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Evaluation of the Preservation of Chitinozoans in a Case Study of the Katian?—Hirnantian Succession, Eastern Precordillera of Argentina

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Abstract: The poor preservation state of chitinozoans recovered from samples of the La Pola (Sandbian–Katian) and Don Braulio formations (Hirnantian-Llandovery), after being processed with standard methods, required significant modifications in processing. The sodium hexametaphosphate technique was used to avoid invasive mechanical procedures. Hence, more complete chitinozoans that preserved original features produced by biological, mechanical, and chemical degradation appeared. The processes that affected the good preservation of the chitinozoan exine are associated with factors inherent to the sedimentary environment, and biological and geological taphonomic effects that occurred in different regions of the Precordillera, added to the important associated tectonic activity. The thermal alteration was also an important factor in the fragile and brittle condition of the organic matter recovered. This transcends the preservation-processing technique relationship to be used. The results obtained from non-standard processing contribute to the paleoenvironmental interpretation of the Pola and Don Braulio successions, which are still widely discussed, and the determination of the most accurate age of the La Pola Formation. The latter will allow us to propose a biostratigraphic correlation between chitinozoan zones and graptolite zones, given the presence of both groups in the succession studied here, and to establish correlations with other successions in the Precordillera and elsewhere.

Keywords: preservation; chitinozoans; glacial event; taphonomy; pyrite; diagenetic processes; late ordovician; Precordillera; argentina

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1. Introduction

The preservation of palynomorphs depends on several factors. These can include the type of environment and climate where the species inhabit, their morphological and chemical characteristics linked to form of life and mode of dispersion, and taphonomic aspects that can modify their original form in the way of the final burial (e.g., type of transport, distance, physical–chemical characteristics of temporary places of deposition and final depocenter). Therefore, we should expect different types of preservation in the same sample due to the specific characteristics of each palynomorph and its taphonomic history (e.g., abrasion during transport, flattened during burial, different tolerances of wall layers (exine) to the various destructive agents such as redox changes, salinity changes, and biogenic corrosion). Besides, diagenetic factors (fossil-diagenesis) can also affect organic matter preservation (physical–chemical transformations such as carbonization, compression, deformation, and diagenetic mineralizations like silicification, carbonation, and pyritization), especially those related to thermal alteration due to buried sediments or even, tectonic, igneous, and metamorphic processes and weathering of rocks (e.g., [1–7]). Finally, rock erosion could bring palynomorphs and other components of organic matter that would be incorporated into a new deposit site within a new cycle of sedimentation (reworked material). For this reason, palynomorphs need to be identified at the species level, if possible, to know the most probable range of time (biochron) that will allow the discrimination between indigenous and reworked taxa in a palynological (or any fossiliferous) analysis [8] (references therein). Hence, the ideal conditions for the excellent preservation of palynomorphs, like those called fossil-lagerstätten (i.e., the assemblages in these types of deposits are probably the closest approximation to the abundance and diversity of the original assemblage), are not as frequent as we would like, being that the inverse, poorly preserved, and thermally-altered associations are more frequent (e.g., [2]). Standard techniques for treating palynological samples have primarily been designed for rocks with ideal preservation states, but those factors should influence the processing method chosen. The term "Standard Technique" is inappropriate as most laboratories have their basic technique modified according to their specific needs and objectives of the research and the particular characteristics of the rock [9].

Concerning chitinozoans, as the group focused on this contribution, their test is made of a highly resistant pseudochitinous substance (e.g., [10] (p. 10), [11] (p. 132)), described as a highly aromatic functional group with less aliphatic components in a pyrolysis and micro-Fourier Transform Infrared (micro-FTIR) analysis [12] and oxygen- or nitrogen-bearing compounds [13]. This composition, shared with range-associated scolecodonts and arthropod cuticles, prevents their autofluorescence [12]. However, the biological affinity of chitinozoans cannot be related with accuracy to any fossil or extant group (e.g., variously attributed to chrysomonad cysts, fungi, a stage in the graptolite life cycle, metazoan (e.g., worm) egg cases, rhizopods (Sarcodina Foraminifera) or relatives of the tintinnids, testate thecamoeba protists, see [3] (and references therein), remaining incertae sedis [12,14]. The occurrence of 'colonial' chains, clusters, and 'cocoons', plus the pseudochitinous composition and wall structure of the test, have led to a general acceptance that the Chitinozoan are probably structures produced by an extinct invertebrate animal or hypothetical creature called 'chitinozoophoran' (e.g., [15]). On the other hand, Tyson [3] stated that almost all the chitin is degraded after only about 150 h. Because of this rapid degradation (which also occurs under anoxic conditions), the chitin content of even Recent sediments is typically small. Most of this is probably derived from mollusk shells and Bryozoan. Despite the exact process of fossilization is unknown, which enables the occasional preservation of chitinous organisms in the fossil record remains enigmatic [3,16].

Their taxonomic classification is mainly based on the external silhouette, and they proved to be very useful in biostratigraphic dating of Early Paleozoic fine-grained sediments [17]. The progressive thermal alteration of the test changes from translucent and amber-colored to brown and finally black (opaque). Hence, they are useful and helpful, especially in those metasedimentary successions (highly tectonic-metamorphosed rocks like green-schist facies) bearing organic matter opaque. However, ongoing palynological analyses have shown that standard techniques are applied by considering their large size, varying from 50 µm to 2 mm in length. It needs to avoid crushing pieces of rocks less than 1 cm. Moreover, for recovering complete specimens of extremely fragile and brittle chitinozoans, standard techniques need modification and adaptation based on the results obtained from different types of rocks [3,18,19]. In this case, direct observation at the surface of the rock slabs and petrological slides constructed along the bedding plane can also be useful [18].

We present a case study on palynomorphs obtained from the Ordovician-Silurian transition of the Precordillera Argentina (Figure 1A,B), including the formations La Pola (Sandbian–Katian?) [20] and Don Braulio (Hirnantian-Llandovery) [21] in the Quebrada La Pola section (Figure 1C–E). The preservation state of chitinozoans has required significant modifications in the process to avoid destroying them and to be able to carry out a morpho-taxonomic analysis and interpretations about the environmental conditions where they lived and determine the taphonomic and post-depositional effects that affected them.

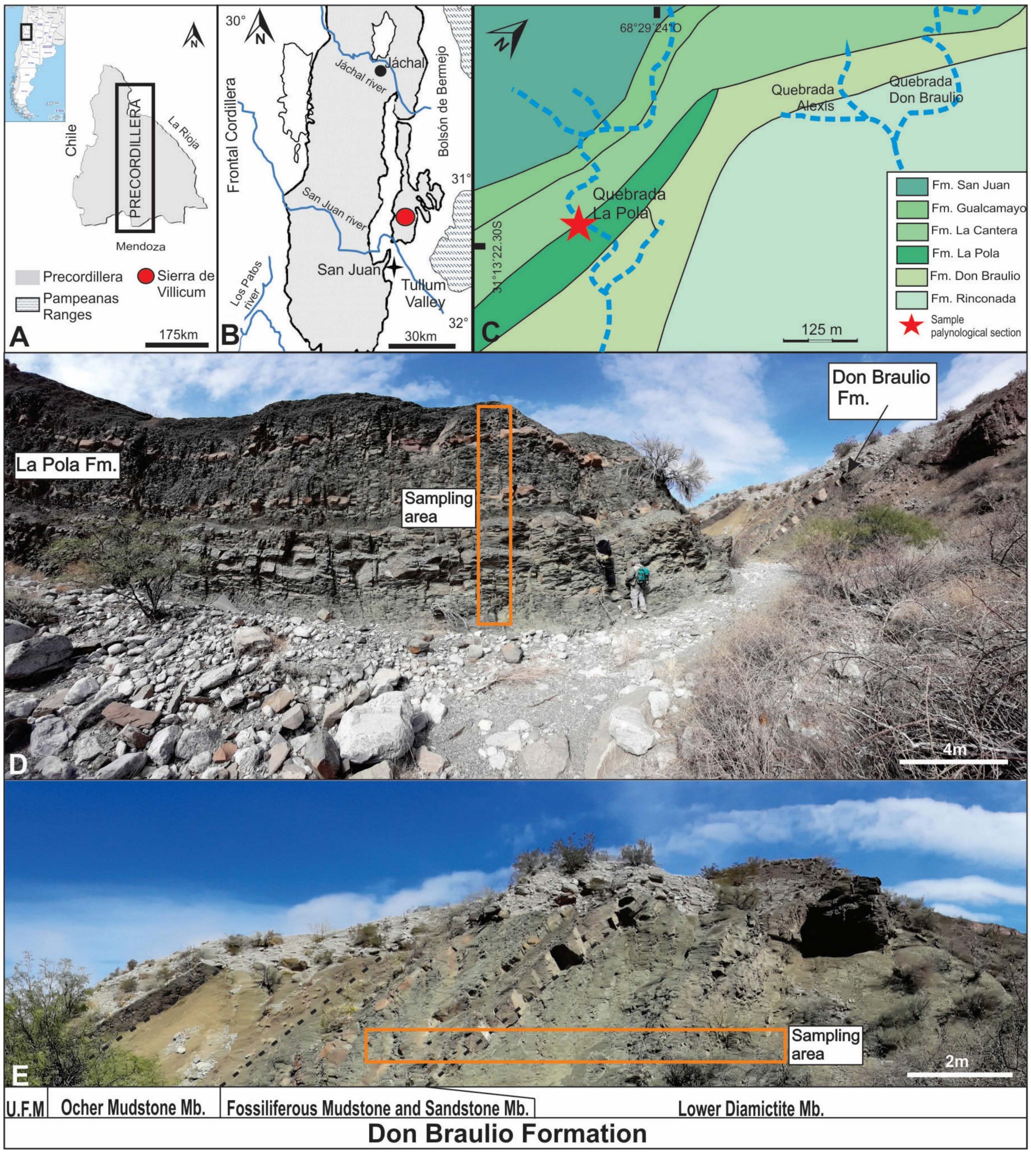

Figure 1. (**A**) Location map of San Juan in Argentina. A rectangle indicates the Precordillera. (**B**) Location of the Sierra de Villicum, Precordillera Oriental of Argentina, San Juan Province. (**C**) Geologic map of the Quebrada La Pola showing the section sampled. (**D**) Panoramic photograph of the sampling area in the upper part of the La Pola Formation. (**E**) Panoramic photograph of the Don Braulio Formation indicating the sampling area. Upper Ferriferous Member (U.F.M).

2. Geological Setting

The Geological Province of Precordillera, Western Argentina [22] is divided into three morpho-structural units. The Eastern Precordillera is characterized as a West-directed thick-skinned fold belt [23]; the Central Precordillera as an East-directed thin-skinned fold belt [24,25], and the Western Precordillera, which is an East-directed thin-skinned fold belt [25,26]. In the Eastern Precordillera, two units have been related to the Late Ordovician glacial event, the La Pola and Don Braulio formations. These units outcrop together only in the Quebrada La Pola section and its surroundings.

The La Pola Formation is interpreted as a relic of the Hirnantian glaciation [20]. It is separated from the underlying La Cantera Formation (Sandbian) by erosional contact, and it is overlain (erosive unconformity) by the Don Braulio Formation, a heterogeneous succession with both allochthonous and autochthonous faunas interbedded. Its lithofacies include blocky paraconglomerates, channels filled with quartz sands, pebbly mudstones, bioclastic sandy conglomerates, graded or massive lenticular sandstones, thin rhythmites, tabular and silty-sandy mudstones (Figures 1D and 2). In the lower and central sections, paraconglomerates indicate the presence of well-developed diamictites. The sequence shows frequent re-sedimentation, where the original materials have characteristics of a high-energy clastic platform (rich in quartz) along with simultaneous accumulations of carbonate, such as reefs [20,27,28].

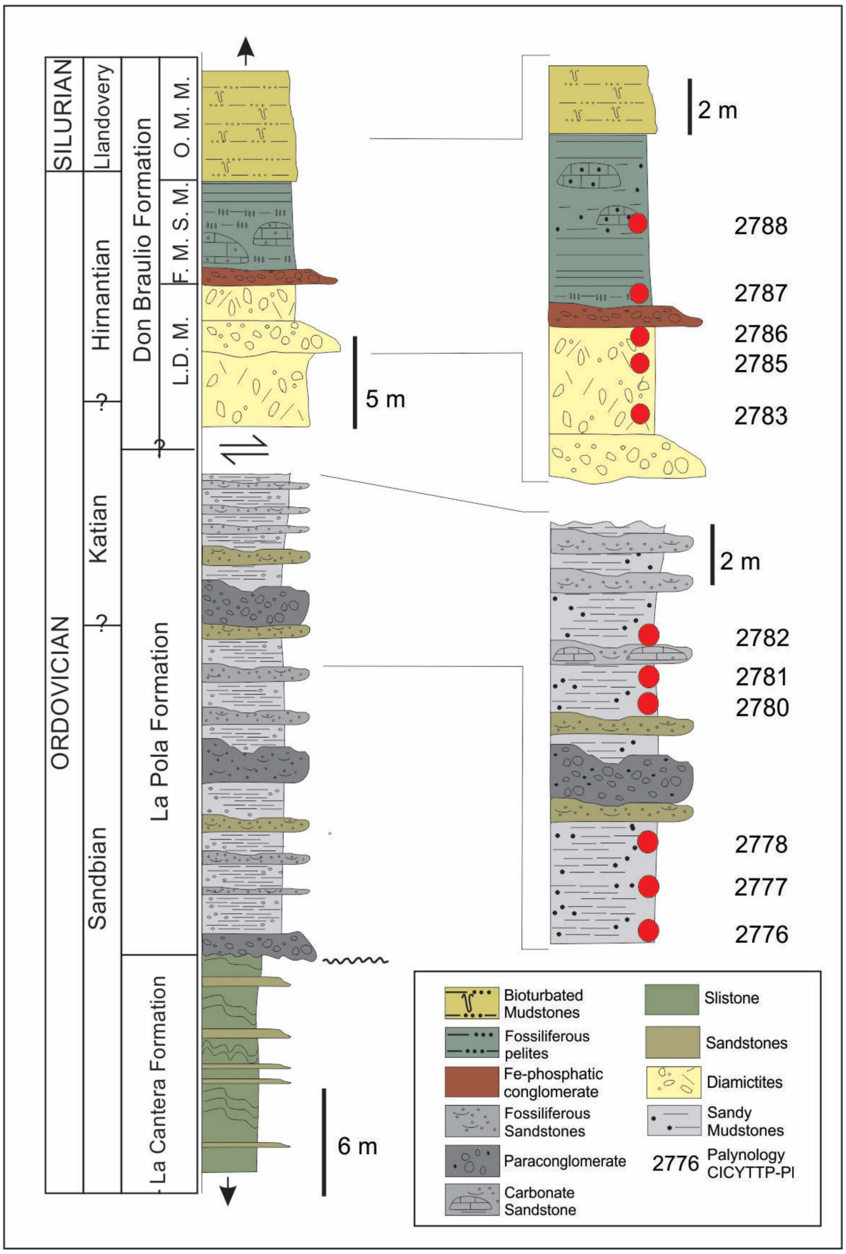

Figure 2. Profile of the Sandbian-Hirnantian succession at La Pola Creek. The sampled levels are shown acronym CICYTTP-Pl. L.D.M (Lower Diamictite Member); F.M.S.M (Fossiliferous Mudstone and Sandstone Member); O.M.M (Ocher Mudstone Member).

A variety of macrofauna (such as colonies of bryozoans, fragments of brachiopods, arthropods, crinoids, and thalli of red algae), mostly of allochthonous (re-sedimented) origin despite fairly good preservation [20], are documented. In the paraconglomeratic blocks, a well-preserved graptofauna of the genus *Dicranograptus* was found [29].

Benedetto [30] notes that the bioclastic levels in the middle of the unit correspond to skeletal concentrations of the mechanical origin (transport, transfer, and/or rework). The bioclasts and quartz sandstone facies were likely re-sedimented from shallower and high-energy environments developed in the eastern part of the basin. However, the author also highlights an autochthonous brachiopod fauna in the upper part of the unit, specifically in the muddy matrix of the diamictites and the calci-limolite facies. A well-preserved conodont fauna was discovered in carbonate sandstone layers in the upper part of the unit by [28]. This fauna was found alongside brachiopods, trilobite spines, bryozoans, crinoids and sponge spicules. Based on paleontological evidence, the La Pola Formation is suggested to be of the Sandbian–Katian age (Middle-Late Caradocian).

The Don Braulio Formation [21] is one of the lowest Upper Ordovician units where well-preserved macrofauna such as brachiopods, trilobites, and graptolites were identified. It is divided into four members [31]: a basal Lower Diamictite Member composed of greenish-gray pebbly mudstone including channel–fill conglomerate structures, followed by a succession of sandstone-bearing plutonic, and carbonate clasts and bioclastic debris to a lesser extent [27,31,32], and they are intercalated with thin to medium-layered sandstones and pebbly mudstones. Occasionally, the sediment contains fossilized remains of brachiopods, bryozoans, and crinoids from the Hirnantian Fauna [31], and well-preserved re-deposited brachiopod association from the Sandbian stage in the La Cantera Formation [33]. This member presents sedimentologic and paleontologic evidence that confirms its relationship with the Late Ordovician Glacial Event [27,32,34–38].

The second member (Fossiliferous Mudstone and Sandstone Member) is paraconformably covered by grayish-green shales and fine-medium-grained sandstones, including fossiliferous calcareous lenses [27,31] (Figure 2). In this member, the trilobites *Dalmanitina sudamericana* and *Eohomalonothus villicunensis* [39], brachiopods of the typical *Hirnantian Fauna* [40–42], a monospecific association of *Metabolograptus* (ex. *Normallograptus*) *persculptus* [43], the bivalves *Modilopsis cuyana* and *Palaeoneila* sp. [44,45], and an assemblage of sponge spicules dominated by hexactins (six rays) including possible pentactins (five rays) and one stauractin (four rays) [46] have been recorded (Figures 1E and 2).

This member is overlain in sharp contact by yellowish bioturbated mudstone, with abundant trace fossils, mainly borings and dwellings, and poorly preserved biserial graptolite structures (Ocher Mudstone Member) [27] (Figures 1E and 2). The Upper Ferriferous Member is a succession of reddish-brown ferriferous sandstone and oolite beds, interbedded with black to dark gray shales and siltstones [47]. Graptolites from the *Atavograptus atavus* Zone [48] and Llandovery palynomorphs [49] have been recorded in these deposits. At its type locality, the Don Braulio Formation lays paraconformably (erosive surface) over the La Cantera Formation and, in turn, is covered (erosive paraconcordance) by the olistostrome of the Devonian Rinconada Formation [31].

3. Materials and Methods

The Sandbian-Hirnantian succession at the Quebrada La Pola, Sierra de Villicum, Eastern Precordillera (31°13′22.30″ S, 68°29′24.00″ W) was analyzed. The rocks came from the upper part of the La Pola Formation and the Lower Diamictite Member of the Don Braulio Formation (Figure 2).

3.1. Sampling

The fieldwork involved systematic sampling, considering the complex facies changes in each mentioned unit. Previously recorded fossil levels, as reported by [28–30,33,50], were also considered.

3.2. Palynological Processing

Subsequently, 16 samples were prepared for palynological analyses in the Palynostratigraphy and Paleobotany Laboratory (LPP) of the CICYTTP Research Center (CONICET-ENTRE RÍOS-UADER). The materials were deposited under acronyms according to the corresponding collection, i.e., CICYTTP-Pl for palynological preparations (Figure 2) and CICYTTP-M for SEM specimens [50,51]. The processing consisted of 11 samples from the

La Pola Formation (CICYTTP-Pl 2776-2782; CICYTTP-M 2776-2782) and from the Don Braulio Formation (CICYTTP-Pl 2783-2788).

The standard methodology (Figure 3) was applied to 20 to 30 g. of sample, crushed into pieces no smaller than 5 mm. It was then immersed in hydrochloric acid (20%) and hydrofluoric acid (45%) (Figure 4A). Due to the high mineral content, the samples were treated again with hydrofluoric acid (HF). The acids were neutralized using distilled water, and residues were sieved through 25 µm and 10 µm meshes (Figure 4B). The excess water was removed from the residue in Falcon tubes by centrifuging for 5 min at 1000 rpm (Figure 4C). The residue was then mixed with alcohol (70%) and centrifuged again before slides were mounted for the palynological analysis using Trabasil NR2 [52] (Figure 4D). Slides were analyzed to evaluate the potential organic matter content, and its features including the presence of palynomorphs, regarded as fertile. Several samples yielded only fragmented organic matter, so they required revision of residues and rocks to decide the application of different methods and try to recover more complete palynomorphs (Figure 4E,F).

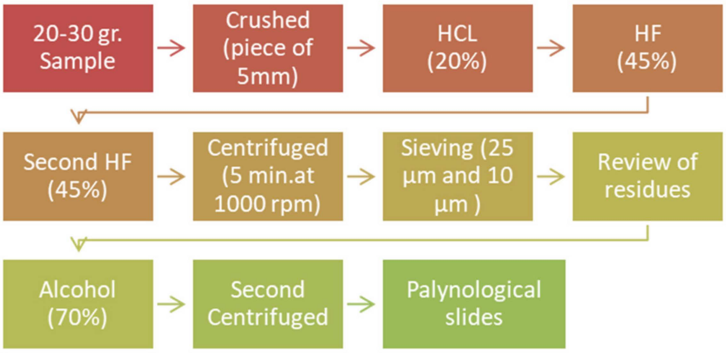

Figure 3. Flow chart summarizing the standard methodology applied in the Palynostratigraphy and Paleobotany Laboratory (LPP) of the CICYTTP Research Center (CONICET-ENTRE RÍOS-UADER), used in processing the samples from the Quebrada La Pola section.

Each residue was observed in a Petri dish under a stereomicroscope (Leica EC3, Leica Macrosystems, Wetzlar, Germany). Palynomorphs and other organic particles were carefully selected using a fine needle. The identified palynomorphs were photographed (Leica 3Mp video camera, Leica Macrosystems, Wetzlar, Germany). Microdroplets were then extracted using a fine glass pipette and placed in temporary preparations with water or glycerin. These preparations were photographed using an optical microscope Leica DM500, equipped with an Amscope 14 Mp video camera and a fluorescence device (LED lamp, filter block for fluorescein ca. 450 nm, Leica Macrosystems, Wetzlar, Germany). The manual concentration of palynomorphs involved repeatedly picking with a pipette to obtain the most significant number of specimens possible for corresponding qualitative and quantitative analyses. Fluorescence assays were carried out using: 1-Temporary slides with glycerin, 2-Temporary slides mounted in water, and 3-Permanent slides with Trabasil NR2.

Afterward, certain palynomorphs were carefully placed onto stubs for photography using an SEM (Jenck Phenom ProX scanning electron microscope, Phenom-World BV, Eindhoven, The Netherlands). This type of microscope operates under a vacuum and does not need metallization (Figure 4G), according to [53]. Additionally, the microscope performed some elemental chemical analyses (EDS).

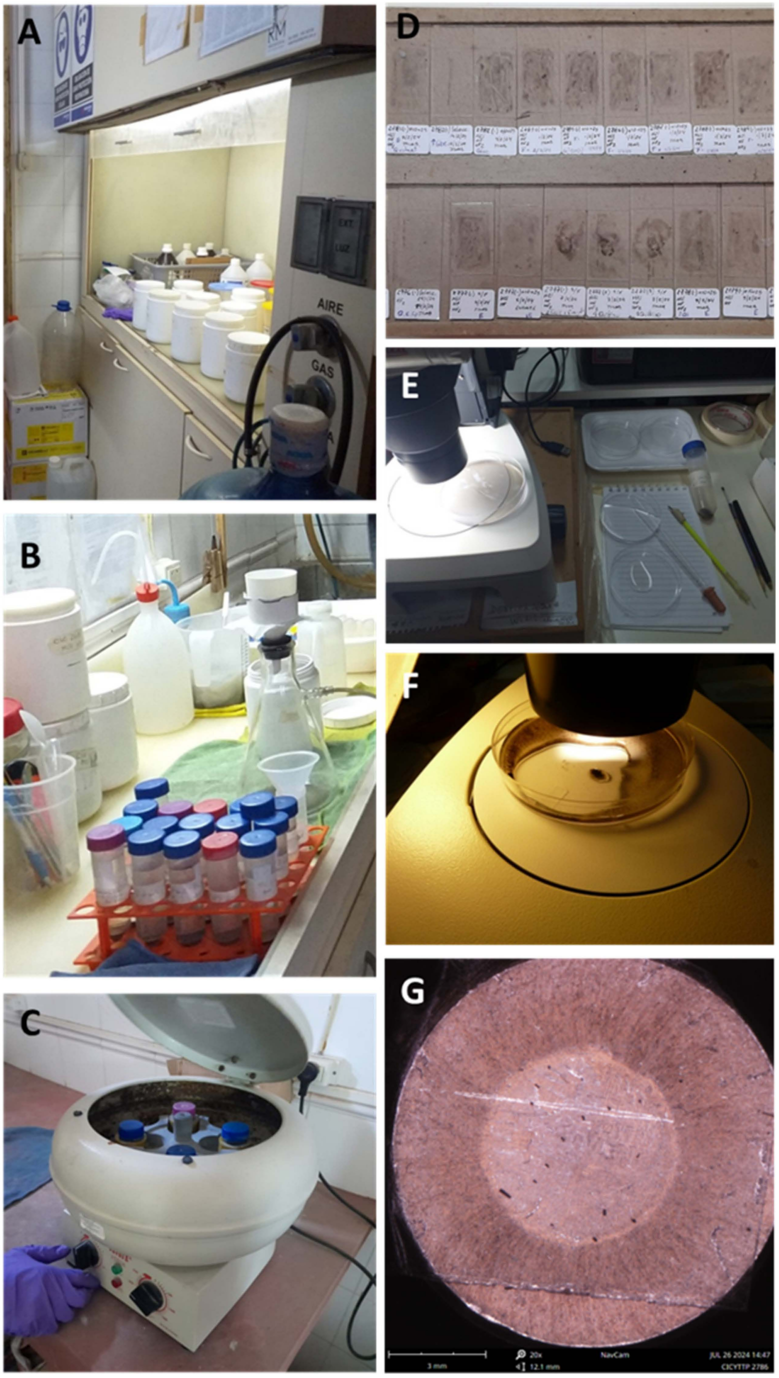

Figure 4. Standard methodology. (**A**) Digestion treatment with HCL and HF. (**B**) Sieving process using 10 and 25 micron filters. (**C**) Remove excess water from the residues by excess centrifugation at 1000 rpm. (**D**) Palynological slides were prepared using Trabasil as an adhesive. (**E**,**F**) Review the residues to analyze the content, abundance, and preservation of the palynomorphs. (**G**) Chitinozoans from CICYTTP-Pl 1786 mounted into a stub for SEM illustration (picture taken into the SEM).

4. Results

4.1. First Analysis of Slides After the Standard Method

During the initial inspection after standard processing, 80% of the palynological slides yielded black remains of possible chitinozoans, graptolites (fragments), and abundant minerals (Figure 5A,B). In some slides, a few poorly preserved chitinozoans were observed to have evidence of biological degradation (Figure 5C,D) and fragmentation lines (Figure 5H,I). Additionally, we found palynomorphs where the organism adhered to the mineral, copying their shape and giving the appearance of biological degradation (Figure 5E,F). There was also chemical degradation by framboidal pyrite and, in some cases, euhedral pyrite (Figure 6). Sample CICYTTP 2782 yielded complete colonies of *Gloeocapsomorpha prisca* Zalessky and almost no whole chitinozoans. Two slides, CICYTTP 2778 and 2780, were infertile/sterile.

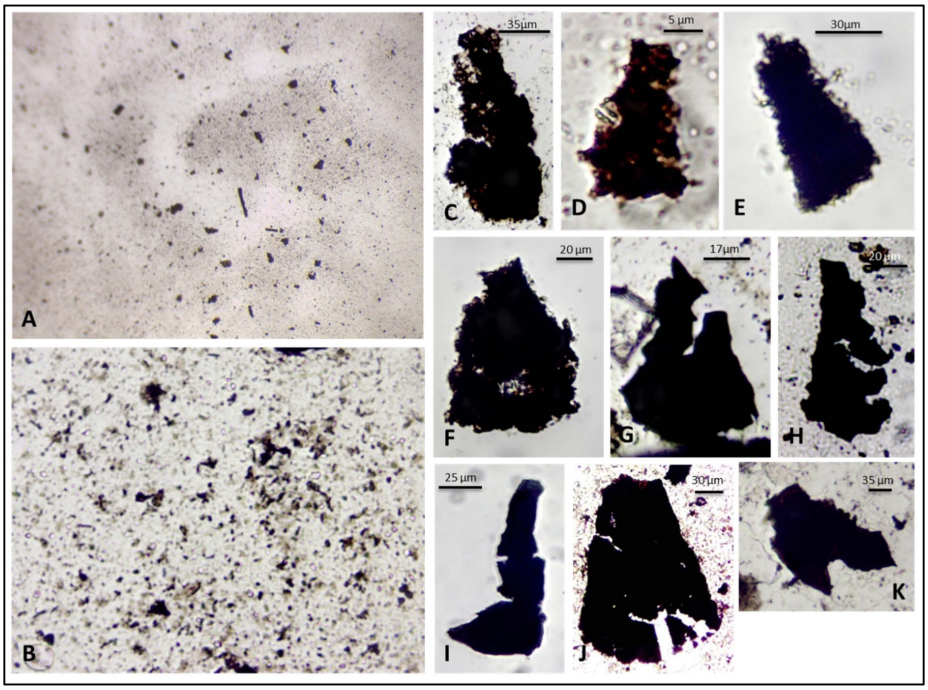

Figure 5. (**A**) Concentration of palynomorphs and fragments of organic matter in examining the residue under a stereomicroscope (CICYTTP-Pl-2777). (**B**) Palynological slide with fragments of chitinozoans processed by standard techniques (CICYTTP-Pl-2777). (**C**,**D**) Examples of chitinozoans with biological degradation (**C**) CICYTTP-Pl-2777 EF N41/1) (**D**) CICYTTP-Pl 2781). (**E**,**F**) Palynomorphs bearing mineral particles still adhered, copying its shape and giving the appearance of biological degradation (**E**) CICYTTP-Pl-2778. (**F**) CICYTTP-Pl-2781). (**G**–**K**) Chitinozoans fragmented after the standard method illustrated in temporary slides with glycerin or water (without coordinates: (**G**) CICYTTP-Pl 2781. (**K**) CICYTTP-Pl-2776. (**I**) CICYTTP-Pl-2785. England Finder coordinates (**E**,**F**): (**H**) CICYTTP-Pl-2783 EF A62/2. (**J**) CICYTTP-Pl-2785 EF D30/1).

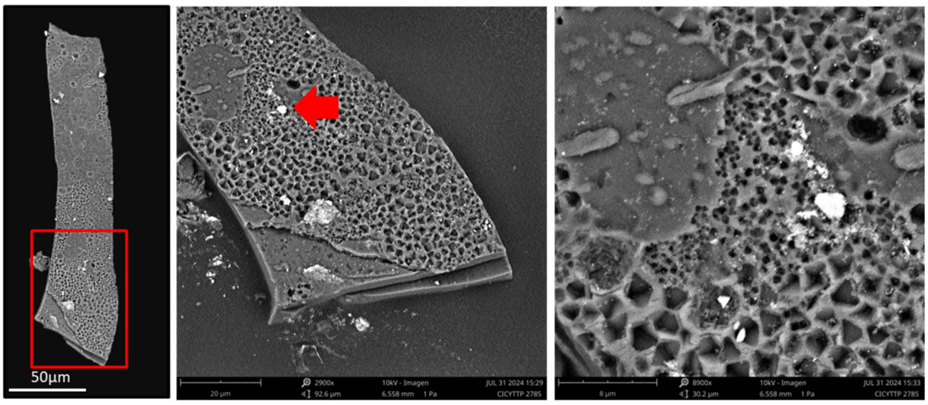

Figure 6. Chitinozoans have chemical degradation caused by framboidal and euhedral pyrite in their external walls (red box). The red arrow indicates framboidal and larger euhedral holes enlarged in the third figure (SEM intensity of energy 10 kV, CICYTTP-M-549 (CICYTTP-Pl 2785).

4.2. Recovery After Applying Modifications to the Standard and the Sodium Hexametaphosphate (Shmp) Methods

A visible fragility in the black-colored walls of the chitinozoans bearing fractures and corrosion confirms the first result obtained from the standard processing (Figure 5G–K). The observation of large chitinozoans and some with 3D relief on the surface of rocks (CICYTTP-Pl-2782) filled with the same sediment that forms the rock supported the application of a less invasive and destructive technique, as the sodium hexametaphosphate [19]. This method is very effective in extracting microfossils, especially calcareous microfossils from calcareous sediments, despite the potential for causing corrosion in calcite [54]. Given the low calcium carbonate content in the Upper Ordovician samples analyzed and the presence of fragile-walled chitinozoans with 3D relief, this technique was modified by applying the dissolved SHMP after a preliminary treatment with neutral detergent (using distilled water). After several weeks, during which the material was smoothly stirred, the grinding, hydrogen peroxide, and heavy liquid ($ZnCl_2$) phases of the [54] method were not followed to prevent additional fracturing and loss of organic material. At the same time, a very gentle process using HCL and HF techniques was applied to the 14 samples. For the former and also, for those in HCl-HF, certain precautions were taken, as follows: (a) despite the high mineralogical content of the samples, the application of a second HF was discouraged, to avoid weakening even more chitinozoan walls; (b) centrifuge to remove excess water in Falcon tubes was avoided, instead decantation and manual extraction of the supernatant was preferred; (c) residues were sieved carefully with 10 and 25 microns sieves; (d) residues were revised in Petri dishes and chitinozoans concentrated manually using a fine brush under a stereomicroscope; (e) pictures were taken before specimens were picked using a fine glass pipette (the use of needles, or rigid materials avoided); (f) surface tension occurring during picking was prevented by using micro washes with distilled water; (g) chitinozoans mounted in temporary slides with water were photographed under a white light optical microscope (Figure 4D); (h) Finally, they were selectively transferred to stubs and photographed in an SEM electron microscope. Elemental chemical analyses (EDS, attached to the SEM) were carried out in small rock fragments picked from the residues bearing chitinozoans and other fossils (graptolites, *Gloeocapsomorpha*). We prioritize the recovery of more complete chitinozoan specimens than a cleaner aspect due to using this technique. The manual concentration of chitinozoans from 14 residues allowed the mounting of new slides, prepared with great care, putting a drop of residue and alcohol over the coverslip on the heat plate and adhering to the slide using Trabasil. This enhanced the qualitative and quantitative analyses of the assemblages and the recognition of slightly fractured specimens identified at the species level.

4.3. Observations Through Residues and Surface of Rocks

Residues and rock samples were thoroughly examined under a stereomicroscope to identify chitinozoans and associated macrofossils (e.g., graptolites). After closely examining the samples, the presence of large chitinozoans with 3D preservation at level CICYTTP-Pl-2782 was observed (Figure 7A–D). Chitinozoans were filled with the same sediment as the rock, having thin, black-colored outer walls (exine) (Figure 7A,B). Some specimens showed wall fractures similar to those observed under a microscope after standard processing. These chitinozoans were found alongside colonies of *Gloeocapsomorpha* on a macroscopic scale, and any characteristics linked to the preservation were annotated (Figure 7E–H).

Therefore, this analysis reveals that relatively well-preserved chitinozoans should be recovered from the samples if they are not destroyed by processing. The high percentage of broken elements in the slides after the standard process and the characteristics of the observed 3D chitinozoans on the surface of some rocks indicate a high degree of fragility (brittle) of the exine walls. This confirms the need to apply other techniques, or the standard modified as explained above, considering the features of in situ exine of chitinozoans (e.g., fractures, brittle and thin thickness, biological and mechanical degradation, and remains of framboidal pyrite).

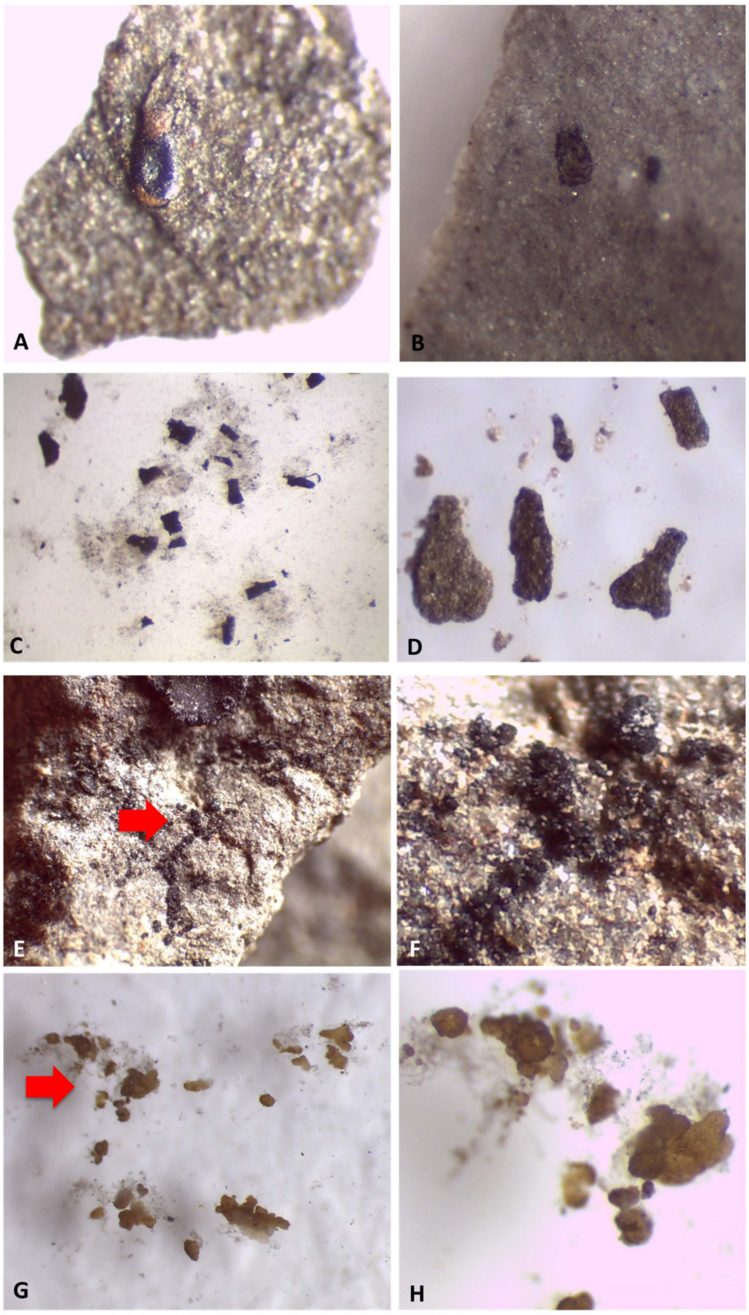

Figure 7. Sample CICYTTP-Pl-2782. (**A**) Chitinozoans with 3D relief on the rock (in situ). (**B**) Chitinozoans recovered after using sodium hexametaphosphate. (**C**) Chitinozoans released after using the modified standard technique. (**D**) Chitinozoans internal mold-cast (mineral 3D) maintaining its shape after the action of sodium hexametaphosphate). (**E**) Colonies of *Gloeocapsomorpha prisca* (red arrow) on the rock. (**F**) Detail of the colonies of *Gloeocapsomorpha* well preserved in the rock. (**G**) Colonies of *Gloeocapsomorpha* (red arrow) recovered after applying the modified standard technique. (**H**) Details of the colonies shown in (**E**).

A significant increase in the recovery of complete or slightly fractured palynomorphs was observed in most of the samples, especially CICYTTP-Pl-2778 and 2780 (Figure 8A,B). There was a significant decrease in unrecognizable broken chitinozoans and those with chemical degradation. The modified techniques allowed us to understand the separation mechanism of the palynomorph from the mineral matrix. In some cases, a strong adhesion occurs (Figure 9A–C), and it is released, leaving part of its wall attached to the matrix

(Figure 9D,E), thereby preserving part of the original morphology of the palynomorph. The detached fraction may exhibit a poor morphology and sometimes can be determined.

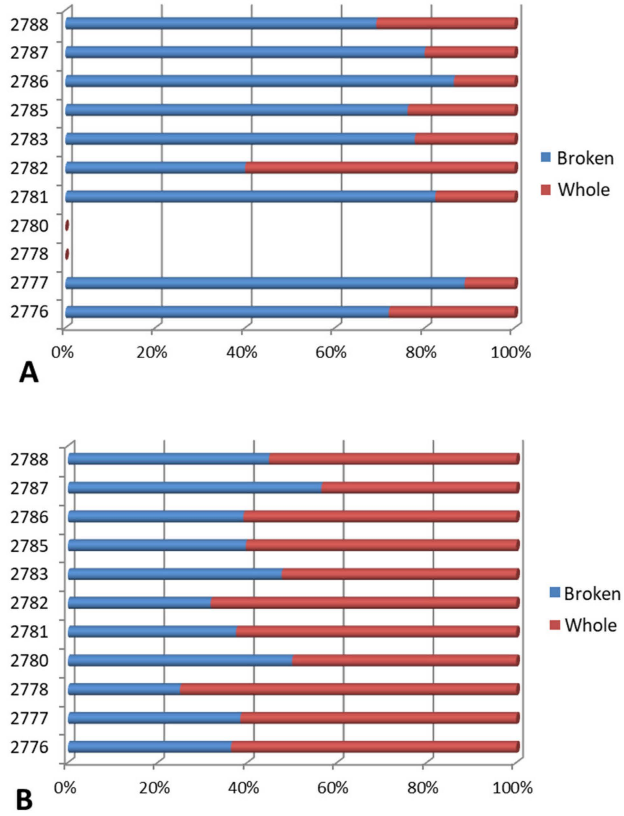

Figure 8. Percentages of whole and broken palynomorphs recovered in each of the samples with the standard technique (**A**) and the modified technique + Sodium hexametaphosphate (**B**).

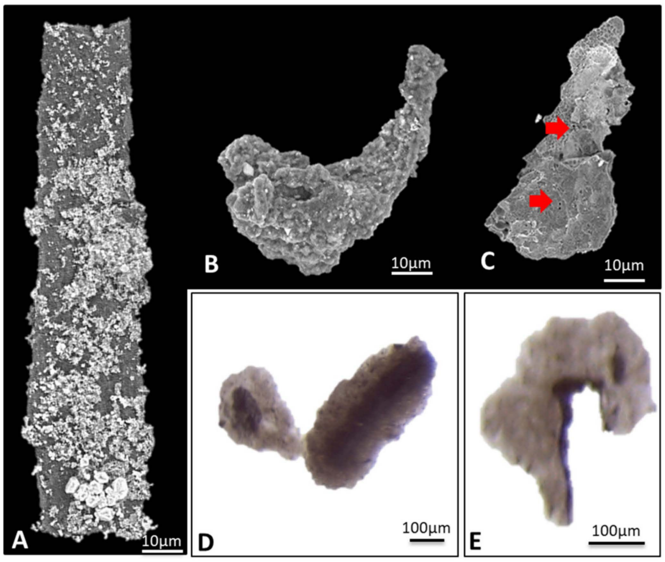

Figure 9. (**A**) SEM image of a chitinozoan with mineral adhered to the wall, CICYTTP-M-550 (CICYTTP-Pl 2777). (**B**) Possible fragment of scolecodont equivalent to internal mold-cast without the fossil wall, CICYTTP-M 551 (CICYTTP-Pl-2778). (**C**) Poorly preserved chitinozoans? with small holes on the exine (red arrow), CICYTTP-M 552 (CICYTTP-M-2786). (**D**,**E**) Chitinozoans (fragmented) are manually detached from masses of AOM (Amorphous organic matter) and minerals from residues in a Petri dish. Notice the shape maintained after the chitinozoan was released (CICYTTP-Pl-2781).

The quantitative comparison between the standard (1) and the improved methods (2) indicated that (1) 80% of the chitinozoans were broken, whereas 20% were whole or slightly fragmented. Approximately 119 chitinozoans with moderate to good preservation were obtained, 4 scolecodonts, 1 cryptospore, graptolite cuticles and colonies of *Gloeocapsomorpha*. (2) the ratio changed to 40% broken chitinozoans and 60% whole or slightly fragmented ones (Figure 10). Although the implementation time is longer, the positive results contributed greatly to the fertility of the samples and a significant increase in the identified specimens at the species level.

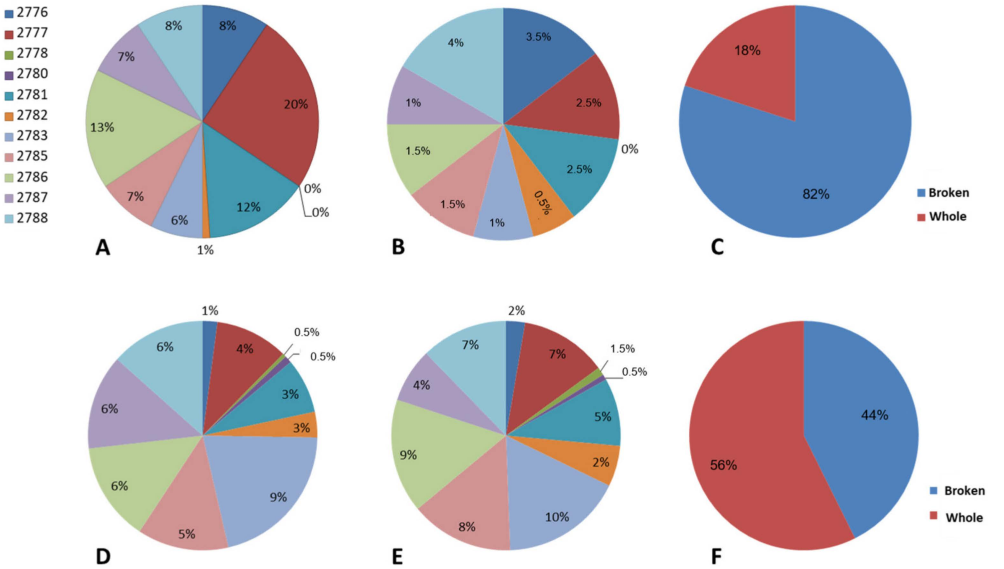

Figure 10. Statistical comparison of the recovered broken and whole palynomorphs in each sample. (**A**–**C**). Standard technique applied (**A**) Broken. (**B**) Whole. (**C**) General overview of the results (based on the total amount of all samples) indicates low recovery of slightly, complete chitinozoans). (**D**–**F**) Sodium hexametaphosphate and modifications to the standard technique (**D**) Broken. (**E**) Whole. (**F**) It shows a decrease in broken palynomorphs and a significant increase in complete and slightly fragmented specimens. Total values (%) of Subfigures (**A**) + (**B**) and (**D**) + (**E**) is 100% (±1 by decimals).

The analysis of new slides and residues revealed the recovery of 265 well-preserved chitinozoans, 20 scolecodonts, abundant graptolite cuticles and colonies of *Gloeocapsomorpha*. A preliminary taxonomic analysis identified 60 species of chitinozoans and 13 forms of scolecodonts. Noteworthy among the recognized species are the following (see [55,56]): *Belonechitina capitata* (Eisenack), *Clathrochitina eisenacki* Taugourdeau, *Conochitina cactacea* Eisenack, *Conochitina chydaea* Jenkins, *Conochitina intermedia* Eisenack, *Conochitina dolosa* Laufeld, *Conochitina simplex* Eisenack, *Cyathochitina campanulaeformis* (Eisenack), *Cyathochitina calix* (Eisenack), *Desmochitina erratica* Eisenack, *Desmochitina minor* (Eisenack), *Desmochitina cocca* Eisenack, *Desmochitina amphorea* (Eisenack), *Demoschitina* aff. *typica* Eisenack, *Eremochitina baculata* Taugourdeau and de Jeckowsky, *Eremochitina brevis* (Taugourdeau and de Jekhowsky), *Euconochitina lepta* (Jenkins), *Lagenochitina baltica* Eisenack, *Lagenochitina deunffi* Paris, *Lagenochitina elongata* (Eisenack), *Lagenochitina obeligis* Paris, *Lagenochitina* cf. *prussica* Eisenack, *Pistillachitina comma* (Eisenack), *Pistillachitina pistillifrons* (Eisenack), *Pistillachitina* sp., *Pogonochitina grandis* Taugourdeau, *Rhabdochitina* sp., *Tanuchitina* (*Conochitina*) *elegans* (Eisenack), *Tanuchitina elongata* (Bouché), *Tanuchitina ontariensis* Jansonius, among others (Figure 11).

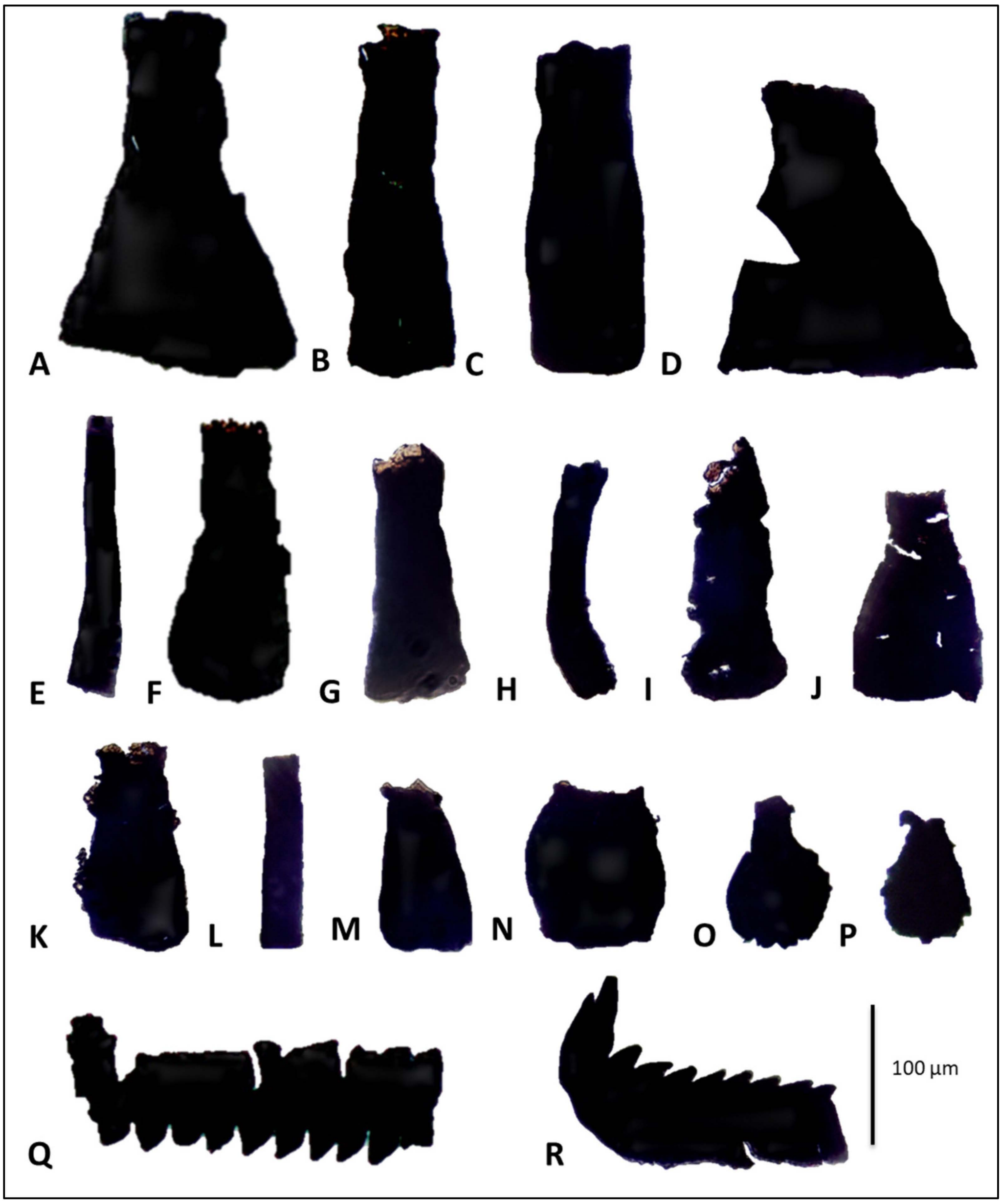

Figure 11. Palynomorphs recovered after applying modifications to the standard technique. (**A**) *Cyathochiitna calix* (Eisenack) (CICYTTP-Pl-2783). (**B**) *Tanuchitina ontariensis* Jansonius (CICYTTP-Pl-2788).(**C**) *Conochitina dolosa* Laufeld, (CICYTTP-Pl-2785). (**D**) *Cyathochitina campanulaeformis* (Eisenack) (CICYTTP-Pl-2785). (**E**) *Pistillachitina* sp. (CICYTTP-Pl-2785). (**F**,**G**) *Belonechitina capitata* (Eisenack) (CICYTTP-Pl-2786). (**H**) *Tanuchitina elongata* (Bouché) (CICYTTP-Pl-2786). (**I**) *Euconochitina lepta* (Jenkins) (CICYTTP-Pl-2788). (**J**) *Pogonochitina grandis* Taugourdeau (CICYTTP-Pl-2776). (**K**) *Conochitina cactacea* Eisenack (CICYTTP-Pl-2788). (**L**) *Tanuchitina* (*Conochitina*) *elegans* (Eisenack) (CICYTTP-Pl-2776). (**M**) *Conochitina intermedia* Eisenack (CICYTTTP-Pl-2785). (**N**) *Desmochitina minor* (Eisenack) (CICYTTP-Pl-2776 EF J49/1). (**O**) *Lagenochitina deunffi* Paris (CICYTTP-Pl-2781). (**P**) *Lagenochitina* cf. *prussica* Eisenack (CICYTTP-Pl-2776 EF O31/0). (**Q**) Scolecodont indet. (CICYTTP-Pl-2781) (**R**) Scolecodont indet. (CICYTTP-Pl-2781). Specimens illustrated in temporary slides except for (**N**,**P**) in permanent slides with England finder coordinates.

The authors agree with several other researchers [9,19,57] that any processing technique used on the samples can significantly impact the recovery of palynomorphs [16,18,58]. Therefore, reviewing the residues at each processing stage is crucial to determine the next step to be applied.

5. Discussion

5.1. Preservation of Organic Matter During Tapho-Diagenetic Stages

Rock samples from this period and geological context have shown a good palynological record in diverse environments related to the glacial and postglacial events [59–66]. Northwestern Argentina has presented a diverse palynological record that allows it to be related to the northern basins of Gondwana [67,68]. At a local scale, in the Eastern Precordillera, De la Puente and Rubinstein [69] present the first palynological data of the Las Vacas and Trapiche formations, given the palynological potential of glacial and postglacial sediments. This section discusses various factors that could have affected the preservation of palynomorphs deposited across the Katian?–Hirnantian sequence of the Eastern Precordillera.

One of the factors affecting the preservation of palynomorphs is taphonomy. The processes involved in taphonomy, such as putrefaction, disarticulation, fragmentation, and abrasion, can occur partially or completely. Throughout the taphonomy process, physical, chemical, and biological agents may or may not act permanently. Previous studies have evaluated the preservation of palynomorphs by comparing taphonomic processes, and sedimentary environments [3,70] (among others). Certain conditions, such as characteristics of the exine of palynomorphs [71], and those of the origin of each taxon, and conditions in the environment of deposition, such as transport distance, grain size, redox process in the water column and bottom facies, rapid/slow sedimentation, absence of bioturbation, acidity, salinity, and later on fossil-diagenesis processes occur and define their state of preservation [3,5,6,16,18,70]. Moreover, large variations in concentration often occur even within the same lithology and over short stratigraphic intervals, suggesting that abundances are also controlled by sediment accumulation rate and ecological and preservational factors (redox conditions, see [3]).

5.2. Grain Size and Energy

The palynomorphs are best preserved in fine-grained sediments such as mudstones, siltstones, micritic limestones, cherts, and coals [6,16,70]. It has been found that there is a relationship between the size of palynomorphs and the grain size of the sediment where they are found. Palynomorphs ranging from 10 to 100 µm are commonly found in fine sediments such as mudstones, silts, and clays. In contrast, palynomorphs in medium to fine sandstones generally have larger sizes, like many chitinozoans [16,70]. However, this relationship does not agree when considering the chitinozoans found in the Katian?–Hirnantian sequence. A statistical analysis has revealed two large groups: (1) sizes between 40 and 100 µm and (2) sizes larger than 100 µm (Figure 11). In the La Pola Formation, the sandy mudstone facies (Figures 1 and 2) yielded better-preserved palynomorphs larger than 100 µm, in line with the theoretical relationship. Although, it also preserved almost 50% of small palynomorphs. This close relationship between small and large palynomorphs could be related to the original sedimentation conditions of the La Pola Formation in a clastic platform with calm, low-energy water environments [28]. A specific case is presented in sample CICYTTP-Pl-2782, where large chitinozoans were found associated with colonies of *Gloeocapsomorpha* and graptolites.

The special thing about this sample is that despite its sandy nature, it managed to preserve a large fauna, but not organisms smaller than 100 µm. This might be because it contains lithofacies closest to the top, which are coarser in grain size (conglomeratic), as reported by some authors for this section [20,30]. We interpret that a rapid burial would have occurred, but smaller palynomorphs were transported to more distant, finer-grained facies (by-pass or sorting effects) from their original environment. The opposite situation is observed in the lower sections of the Don Braulio Formation (CICYTTP-Pl-2783-2786).

In the diamictites, 80% of the palynomorphs preserved are larger than 100 µm (Figures 11 and 12). The mudstone matrix of these facies from where the chitinozoans were extracted would have been deposited as subaqueous mass deposits due to the continuous contribution of fine material to the platform by thawing [37]. Their preservation is linked, probably thanks to their transport embedded in these fine-grained matrices to more distant facies. The upper member (CICYTTP-Pl-2787-2788), of the unit composed of grayish-green shales and fine-medium-grained sandstones, including fossiliferous calcareous lenses, would be associated with the formation of a shallow extra-glacial platform, where an abundant fauna thrived [27,72]. These levels exhibit large chitinozoans and scolecodonts. The weak currents and fine granulometry contributed to the basin in both cases, increasing the likelihood of finding large chitinozoans and a few more taxa with greater diversity. The lack of acritarchs is not well understood, but likely, cold waters or specific physical and chemical conditions of the environment (e.g., fine particles precluding photosynthesis) may be responsible.

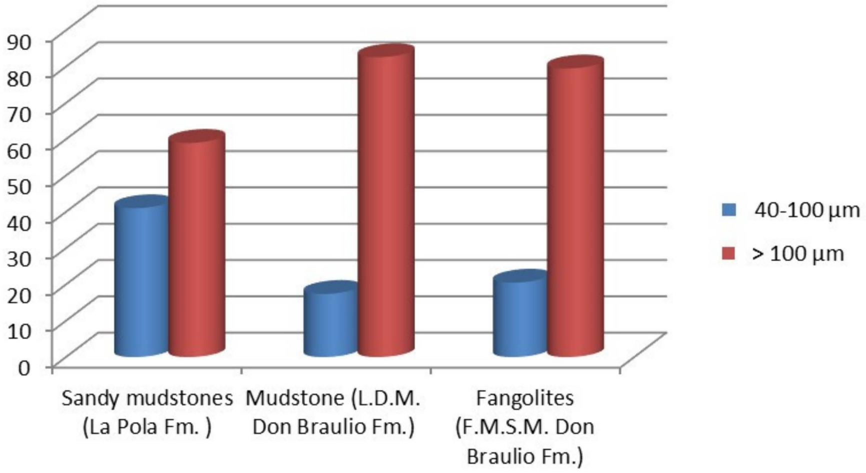

Figure 12. Percentage distribution of chitinozoan sizes in the La Pola Formation, Lower Diamictite Member (L.D.M), and the Fossiliferous Mudstone and Sandstone Member (F.M.S.M) of the Don Braulio Formation.

As a summary, from our analysis, the exine of chitinozoans in our samples was not affected completely by mechanical fragmentation despite high-energy episodes that occurred during the deposition of both units [20,28]. More chitinozoans from the Hirnantian Don Braulio Formation than the La Pola Formation that survived the effects of destructive agents were more resistant to acid digestion treatment in the laboratory, exhibiting better preservation (probably due to thicker exine and slightly less thermally altered). SEM pictures were helpful in comparing the degree of fragility of their walls.

5.3. Redox Changes

Concerning the preservation of palynomorphs, the nature of the chitinozoan exine (the inner layer (=ectoderre) and the outer layer (=periderre) [16,18] plays an important role during the taphonomy process [71]. The origin of the fragility of walls may be due to problems with the amount of pseudochitin and varied thicknesses of the exine of each taxon. Hence, under oxidation conditions, the exine would thin, and changes would occur in its structure, increasing its fragility and ending in the loss of organic matter. For this reason, any organic matter particle requires a dysoxic-anoxic environment, favored by euxinic conditions [3,5,16,18,71]. In the case of framboidal pyrite, structures developed in organic matter associated with cyanobacterial activity at the water-sediment bottom surface under anoxic conditions, even until the early diagenesis stages. In euxinic environments, they form in the water column during the decantation to the seafloor [73–79]. Under specific conditions, the replacement process by iron, for example, can become part of fossils in the form of pyrite, siderite, or limonite. This can benefit organisms with chitinous exoskeletons that are faster degraded [3]. However, exines are corroded by pyrite (framboidal and euhedral forms), and damages can be severe to prevent the identification of the palynomorph. In our samples, the exine of chitinozoans was affected by framboidal pyrite (Figure 6), especially in some levels from the Don Braulio Formation (Figure 2, CICYTTP-Pl-2785-2788), along with its association with euhedral crystals. This supports euxinic conditions and sedimentation in anoxic, gentle, and calm bottom waters [3,16]. Under anoxic conditions and sufficient availability of Fe and organic productivity, the pyrite content would increase in organic matter [80–83]. This is the first time this process of pyrite formation is documented for this unit and contributes to the interpretation of environmental conditions of the upper part of the lower member of the Don Braulio Formation (Figure 2, CICYTTP-Pl-2785, 2786) during the initial postglacial transgressive pulse [84].

5.4. Biological Degradation (Bioturbation)

Biological degradation, also known as bioturbation, plays a central role in almost all diagenetic processes [85]. This degradation, caused by bacteria and fungi, can occur during initial deposition and burial or after the rock has been exposed on to the surface [6]. Considering that some pits and diverse circular perforations occurring in some individuals (Figure 9C) have no physiologic meaning, they are probably due to parasitic micro-organisms (e.g., fungi, bacteria) before or during the complete fossilization process [18]. The low percentage of chitinozoans with biological degradation indicates a low activity of fungi and aerobic bacteria [18], also supported by the pyrite formation under anoxic/dysoxic conditions [3].

5.5. Diagenetic Processes

Our chitinozoans also provided information on the early diagenetic history of the sediments. Their organic wall remains elastic for a long time, so they are susceptible to flattening during the compaction of sediments. When the interstitial water escapes from argillaceous deposits because of sedimentary overloading related to rapid subsidence, flattening is especially favored [18]. Instead, early lithification on some types of rocks, like chert, limestone, phosphate, and ironstone, before the basin loading [18] may preserve chitinozoans (and other fossils) in full relief. This phenomenon was noticed in the upper section of the La Pola Formation and Don Braulio Formations (CYCITTP-Pl-2781, 2785, 2786, 2788; Figures 2 and 6). We cannot confirm this. However, similar conditions have been observed in the Devonian related to the diagenesis of palynomorphs coated by pyrite, whose process allows preservation in different environments and moments of the lithification of the rock (see [86]).

Another process observed in chitinozoans was the mineral adhesion that mirrors their shape, known as encrusting [87], where elements are preserved due to a coating of surrounding mineral material, such as calcareous, phosphatic, ferruginous, or detrital material. Although this kind of fossilization enhances durability and resistance to decay (by oxidation), it leads to the loss of morphological characteristics. Smooth-walled chitinozoans observed herein commonly exhibit this type of preservation (Figures 6 and 9A). During fossilization, the high silica content in the waters of the platforms likely led to the precipitation of silicates, which reacted with the exine of the chitinozoans, resulting in adhesion and a mummification effect similar to that seen in some siliceous sponges [87].

Finally, the increase in temperature during burial produced a progressive darkening of the organic walls (fossil-diagenesis stage). These changes correspond to new molecular equilibria (i.e., progressive increase in the carbon ratio = maturation of the organic matter). This means a highly aromatic functional group with fewer aliphatic components in the wall of chitinozoans, as demonstrated by [12,14]. In addition, exine becomes more brittle, increasing its fragility as it was corroborated in most of our assemblages from the La Pola, whereas slightly less so in those of the Don Braulio formations.

Therefore, the low palynological content of mostly fragmented chitinozoans, graptolites, and scolecodonts recovered after the standard processing herein was not due exclusively to biological degradation (fungi, bacteria, pyritization) processes. Despite that, pulses of energy activation in the surface-water bottom could have likely increased the amount of dissolved oxygen in the waters, thus promoting bioturbation [84]. The thermal alteration was also an important factor in the fragile and brittle condition of the organic matter recovered.

5.6. Rework of Deposits and Taxa

To identify rework in palynological associations, the state of preservation, diversity, and thermal maturity of palynomorphs are often compared at a glance. Nonetheless, these conditions are not always evident enough to discriminate the indigenous taxa from those reworked of older rocks. The present study indicates that similar preservation and thermal maturity features are observed in our palynoassemblages analyzed. The La Pola Formation′s geological conditions and the Braulio Formation′s lower members involve tectonic activation and eustatic changes during and after their deposition [85] (references therein). This instability was responsible for the rework of some levels, whose re-sedimentation, especially in diamictite deposits, may have given rise to mixtures of palynomorphs of the Sandbian into the Katian or even into the Hirnantian units.

On the other hand, it has been demonstrated that chemical damage prevented a good recovery of palynomorphs, preserving their original preservation state in the rock. In most cases, the mechanical fragmentation of palynomorphs produced by higher-energy environments cannot be differentiated if chemical damage prevails. Therefore, the more careful process applied to all the samples enabled the recovery of more complete palynomorphs at almost all levels. The assignment of all specimens to the specific level is mandatory to correctly identify the origin of the taxa (indigenous, reworked) and their stratigraphic range [8,88]. Fluorescence was indicated as a potentially helpful tool for discriminating "native" and reworked palynomorphs [2,4]. However, the mixture of autochthonous and reworked palynomorphs is not discriminated by evaluating autofluorescence (color, intensity) as demonstrated by [7]. This is because fluorescence has shown that when palynomorphs fluoresce with higher intensities, it indicates a good preservation state. The lack of fluorescence in palynomorphs exposes preservation effects linked to their taphonomic and diagenetic history but not their recycling. Furthermore, chitinozoans and scolecodonts are not fluorescent due to their chemical composition.

Therefore, species identification and their most probable ranges will help clarify whether rework taxa are present or absent in the Katian?–Hirnantian sequence. The authors of this contribution are currently conducting this study.

6. Conclusions

The chitinozoans found in the Late Ordovician (Katian?–Hirnantian) of the Argentine Precordillera are often dark and fragile, making them difficult to study. To improve this, we have successively introduced several changes to the standard techniques based on rock characteristics and the poor preservation of scarce chitinozoans recovered in the first attempt. Given the observed palynological preparations, some samples were even considered sterile due to the lack of complete palynomorphs. The revision of residues and their rocks under a stereomicroscope was important in deciding the application of different methods to each sample.

The sodium hexametaphosphate technique was applied to avoid invasive mechanical procedures, and a slightly modified HCl and HF method was also applied without using second and third HF rounds, centrifuge to neutralization, HCl boiled, oxidation, and heavy liquid separation. This favored the recovery of more complete palynomorphs, concentrated manually and pictured under the stereomicroscope. Specimens selected were mounted with water (or glycerin) to take pictures in the white light microscope and then transferred to stubs for SEM illustration. We observed the exines of chitinozoans and preserved morphological features produced by biological, mechanical, and chemical degradation during the taphonomic and fossil-diagenesis phases.

The first results from the standard method revealed that 80% of the chitinozoans were broken, whereas 20% were whole or slightly fragmented. Approximately 119 chitinozoans with moderate to good preservation were obtained, 4 scolecodonts, 1 cryptospore, graptolite cuticles, and colonies of *Gloeocapsomorpha prisca*. After applying the modified techniques, the ratio changed to 40% broken chitinozoans and 60% whole or slightly fragmented ones. This resulted in a significant increase in the identified specimens at the species level, with 265 well-preserved chitinozoans, 20 scolecodonts, abundant graptolite cuticles, and colonies of *Gloeocapsomorpha*. A preliminary taxonomic analysis identified 60 species of chitinozoans (e.g., *Belonechitina capitata*, *Conochitina chydaea*, *Conochitina dolosa*, *Cyathochiitna calix*, *Euconochitina lepta*, *Lagenochitina baltica*, *Lagenochitina deunffi*, *Lagenochitina obeligis*, *Lagenochitina* cf. *prussica*, *Tanuchitina ontariensis*) and 13 forms of scolecodonts.

This study reinforces that the preservation-processing relationship technique is to be used despite it being more time-consuming, as it proved highly effective for siliciclastic samples and resulted in a high percentage of complete material.

Several taphonomic, diagenetic, and tectonic processes affected the preservation of chitinozoans recovered from the La Pola and Don Braulio formations. They disturbed the original composition of the assemblages. However, the recovery of diverse chitinozoans in this study still reflects the original conditions of their environments, particularly the original environment of the diamictites of the Don Braulio Formation. This contributes to the basins′ paleoenvironment interpretation and paleogeographic evolution during the Late Ordovician in Precordillera and will improve litho-biostratigraphic correlations with the graptolite zones in future contributions.

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