K-M) were attacked by the caterpillar of the *Agraulis vanillae maculosa* butterfly. C-J. Different stages of the life cycle of the butterfly.

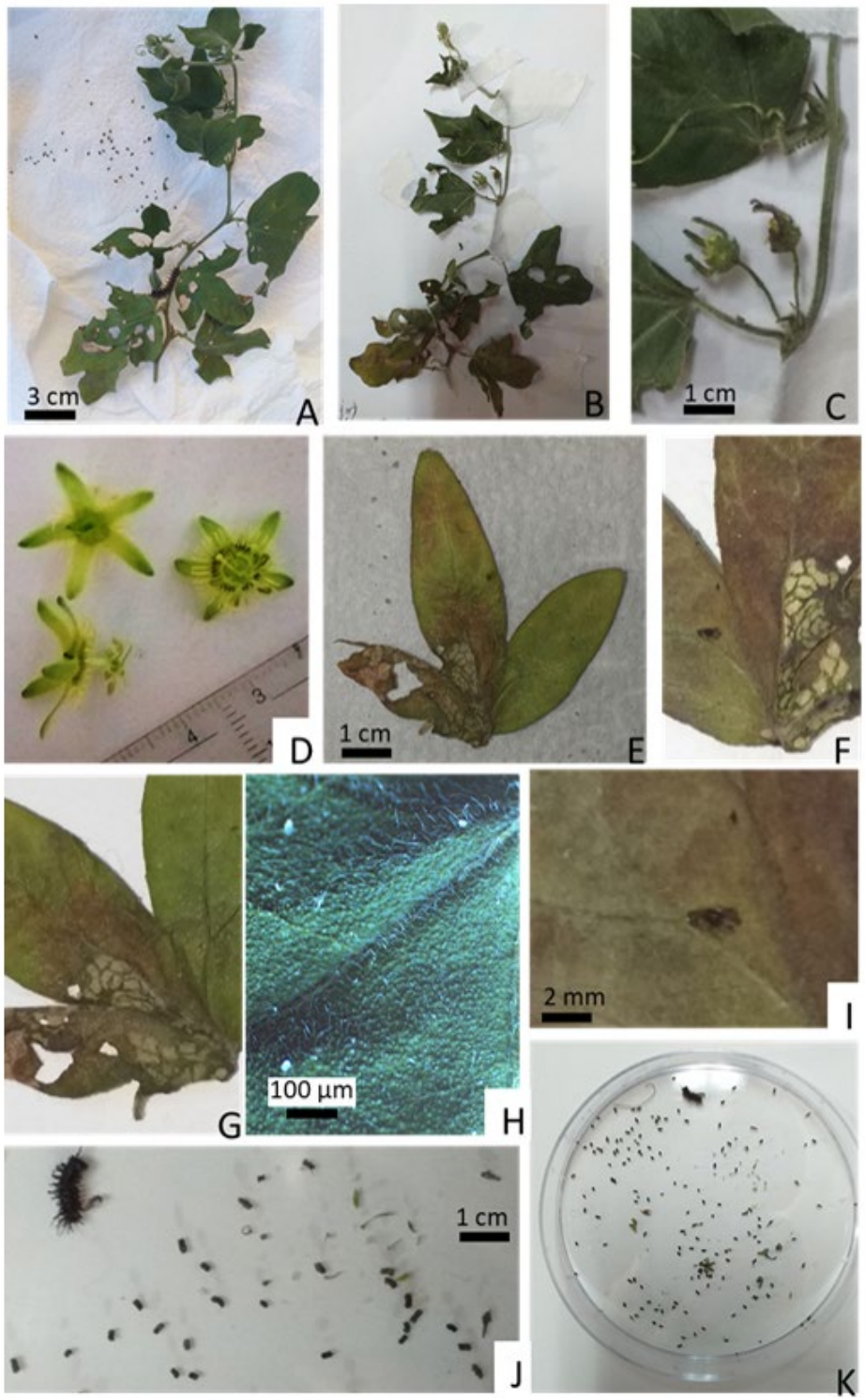

Figure 2. A-I. Plant fragment of *Passiflora suberosa.* A. Plant fragment in the box with the caterpillar and excrements accumulated during 7 days in 2023. B. Plant cataloged and herborized (Exsiccata LPPH 112 2023). C. Enlargement of the flowers eaten by the caterpillar. D. Flowers of the *P. suberosa*. E-G. Leaf with damages produced by the caterpillar. H. Detailed of the surface of the leaf-bearing hairs (trichomes) especially close to the main veins (stereomicroscope picture). I. Possible oviposition mark. J-K. Caterpillar of the *Agraulis vanillae maculosa* butterfly and its excrements in a Petri dish.

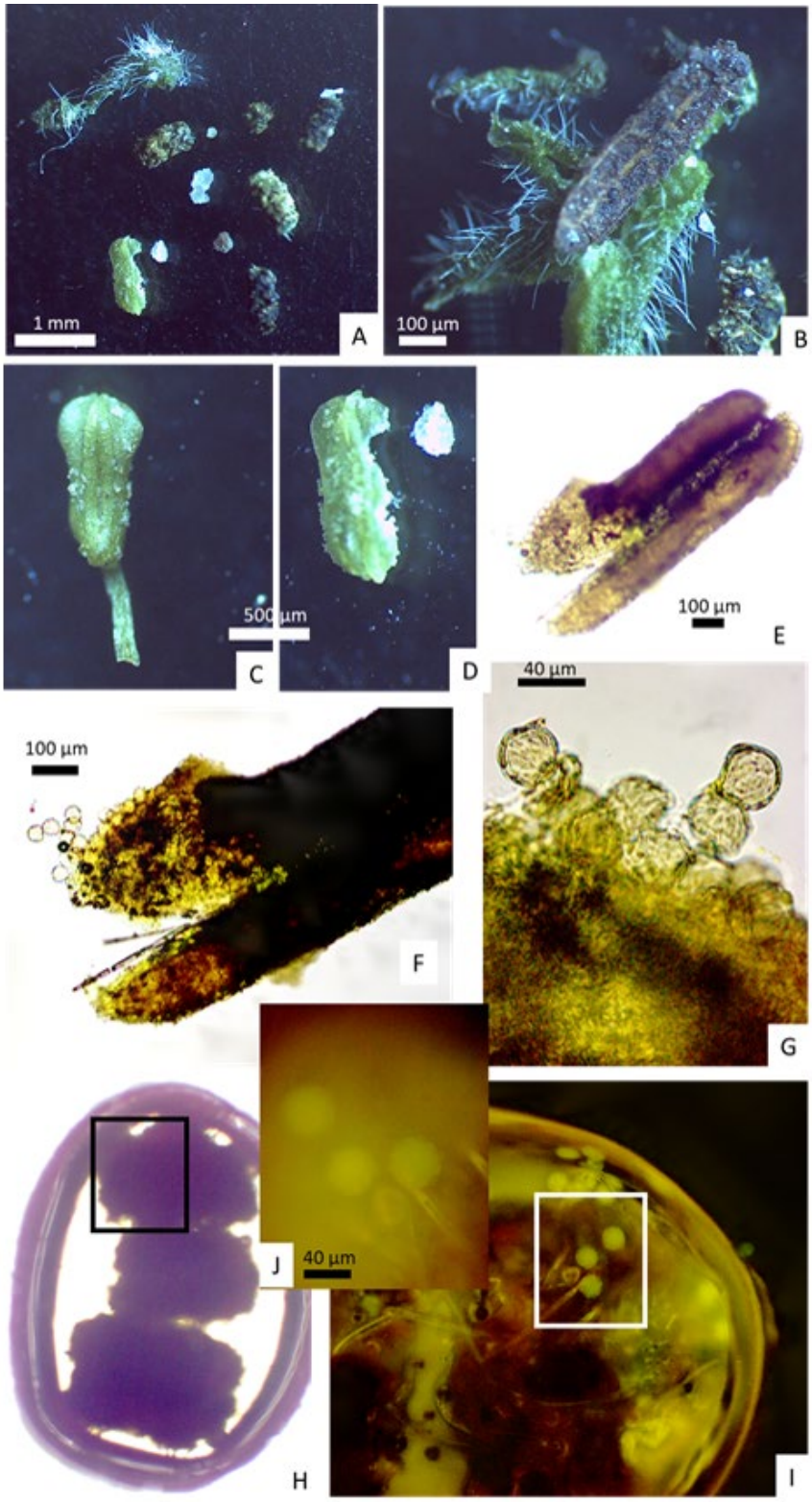

Figure 3. A-D. Excrements and a few organic particles are illustrated in Fig. 2 (J-K), among them two anthers with pollen grains and a leaf portion with trichomes. E-J. Anter with pollen grains (E-G) and excrements bearing pollen grains (H-J), mounted in slide with water illustrated under the microscope with white (E-H) and fluorescent lights (I-J).

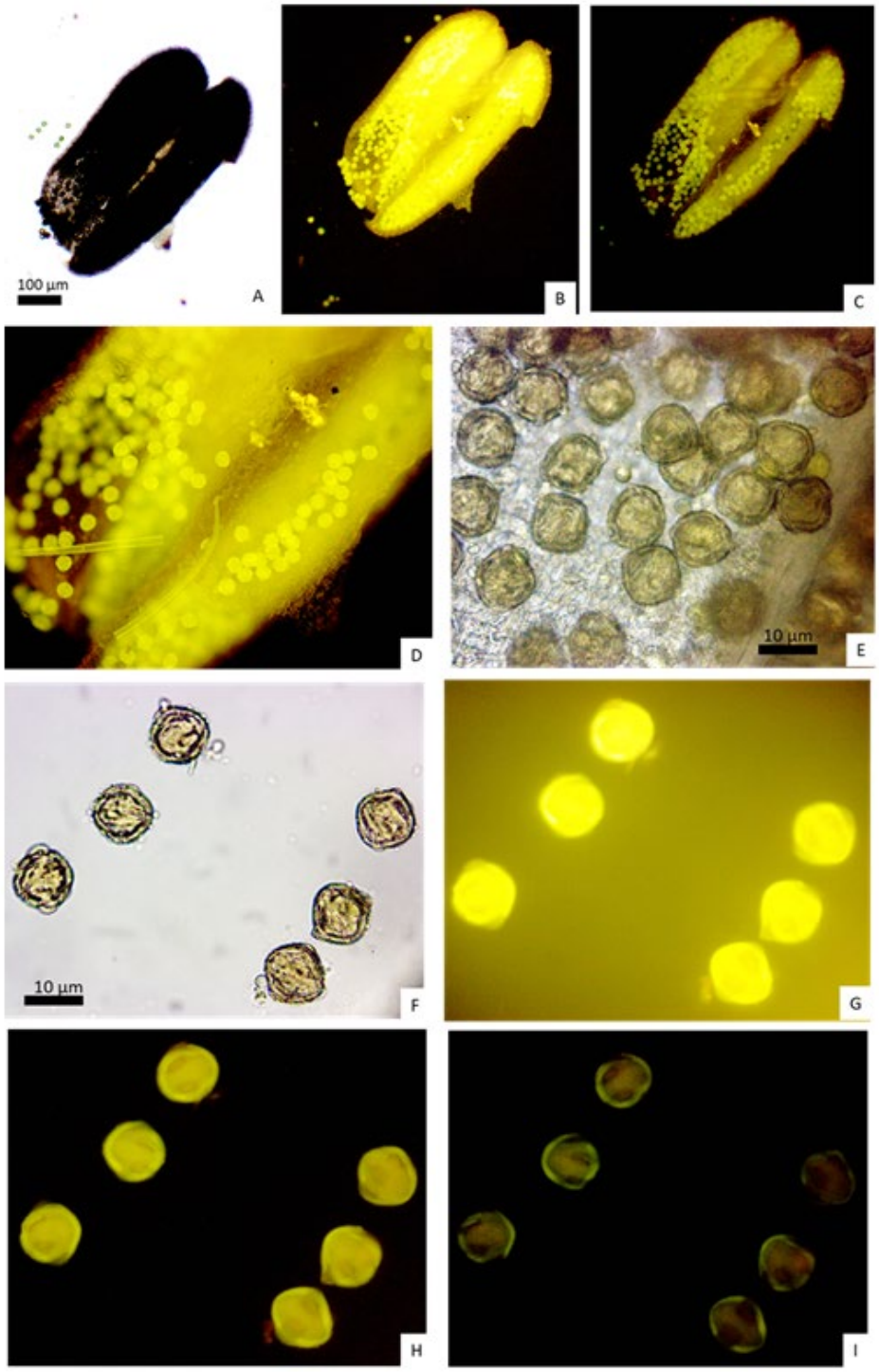

Figure 4. A-I. Anther (A-D) and pollen grains (E-I) of *P. suberosa* (LPPH 112 2023) obtained from not treated excrements. A. x4 magnification under white light microscope. B-C. Fluorescence color with 3G and 2000 ms and 700 ms. D. Pollen in anther (x10 magnifications) with 3G and 370 ms. E-I. Pollen grains (x40 magnification). G-I. Fluorescence color with 3G and 2000 ms, 700 ms, and 350 ms respectively.

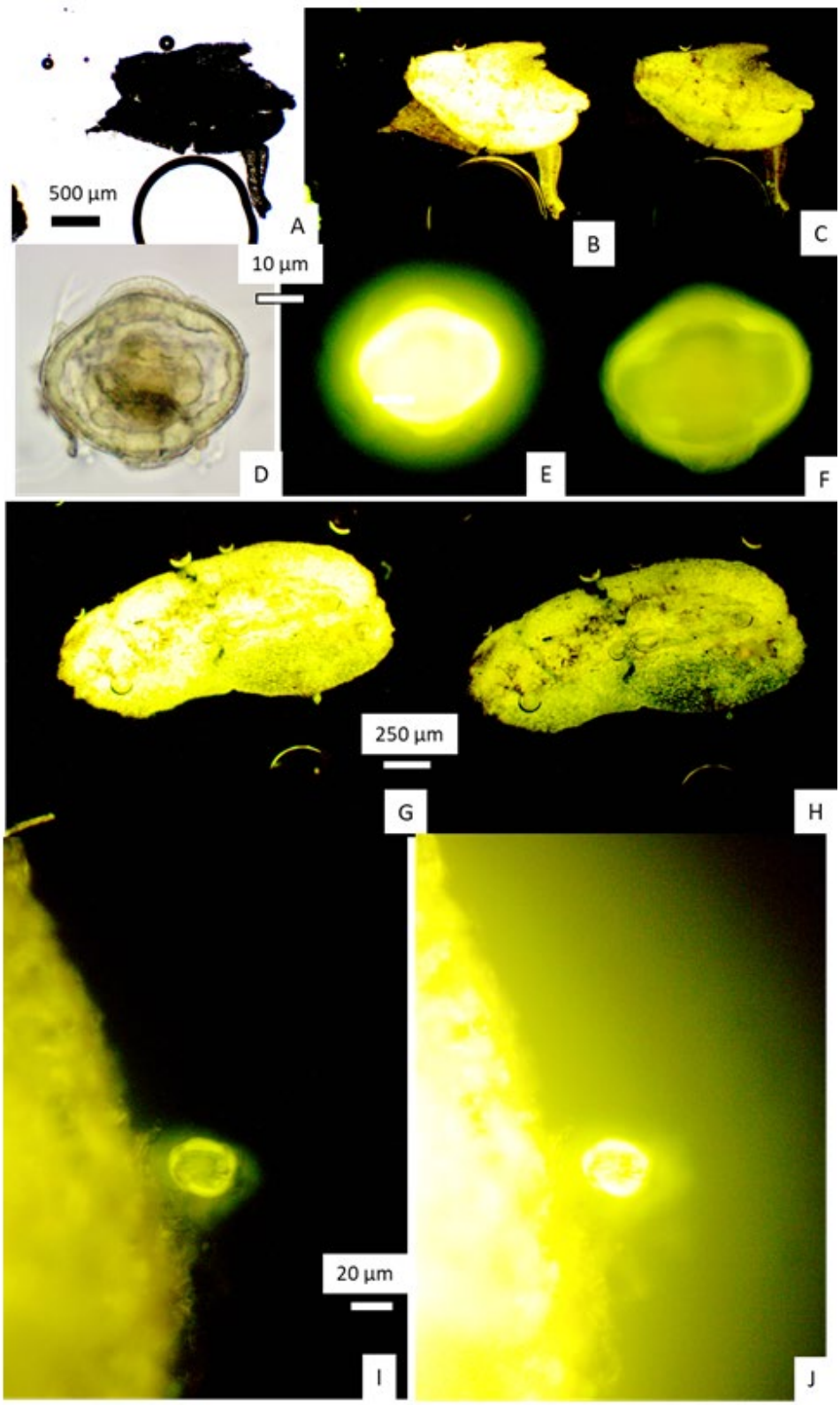

Figure 5. Pollen grains mounted in a slide with glycerin directly taken from the flower anther (LPPH 112 2022). A-C. Anther (x100). B. 2000 ms, 3G. C. 700 ms, 3G. D-F. Pollen grain (x100). E. 2000 ms, 3G. F. 700 ms, 3G. G-J. Anther. G-H. (x4). G. 2000 ms, 3G. H. 700 ms. I-J. (x40). I. 2000 ms, 3G. J. 700 ms, 3G.

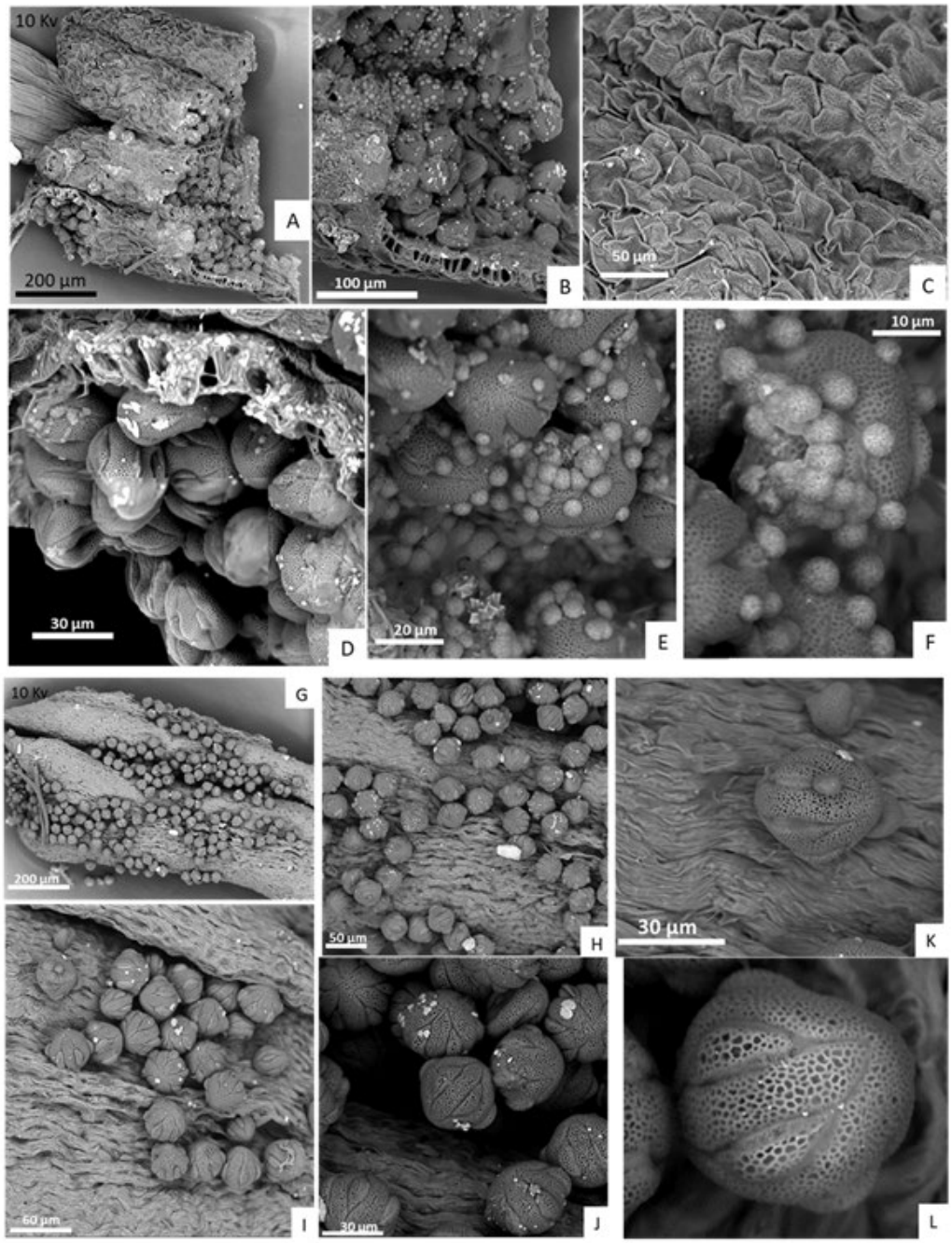

Figure 6. Anthers mounted in the stub (see also Fig. 13A). A-F. Anther-specimen showing external cells in surface (C) and internal arrangement (B, D) as well as pollen grains bearing cells from the tapetum (E-F). G-L. Anther-specimen bearing pollen grains showing the hexa-colpi apertures.

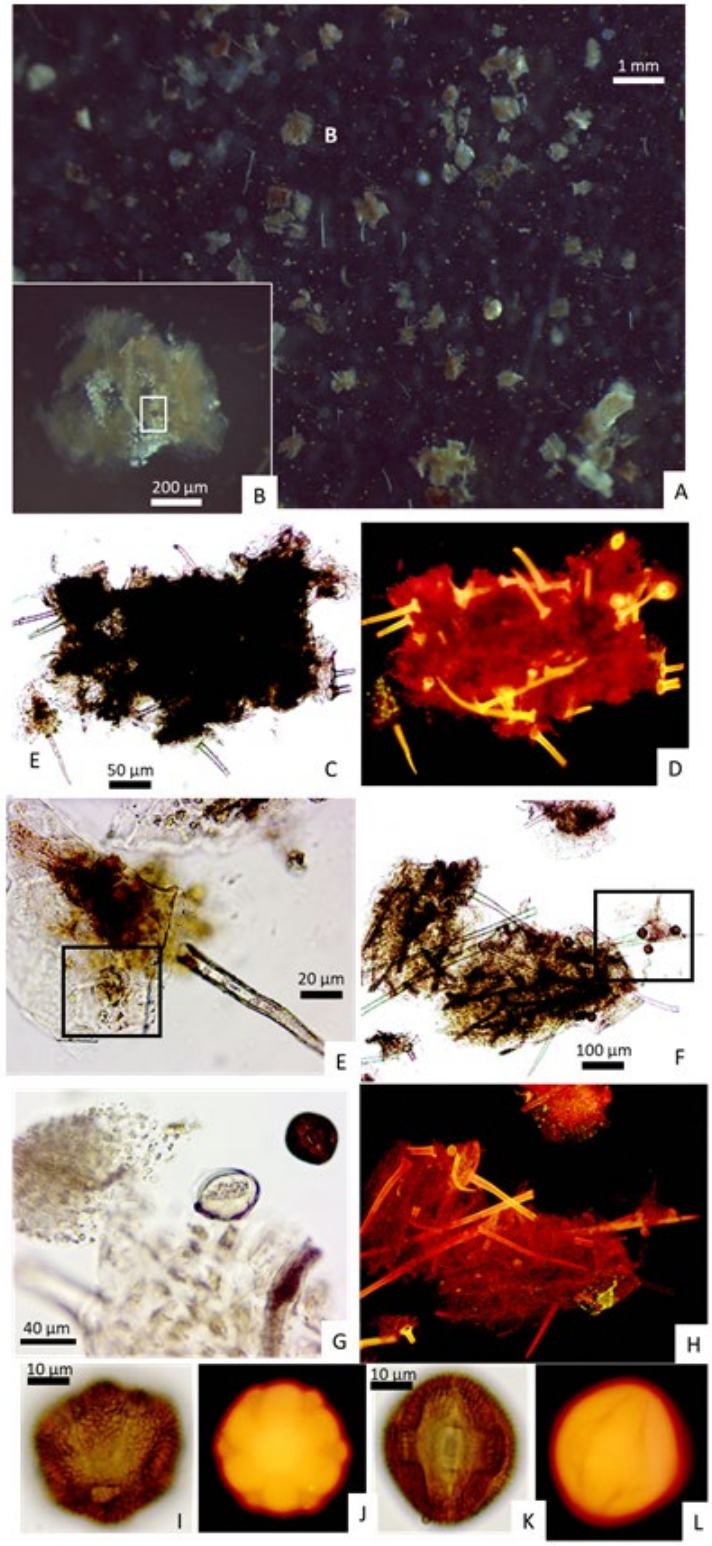

Figure 7. A-L. The residue of the excrements after their disaggregation applying HCl boiled. A-B. Organic matter illustrated in Petri dish with stereomicroscope. In the background, there are lots of dispersed pollen grains. C-E. Leaf fragment with trichomes and stomata in the cuticle (E) illustrated in the microscope with white (C, E) and fluorescent lights (D). F-L. Leaf fragment with trichomes and pollen grains (G, I-L), illustrated in the microscope with white and fluorescent lights (H, J, L).

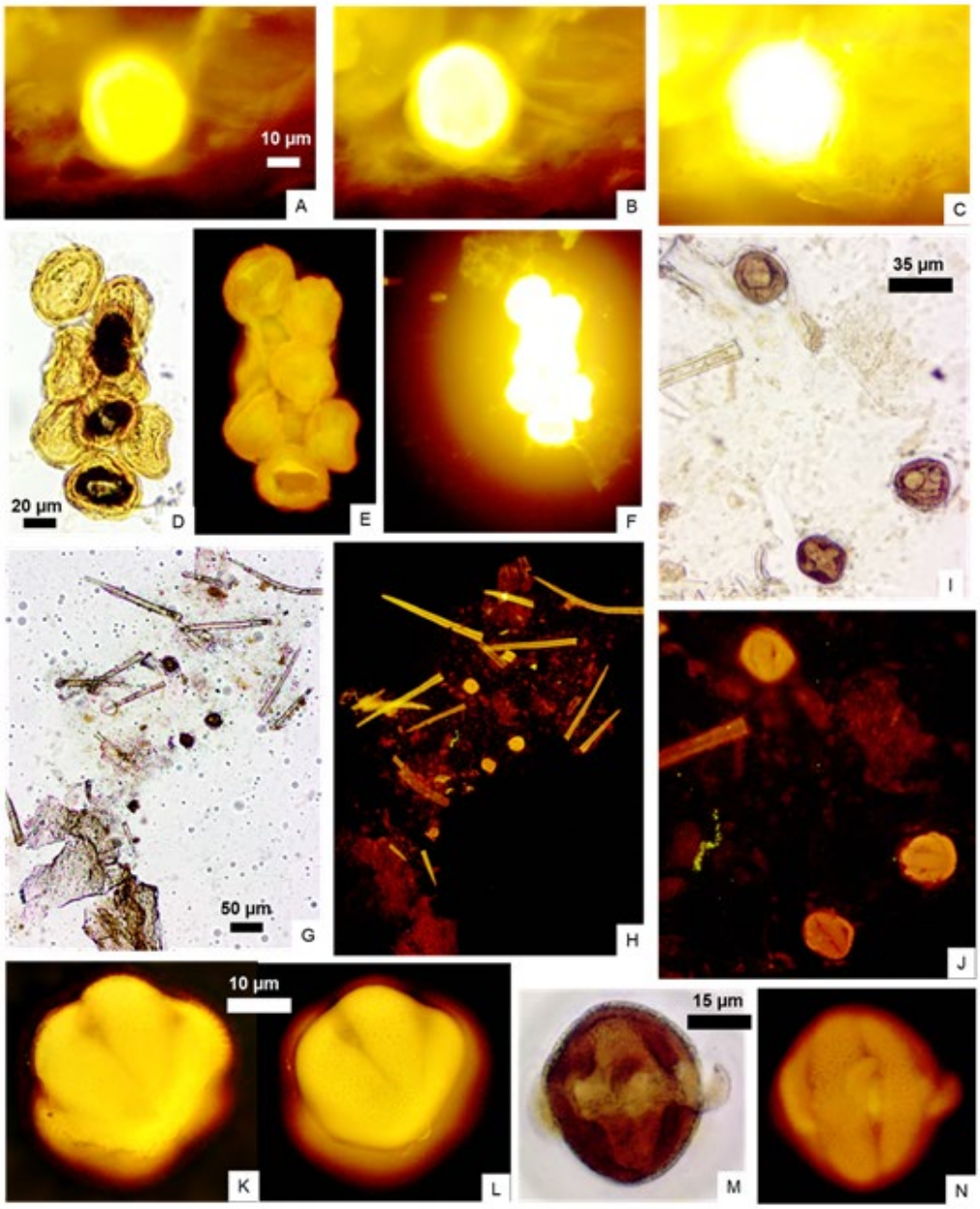

Figure 8. Leaf fragments with trichomes and pollen grains (LPPH 112 2023) from excrements after their treatment with nitric acid and ammonium (2 minutes), washed in a centrifuge and treated with HCl boiled, washed once again and mounted with glycerin. The parameters are detailed in the illustrated specimens with white and fluorescent lights. A-C. Pollen grain (x100). A. 1000 ms, 2G. B. 1000 ms, 3G. C. 2000 ms, 3G. D-F. (x40). E. 380 ms, 2G. F. 2000 ms, 3G. G-H. (x10). H. 2000 ms, 3G. I-J. Magnification (x 40) of the image in G. J. 2000 ms, 3G. K-L. (x100), 2000 ms, 3G. M-N. (x100), 2000 ms, 3G. Cuticles, trichomes, and pollen grains revealed a fluorescent darker yellow color bearing a red halo.

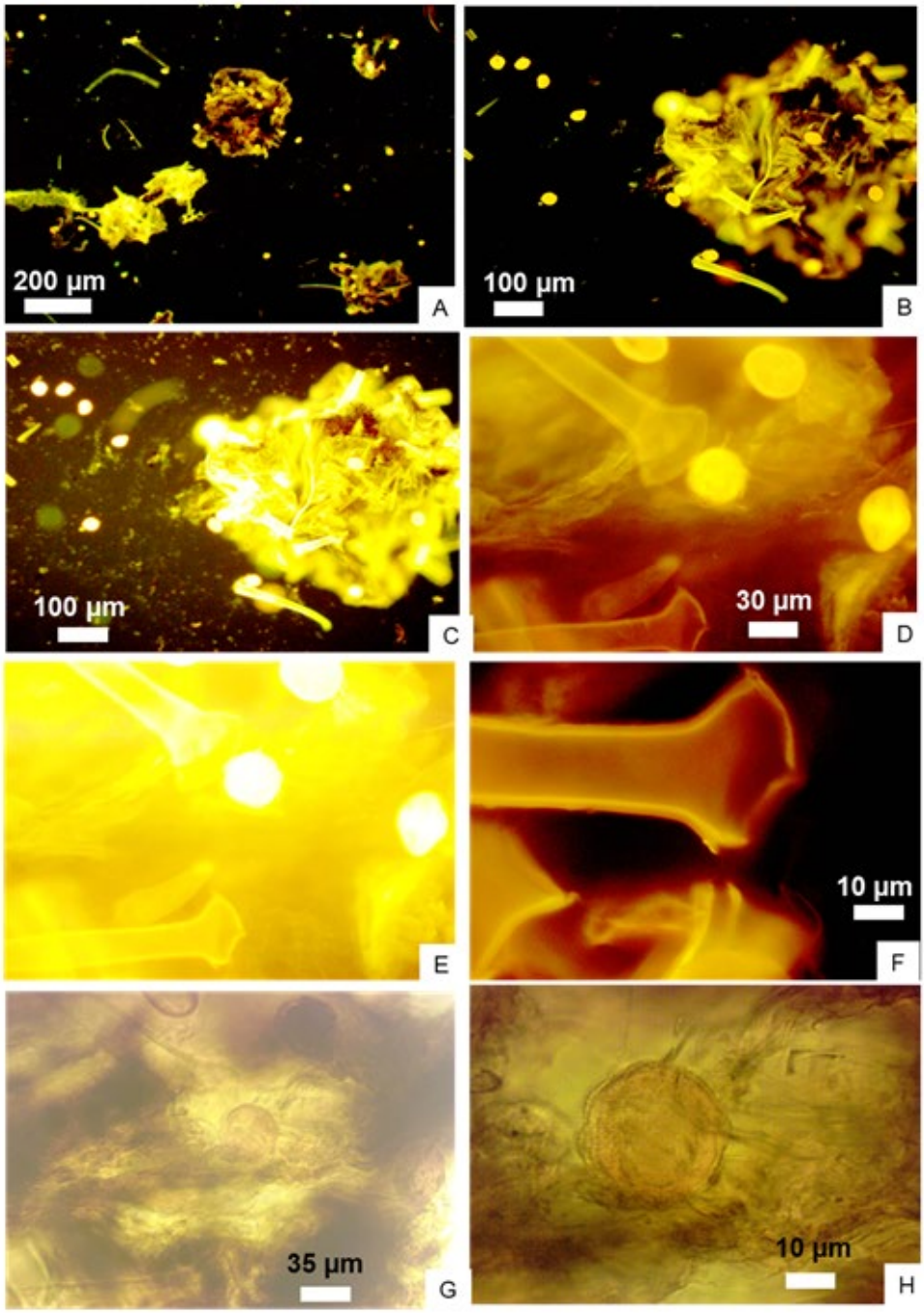

Figure 9. Excrements bearing pollen grains and other passionflower remains (LPPH 112 2023), after acetolization mounted with glycerin. The parameters are detailed in the illustrated specimens with white and fluorescent lights. A. (x4), 2000 ms, 3G. B. (x10), 1000 ms, 2G. C. (x10), 2000 ms, 3G. D-E (x40). D. 750 ms, 3G. E. 2000 ms, 3G. F. (x100) 1000 ms, 2G. G-H. A pollen grain in D-E is poorly seen under the white light microscope. G. (x40) and H. (x100). Cuticles, trichomes, and pollen grains revealed a fluorescent darker yellow color bearing a red halo.

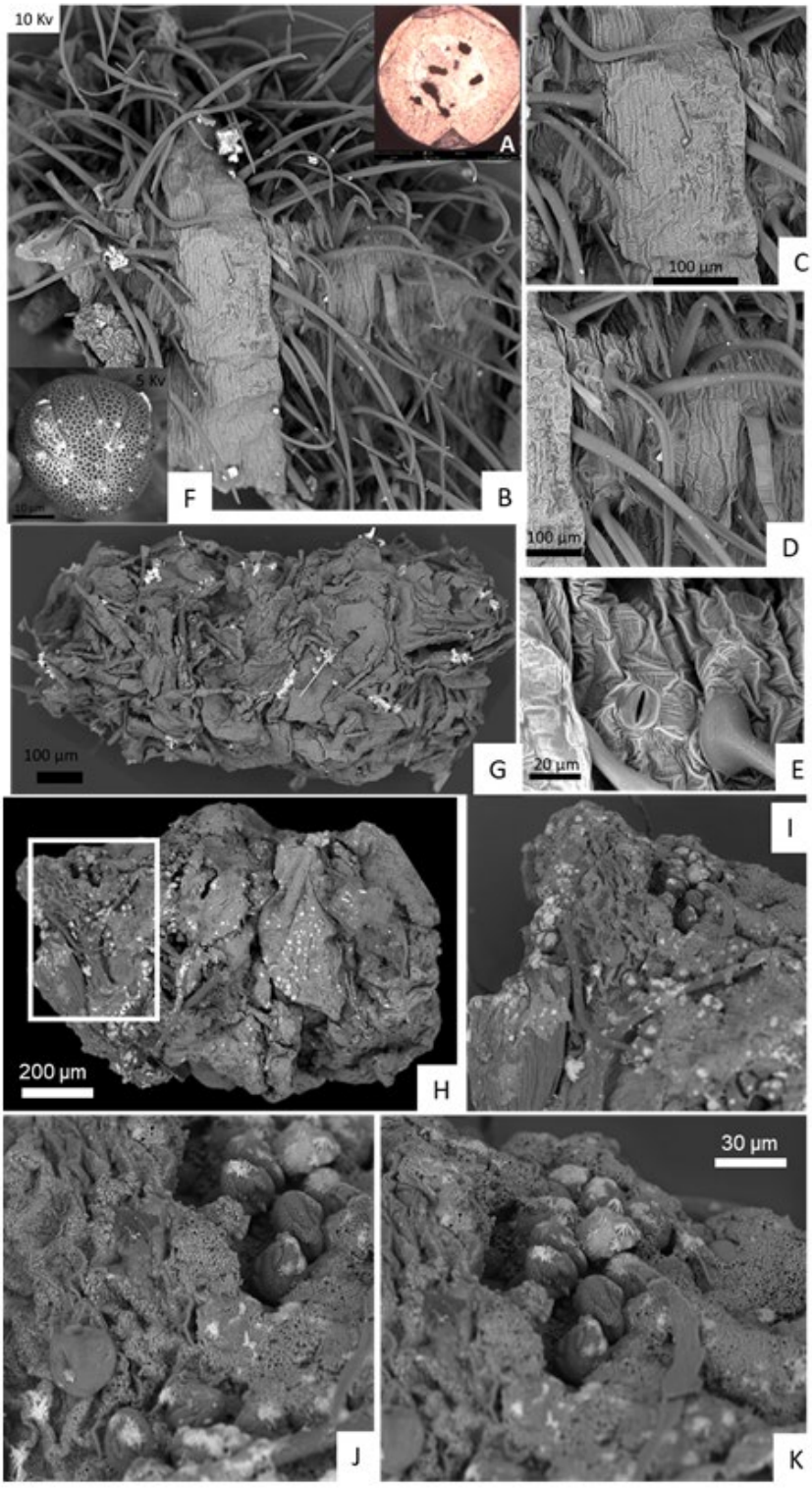

Figure 10. A. Stub showing specimens of excrements (1) and anthers (2) of *P. suberosa*. B-K. Three excrement specimens with different content. B-F. Excrements bearing cuticles with stomata (E) and trichomes (B, C, D) and one pollen grain (F). G. Another specimen with varied particles of organic matter derived from *P. suberosa*. H-K. The third specimen shows cuticles, trichomes, and embedded pollen grains detailed in J-K.

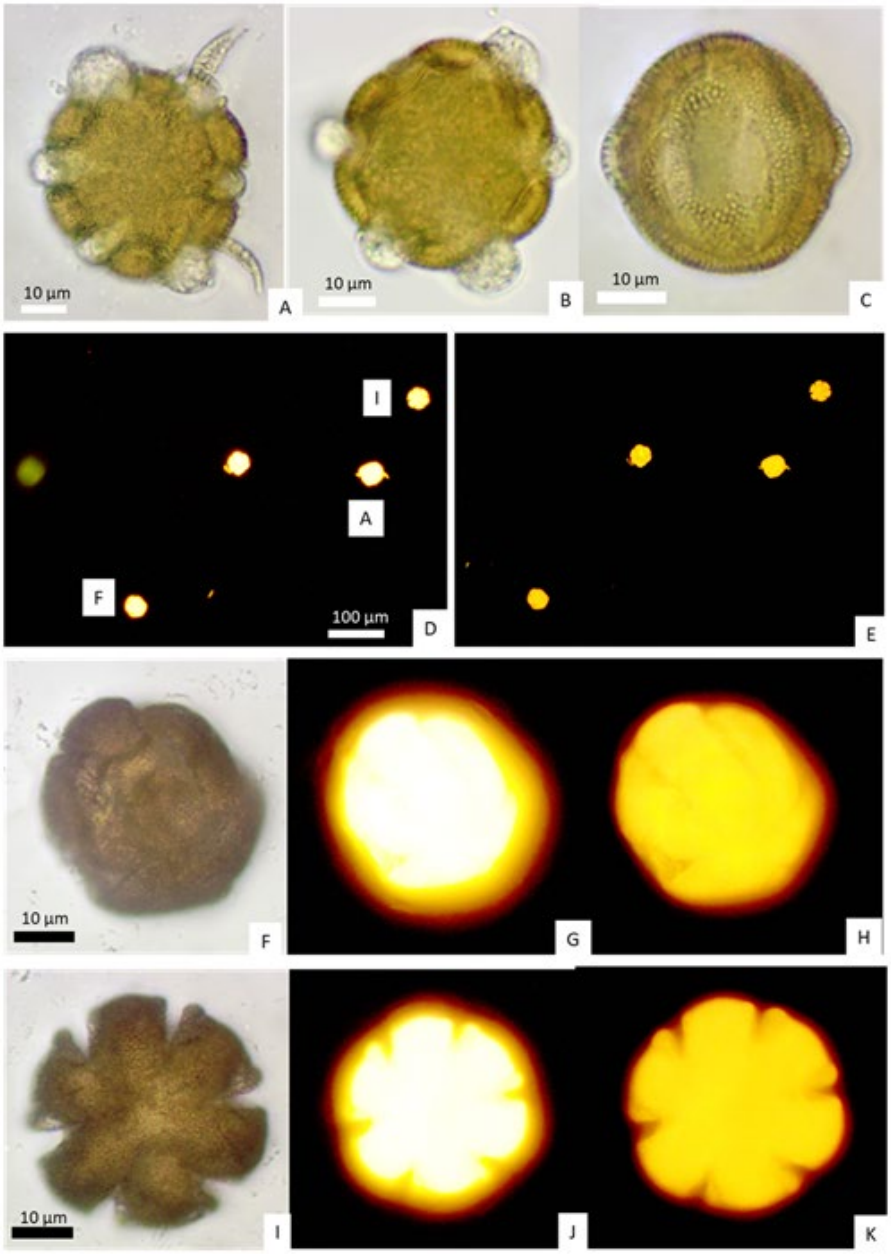

Figure 11. Pollen grains from the anther of the flower (LPPH 112 2022) were treated with HCl boiled and mounted with water (A-C, x100 magnification) and glycerin (D-K). Despite some specimens maintaining the cell content as seen in A-C, others lost it when treated with HCl boiled (an easier faster, and less risky method than acetolysis). Also, they show a shift in fluorescence color of a darker yellow/orange encircled by a red annulus (halo) using both 700 ms and 2000 ms and 3G parameters of the video camera. (x10). D-E. D. 2000 ms, 3G. E. 700 ms, 3G. (x100). F-K. G and J. 2000 ms, 3G. H and K. 700 ms, 3G. This color differs from the pollen mounted directly from the flower illustrated in Fig. 5.

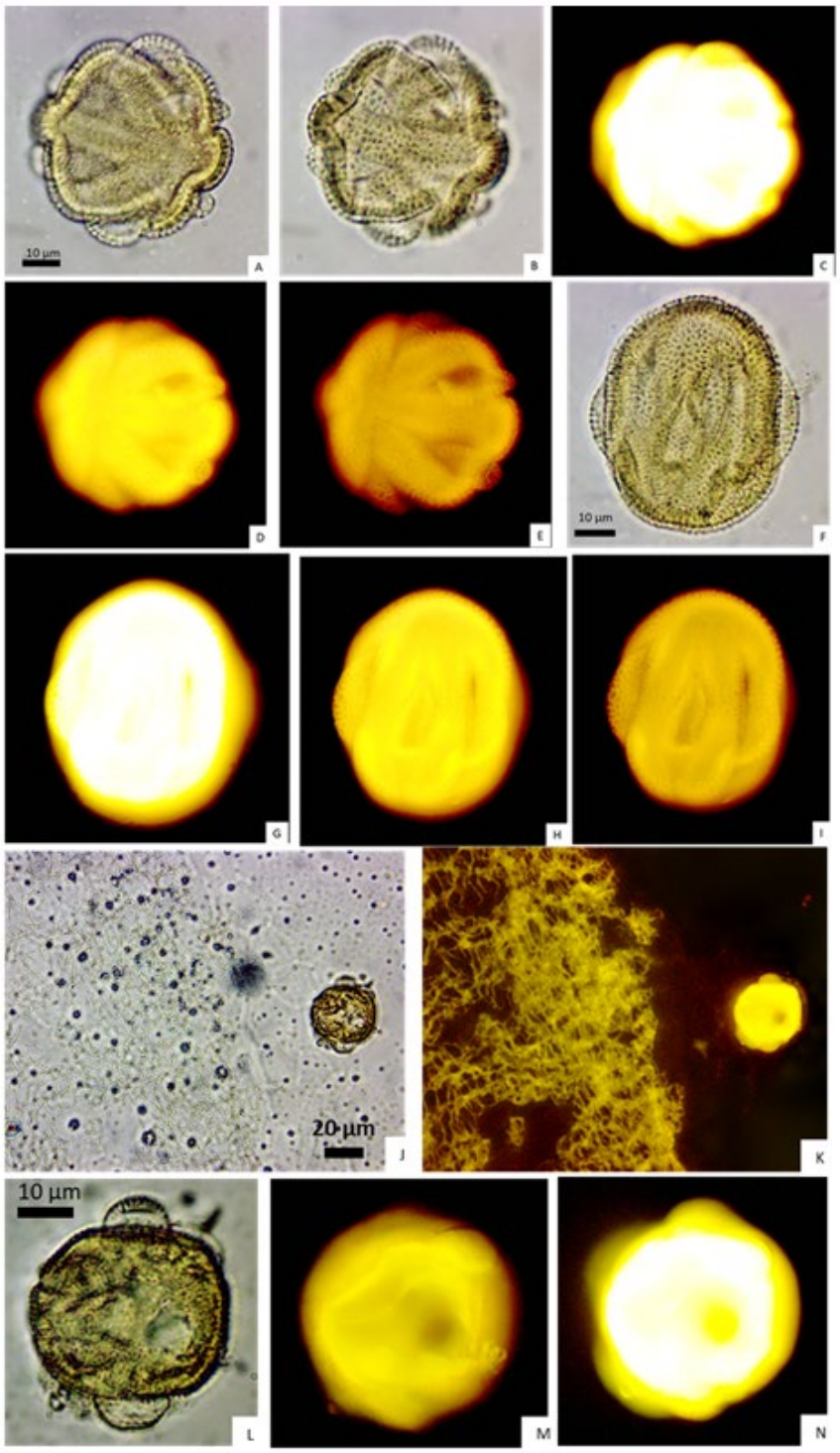

Figure 12. Pollen grains from the anther of the flower (LPPH 112 2022) were treated with acetolysis and mounted with glycerin and illustrated with white (A-B, F, J, L) and the remaining with fluorescent lights under the following parameters: (x40), K. 1100 ms, 3G. (x100), C. 2000 ms, 3G. D. 700 ms, 3G. E. 350 ms, 3G. G. 2000 ms, 3G. H. 700 ms, 3G. I. 350 ms, 3G. M. 700 ms, 3G. N. 2000 ms, 3G. It is noticed in the cuticle in K and all pollen grains a fluorescent darker yellow color bearing a red halo.

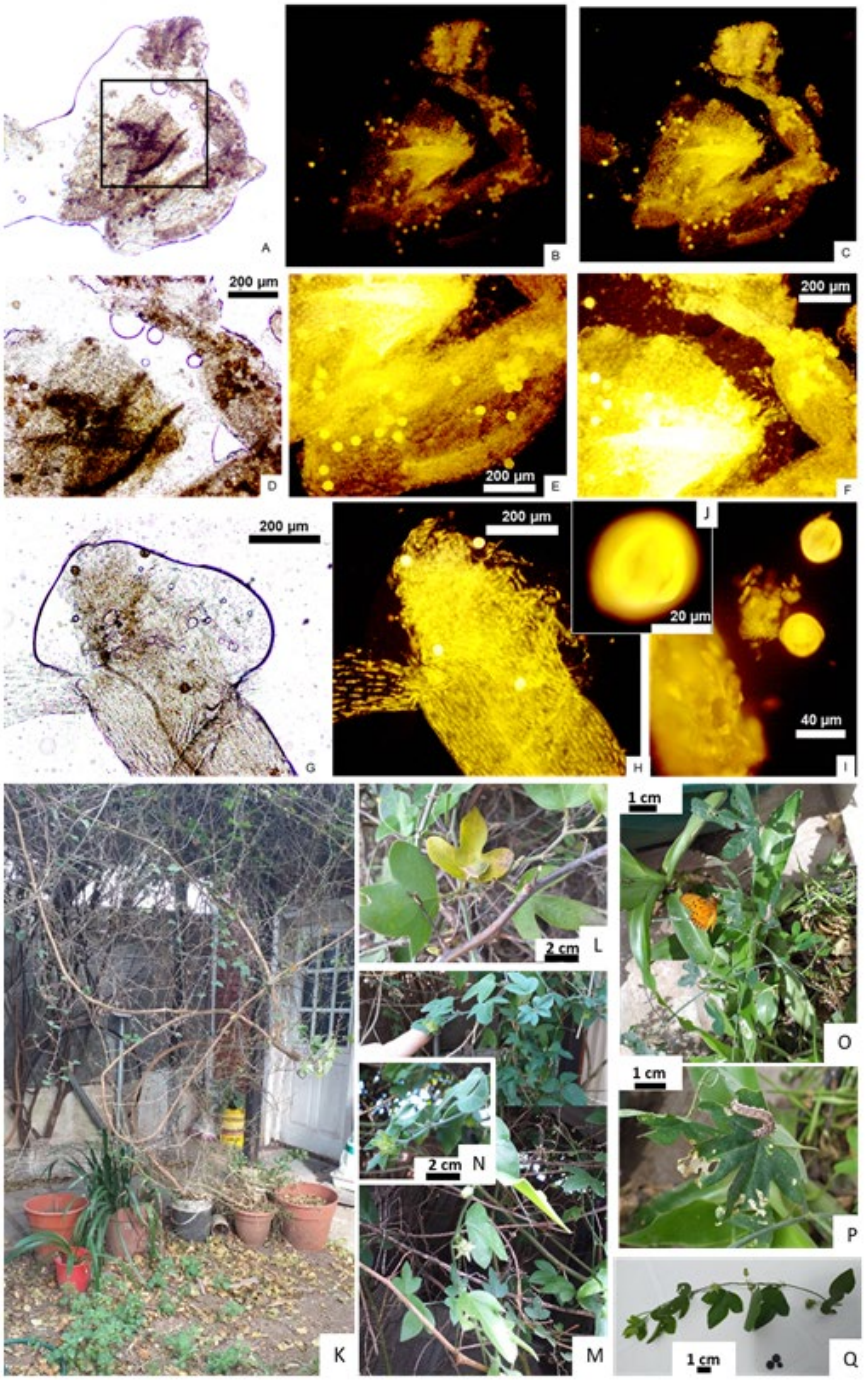

Figure 13. Anthers of flowers bearing pollen grains (LPPH 112 2023) oxidized with nitric acid and ammonium (2 minutes). After washes in a centrifuge, HCl boiled was applied, and after washes again, slides were mounted with glycerin. The parameters are detailed in the illustrated specimens with white and fluorescent lights. A-C. (x4). B. 1200 ms, 3G. C. 2000 ms, 3G. D-F (x10), areal zone mark in A. E-F. 1200 ms, 3G. G-H. (x 10), 1200 ms, 3G. I. (x40) 1200 ms, 3G. J. (x100), 1200 ms, 3G. It is noticed in the cuticle of anthers and pollen grains a fluorescent darker yellow color bearing a red halo. K-Q. Garden with *Passiflora suberosa* vines from where flowers (N-M and Q) and soil samples were taken. L. Insect over a leaf of *P. suberosa*. O-P. Butterfly and caterpillar on *P. suberosa*. Q. *P. suberosa* fragment with flowers and fruits.

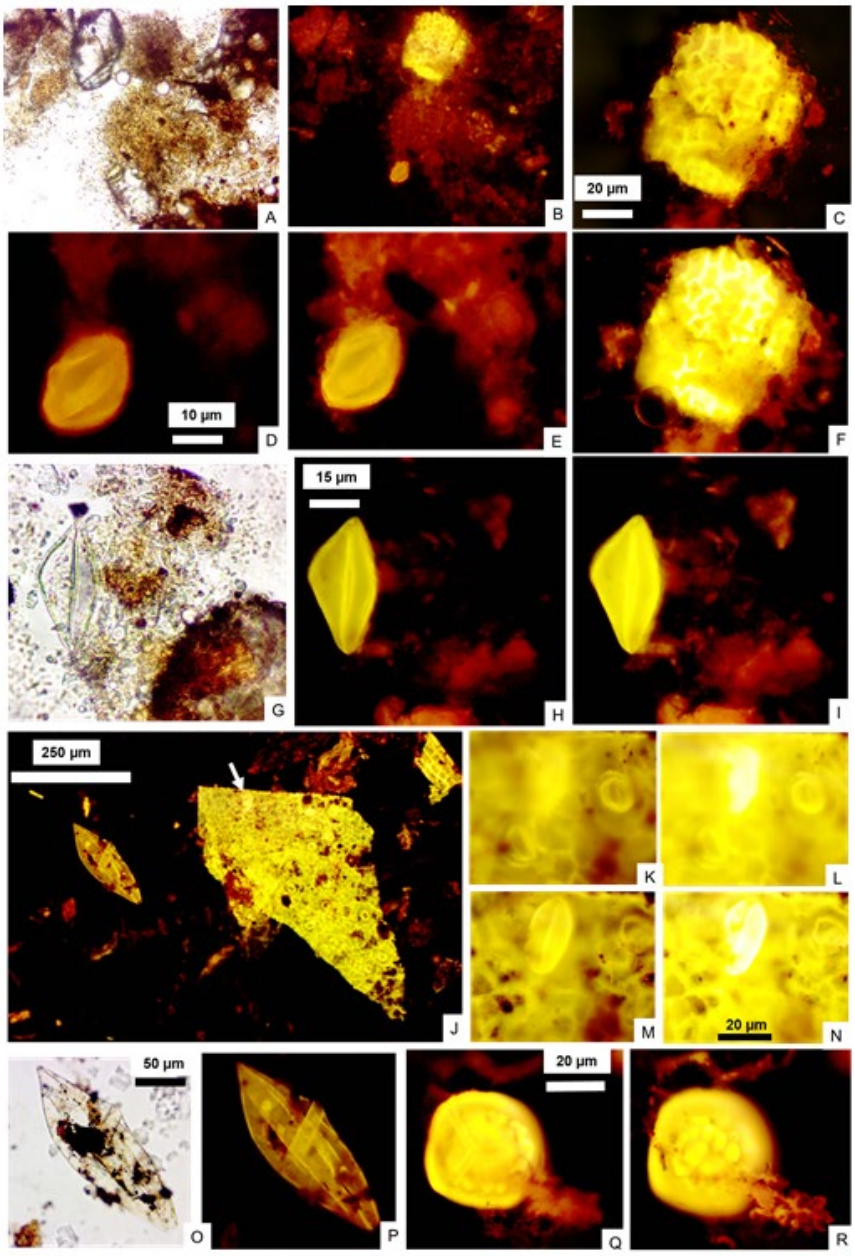

Figure 14. Palynomorphs from a soil sample of the garden (CICYTTP-Pl 2768), after being processed with HF and HCl boiled, washed, and mounted in glycerin (A-I), and then, acetolysis was applied and also mounted in glycerin (J-R). The fluorescence of palynomorphs is compared considering parameters used detailed in each illustration. A-B. Two pollen grains (x40), not visible under white light but with fluorescence are identified as poorly preserved *P. caerulae-edulis* (C, F) and *P. suberosa* (D-E) types. C-D. (x100), 1200 ms, 3G. E-F. (x100), 2000 ms, 3G. G-I. Palm pollen grain (*Syagrus romanzoffiana* type, x100) well-seen in fluorescence light (H, 1500 ms, 3G. I, 2000 ms, 3G). J. Palynomorphs (arrow indicates a palm pollen over cuticles illustrated in K-N), and cuticles (x10) are revealed under fluorescence light (1200 ms, 3G). K-N. Palm pollen grain (*Syagrus romanzoffiana* type, x100) over a plant cuticle (K, M. 1200 ms, 3G. L, N. 2000 ms, 3G). O-P. Arthropod egg (2000 ms, 3G). Q-R. *Lophosoria* type spore (Monilophyte), proximal (Q) and distal (R) faces (x100, 2000 ms, 3G).

Figure 15. A-N. *P. caerulae* consumed by caterpillars (march 2024, Diamante). I and I*. Pupa phase (chrysalis) c. 2 cm in length.

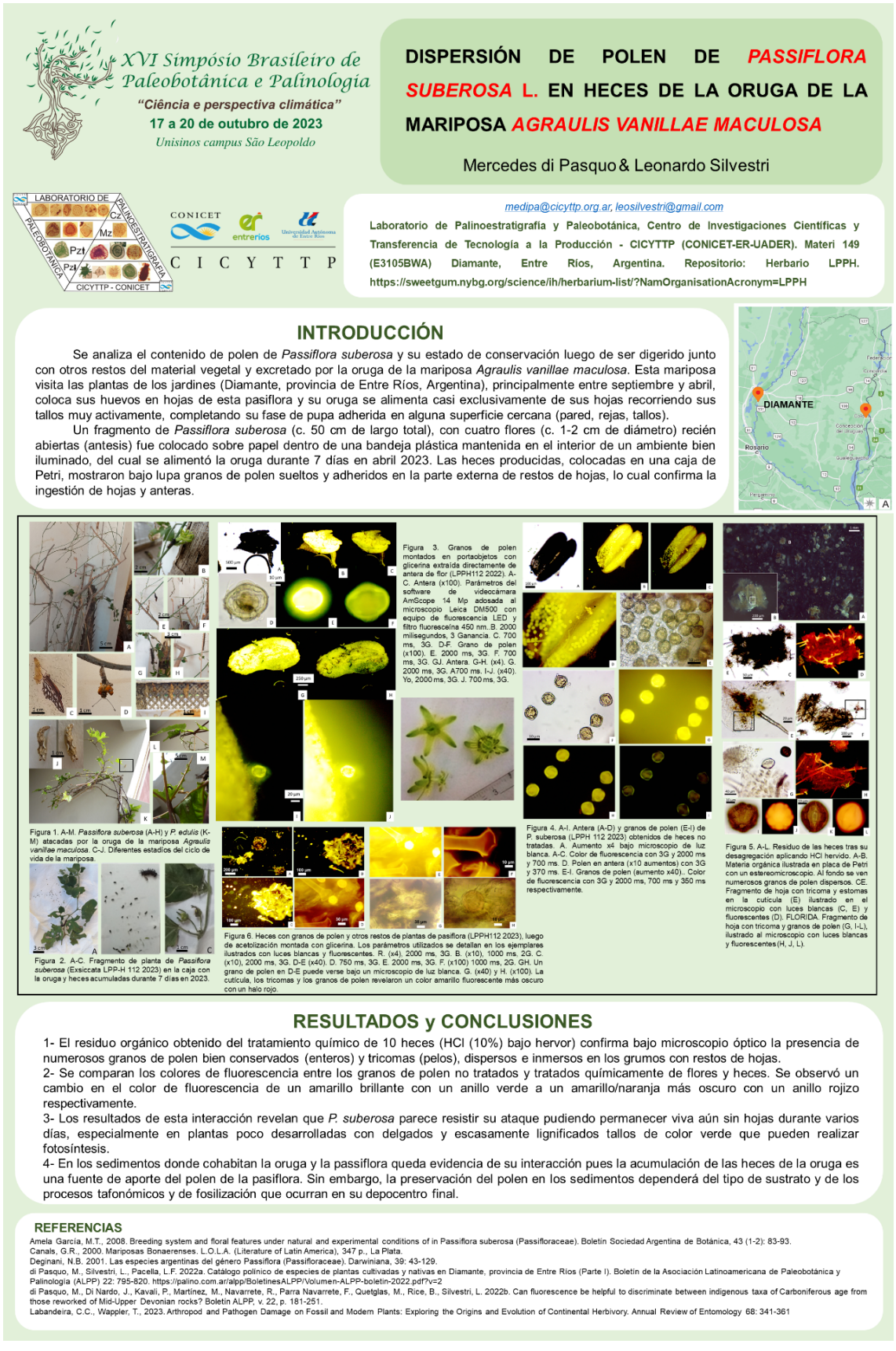

Figure 16. Poster presented at the XVI Simpósio Brasileiro de Paleobotânica e Palinologia (So Leopoldo). https://www.even3.com.br/xvisbpp2023