UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

CENTRO DE CIÊNCIAS HUMANAS E NATURAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Unraveling the Crato Formation's preservation and paleoenvironment through the lens of insect taphonomy

Arianny Pimentel Storari

Vitória, ES

Maio, 2024

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Orientadora: Taissa Rodrigues Marques da Silva

Tese submetida ao Programa de Pós-Graduação em Ciências Biológicas (Biologia Animal) da Universidade Federal do Espírito Santo como requisito parcial para a obtenção do grau de Doutora em Biologia Animal

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“Unraveling the Crato Formation's preservation and paleoenvironment through the lens of insect taphonomy”

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas do Centro de Ciências Humanas e Naturais, da Universidade Federal do Espírito Santo, como requisito parcial para obtenção do Grau de Doutora em Biologia Animal.

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"It seems to me that the natural world
is the great source of excitement;
the greatest source of visual beauty;
the greatest source of intellectual interest.
It is the greatest source of so much in life
that makes life worth living."

– David Attenborough

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Resumo

A Formação Crato (Grupo Santana, Bacia do Araripe, Brasil) é uma famosa unidade fossilífera do Cretáceo Inferior, considerada um Konservat-Lagerstätte, que preserva insetos em abundância e grande diversidade, e que também são, muitas vezes, excepcionalmente bem preservados tanto em escala micro quanto macro. Esta unidade representa um paleoambiente lacustre e, portanto, também é importante para o estudo dos insetos aquáticos e sua interação com esse ambiente passado. Até o momento, apenas algumas investigações analisaram a tafonomia geral dos insetos da Formação Crato, mas nenhuma utilizou dados atualísticos, que aplicamos no terceiro capítulo desta tese. A primeira parte da tese apresentada aqui consiste em um artigo publicado contendo a primeira descrição detalhada de proventrículos fossilizados de nove espécimes de Grylloidea (Orthoptera: Ensifera) da Formação Crato. Com base nas assinaturas preservacionais dos espécimes, trazemos novos dados morfológicos para explorar as diferenças entre táxons extintos e viventes, o que dá uma visão sobre a evolução e desenvolvimento fisiológico de Orthoptera, mas principalmente, sobre sua excepcional preservação nesta unidade fossilífera. A segunda parte desta tese apresenta um manuscrito com os resultados sobre modos de preservação de efêmeras (Ephemeroptera) e grilos (Orthoptera) da Formação Crato. Apresentamos também os modos de preservação de efêmeras dos calcários de Solnhofen (Jurássico Superior, Alemanha) para comparação. A maioria dos espécimes do Crato possui diversos caracteres externos e internos conservados, e são preservados pela substituição da cutícula e dos órgãos internos por óxidos de ferro após piritização, enquanto os fósseis de Solnhofen são preservados como impressões. Na maioria dos fósseis de Solnhofen, sua morfologia em escala micrométrica é suprimida pelo crescimento mineral grosseiro de cristais de calcita. Em geral, os fósseis de Solnhofen são completos, totalmente articulados, mas sem replicação em escala submicrométrica da morfologia externa e interna, e são extremamente mal preservados se comparados aos insetos da Formação Crato. Por fim, o terceiro manuscrito apresentado nesta tese exibe os resultados de experimentos realizados para determinar a natureza dos processos e padrões tafonômicos que causaram o estado de preservação observado nas larvas e adultos de efêmeras e larvas de libélulas da Formação Crato. Nossos resultados mostram indícios de que carcaças de larvas de Hexagenitidae sofreram pouca perturbação, típica de uma assembleia autóctone. Notamos também que os espécimes fósseis adultos com sinais de longa decomposição eram extremamente raros. Os fósseis de libélulas

analisados estão preservados em uma posição característica que indica transporte mínimo após a morte, o que descobrimos após experimentos com larvas modernas. O nível de desarticulação dos fósseis também sugere que as larvas de libélulas da Formação Crato não foram transportadas por longas distâncias. Finalmente, os nossos experimentos mostraram que, quando pequenos insetos, como as efêmeras, morrem em condições subaéreas, há poucas possibilidades de superar a tensão superficial e afundar. Por isso, consideramos que biofilmes microbianos na superfície da água atuaram durante o afundamento de carcaças na Formação Crato.

Palavras-chave. Tafonomia de insetos; preservação; Ephemeroptera; Orthoptera; Odonata; Solnhofen.

Abstract

The Crato Formation (Santana Group, Araripe Basin, Brazil) is a well-known Konservat-Lagerstätte that preserves a great number of various insect taxa that are exceptionally well-preserved both in micro and macro scales. This unit represents a lacustrine paleoenvironment, and therefore, is also important for the study of aquatic insects and their interaction with this past environment. To date, only a few investigations have analyzed the general insect taphonomy of the Crato Formation so far, yet none used actualistic data, which we apply in the third chapter of the thesis. The first part of this thesis consists of a published paper containing the first detailed description of fossilized proventriculi from nine Grylloidea (Orthoptera: Ensifera) specimens of the Crato Formation. Based on the morphological and preservational signatures of the specimens, we bring new morphological data to explore the differences among extinct and extant taxa, that gives insight into the evolution and physiological development of Orthoptera, but mostly, about their exceptional preservation in this unit. The second part of this thesis presents a manuscript with the results on preservational modes of mayflies (Ephemeroptera) and crickets (Orthoptera) of the Crato Formation. We also present the modes of preservation of mayflies from the Solnhofen limestones (Upper Jurassic, Germany) for comparison. The majority of Crato specimens have several conserved external and internal microfeatures, and are preserved by replacement of the cuticle and of internal organs by iron oxides after pyritization, while the Solnhofen fossils are

preserved as impressions. In most Solnhofen fossils, their micron-scale morphology is obliterated by coarse mineral growth of calcite crystals. In general, Solnhofen fossils are complete, fully-articulated, but with no submicron-scale replication of both external and internal morphology, and are extremely poorly preserved if compared to the insects of the Crato Formation. Lastly, the third manuscript presented in this thesis brings the results of experiments performed to determine the nature of the taphonomic processes and patterns which caused the state of preservation seen in the larvae of mayflies and dragonflies and adult mayflies preserved in the Crato Formation. Our results with experimentation show indications that carcasses of larval Hexagenitidae suffered little disturbance, typical of an autochthonous assemblage. We also noticed that adult fossil specimens with signs of longer decay time were extremely rare. The fossil dragonflies analyzed are preserved in a characteristic position that indicates minimal transport after death, which we find out after experiments with modern larvae. Data on disarticulation also suggest that the dragonfly gomphid larvae were not transported for long distances. Finally, our experiments have shown that when small insects, such as mayflies, die in sub-aerial conditions, there are few possibilities of overcoming the surface tension and sink. Thus, we believe microbial biofilms on the surface of the water were acting during carcass sinking in the Crato Formation.

Keywords. Insect taphonomy; preservation; Ephemeroptera; Orthoptera; Odonata; Solnhofen.

Objectives

The general goal of this thesis was to examine and describe taphonomic aspects of prominent groups of fossil insects from the Crato Formation, Araripe Basin, based on actualistic, biostratinomic, and geochemical data.

The specific goals of this thesis were:

1. Describe exceptionally well-preserved microstructures and internal organs in insect fossils from the Crato Formation;
2. Geochemically characterize insect specimens from the Crato Formation, and identify fossilization trends;
3. Characterize the preservation of insects from the Crato Formation through comparison with fossils from the same taxonomic group from the Solnhofen limestones;
4. Evaluate biostratinomic signatures in carcasses of extant aquatic insects (Ephemeroptera and Odonata);
5. Evaluate how biostratinomic processes influenced the preservation of aquatic insect fossils from the Crato Formation (Ephemeroptera and Odonata).

General Introduction

Fossil insects

Insects are the most diverse group of animals on Earth today, and their fossil record, starting from the Carboniferous, also has impressive numbers of approximately 25 thousand species described (Jarzembowski and Ross, 1996; PaleoBioDB, 2024). Fossil insects are extremely important for evolutionary research since they provide the only direct record of extinct lineages; reveal patterns and timing of extinctions and radiations; and, therefore, assist in reconstructing the phylogeny of groups (Grimaldi, 2009). Particularly for the field of taphonomy, the study of insect preservation is important since their small size and durable cuticle have allowed them to be preserved in many ways, varying more than vertebrates (Grimaldi and Engel, 2005). The modes of preservation are usually compressed impressions of the resistant cuticle, although isolated wings are also easily recovered. In other cases, insects can be preserved as three-dimensional concretions, which are replicas of the original animal (Grimaldi, 2009).

The common denominator among most fossil insects is the preservation in a lake paleoenvironment. Even amber, or fossilized tree resin, requires an aqueous lacustrine or brackish environment to be preserved (Grimaldi and Engel, 2005). There are some important exceptions to the lacustrine fossil insect theme, the most famous being the Late Jurassic limestones of Solnhofen (Altmühltal Formation), Germany, which are marine (Barthel et al., 1990). Regardless, the study of lacustrine depositional units, since almost universal for the preservation of insects, helps set generalizations and/or models for insect taphonomy. The Early Cretaceous Crato Formation of Brazil holds one of the most exceptionally well-preserved non-amber Mesozoic fossil insects; in this project, their insect fossils are comprehensively studied.

Crato Formation's insect diversity

The focus of this thesis, which is addressed in all its chapters, is one of the most remarkable fossiliferous deposits in the world, the Crato Formation (Carvalho and Santos, 2005). Lithostratigraphically, this unit comprises the base of the Santana Group, a post-rift unit of the Araripe Basin in northeast Brazil, and is of Aptian age (Lower Cretaceous) (Assine et al., 2014). It represents a lake complex paleoenvironment (Mabesoone and Tinoco, 1973), containing fossils of algae, plants, arthropods, mollusks, fish, amphibians, crocodilians, pterosaurs, dinosaurs, and

birds, all of which are usually preserved compressed in its limestones (Bechly, 2007; Saraiva et al., 2021), but central to this thesis, this geological unit preserves one of the most diverse Cretaceous entomofaunas, standing as the only major Cretaceous insect deposit from South America, as well as the biggest and most diverse of all known units from Gondwana (Carvalho and Santos, 2005; Grimaldi and Engel, 2005).

Ribeiro et al. (2021) synthesized the total diversity of macrofossils of a given succession of the Crato Formation, evidencing that approximately 80% of its macrofossil composition was of hexapods. The latest review on the total diversity of Crato insects performed by Moura-Júnior (2018) recorded 379 insect species described, belonging to 121 families, and this number most probably already increased after six years. The Crato entomofauna have representatives of many insect orders (Class Insecta), in an unusual mix of aquatic and terrestrial forms, mainly composed of the orders Ephemeroptera, Orthoptera, Hemiptera, Blattodea, Odonata, and Neuroptera. But representatives of Isoptera, Mantodea, Phasmatodea, Dermaptera, Hymenoptera, Diptera, Trichoptera, Lepidoptera, Coleoptera, Megaloptera, Mecoptera, and Raphidioptera are also recorded. The basal wingless Zygentoma order is also present within Insecta representatives (Bechly, 2007; Menon and Martill, 2007; Moura-Júnior et al., 2018). Finally, the Entognatha hexapods (non-Insecta) are represented by Diplura (Bechly, 2007).

There are some peculiarities within the composition of the Crato paleoentomofauna still missing some explanation, like the complete absence of any Plecoptera (adults and larvae), considering the distinct abundance of virtually all other aquatic insects, including groups of Ephemeroptera and Odonata that commonly share the same habitat with plecopterans in the neotropics (Bechly, 2007). Another inexplicable missing group is the hemipteran Gerridae, commonly known as water striders, which are extremely common in any water setting in recent times in South America (Lancaster and Briers, 2008).

Insect taphonomy

Although details on the fossil diagenesis of insects are still to be better understood, remarkable progress has been made in insect taphonomy in the last few years (Zhehikhin, 2002; Martínez-Delclòs et al., 2004; Archibald and Makarkin, 2006; Smith and Moe-Hoffman, 2007; Mancuso et al., 2007; Ilger, 2011; Thoene-Henning et al., 2012; McNamara et al., 2012; Wang et al., 2012; Pan et al., 2014; Barling et al., 2015; Greenwalt et al., 2015; Osés et al., 2016; Anderson

and Smith, 2017; Dias and Carvalho, 2020, 2022; Storari et al., 2021, 2024). Taphonomy literally means “the laws that drive death" (Efremov, 1940), and it is the study of how much fossilization affects the quantity and quality of biological information present in fossils, and one of its main objectives is to quantify the influence of the environment on the degree of destruction of fossils (Erthal et al., 2016).

Taphonomy traditionally involves two fields of research: Biostratinonomy and Fossildiagenesis. Biostratinonomy consists of the sedimentary history of organic remains, mainly including the processes of necrolysis, fragmentation, disarticulation, orientation, and selection (Zuschin et al., 2003; Araújo-Júnior and Bissaro-Júnior, 2017; Datta et al., 2020). Insect carcasses that survive the biostratinomic processes may be buried and subsequently affected by a number of other processes: mineralization, flattening, deformation, and reworking. These latter processes are part of another field of study of taphonomy, namely Fossildiagenesis (Martínez-Delclòs et al., 2004). Fossildiagenesis comprises the chemical processes initiated after the death of the organism that will be responsible for the type of fossilization of the organism (Briggs, 1995). Organisms can be preserved exceptionally, when including the hard and soft parts (Vega et al., 2015). But in most cases, preservation occurs with alteration of the hard parts, and the soft parts decompose (Vega et al., 2015). During fossilization, the hard parts can remain unmodified, preserving in the fossils the minerals that originally constituted them, or undergo some type of alteration, such as carbonification, recrystallization, replacement, permineralization, and formation of concretion (Medeiros, 2004). Decay-resistant tissues (or hard parts) may survive to be preserved as organic material when diagenetic changes result in their transformation to more stable molecules than the original ones, while the preservation of labile tissues (way less resistant than hard parts) requires replication by authigenic minerals before the loss of morphological detail (Briggs, 1995). The type of mineral involved depends on the chemistry of the environment and on the prevalent microbial processes (Allison, 1988).

Another field of Taphonomy is Actualistic Taphonomy or Actuotaphonomy, which develops comparative studies between fossil records and their modern correlates, which may include results of experiments on the preservation and decomposition of animal and plant carcasses (Ponciano, 2015; Iniesto et al., 2016). For example, it has been observed that spider leg flexion can be used as a proxy to measure salinity (Downen et al., 2016), and that if left completely undisturbed, a fly carcass can remain without disarticulating for almost a year (Martínez-Delclòs

and Martinell, 1993). The first studies on insect biostratinomy using modern fauna as a proxy were published by Schäfer (1972) and Lutz (1984). Schäfer (1972) described the necrolysis and biostratinomy of the extant *Libellula quadrimaculata*, and Lutz (1984) described the disarticulation of cockroach elytra. Examples of important studies on actuotaphonomy of insects to mention are those of Martínez-Delclòs and Martinell (1993), Lutz (1997), Rust (1998), and Duncan et al. (2003). Of special interest to this thesis is the study of actuo necrolysis, since soft tissues of all organisms are quickly degraded by bacteria and fungi, which may completely destroy a carcass in a few days, depending on its size, the water temperature, and other environmental factors (Martínez-Delclòs et al., 2004). Martínez-Delclòs and Martinell (1993) studied the death and necrolysis of cockroaches, crickets, earwigs, terrestrial hemipterans, mayflies, flies, wasps, ants, beetles, snakeflies, lacewings, and butterflies in aquatic environments to determine the taphonomic processes that influenced insect fossilization. The results of this study are important because they indicated that different types of insects behave in varied ways when they enter water. Experimental observations made by Duncan et al. (2003) show that the sinking of an insect carcass is usually slow and vertical, even for insects enveloped by algae or fungi, and that normally only insects trapped by cyanobacterial mats in the water column exhibit varied positions.

Thesis justificative

Extrinsic environmental factors affect insects that may fossilize, such as water depth, density, and salinity, winds, climate, and type of sediment (Larsson, 1975; Wilson, 1988; Lutz, 1997; McCobb et al., 1998; Tischlinger, 2001; Martínez-Delclòs et al., 2004; Mancuso et al., 2007; McNamara et al., 2012; Smith, 2012; Thoene-Henning et al., 2012; Wang et al., 2012). Apart from extrinsic factors, characteristics of the insect itself (intrinsic factors - e.g., insect size, body shape, robustness, wing structure, and wing surface-to-body mass ratio) are important to contribute to the quality and representation of fossil insect groups. Due to that, taphonomic variability may occur and affect the preservation of insects at different taxonomic levels, and further influence the reconstruction of their paleoecology and paleodiversity. More detailed investigations of specific taxonomic groups at specific lithostratigraphic units are, therefore, important to explore taphonomic variability and to understand the controlling factors (intrinsic and extrinsic) for a given taxonomic group and geological unit.

Since different taxonomic groups of insects may have different taphonomic processes, leading to contrasting preservation patterns and taphonomic bias, the thesis focused on key-groups, such as the most abundant of the unit, Ephemeroptera, together with Orthoptera and Odonata that are also representative of the Crato Formation. In a previous taxonomic analysis of Hexagenitidae types (described based on *Hexagenites* from the Solnhofen limestones of Germany), the author noticed many divergences in the preservation of those German fossils about what is stated in the literature. This situation started the wish to deeply investigate the preservation of Hexagenitidae of both Crato and Solnhofen in parallel, and test if insects of these units indeed share similar modes of preservation, as known for their vertebrates (Fielding et al., 2005). In general, for the whole insect fauna of the Crato Formation, most studies focused only on taxonomy (with exception of orthopterans recently - Barling et al., 2015; Osés et al., 2016; Bezerra et al., 2020a; Dias and Carvalho 2020; Mendes et al., 2020), leaving the fossildiagenetic processes that act on their preservation and their relation with the paleoenvironment of Crato unexplored. In this context, Hexagenitidae fossils from Crato and Solnhofen are ideal for comparative studies, which can lead to generalizations, because they present the same overall morphotype and are abundant in both units.

Finally, the use of less-destructive techniques for the study of the fossil record is proving to be very useful worldwide. Spectroscopy techniques, like X-ray spectroscopy and Raman, elevated paleontological studies to new levels of complexity (Schopf e Kudryavtsev, 2009; Fairchild et al., 2012). These are used to reveal chemical and mineralogical traits of the fossil samples to paleoenvironmental and paleobiological approaches, which are commonly applied abroad, yet in Brazil, and especially within the studies of the Araripe Basin, these same techniques are only starting to be more widely applied, with novel research published no more than 5 to 10 years ago (Barling et al., 2015; Osés et al., 2016; Bezerra et al., 2018; Barling et al., 2020; Bezerra et al., 2020a,b; Dias and Carvalho 2020; Iniesto et al., 2021; Dias and Carvalho, 2022; Bezerra et al., 2023; Dias et al., 2023). Paleontology is naturally multidisciplinary, however, traditionally, projects on the taphonomy of the Araripe Basin fossils do not tend to combine multiple knowledge areas. Here, within the central theme of paleoentomolgy, the three areas of taphonomy are combined in a novel way, namely biostratinomy, fossildiagenesis, and actuotaphonomy.

Thesis organization

As this thesis is multidisciplinary, clear boundaries were established for each chapter (i.e. manuscripts) to maintain achievable goals, for that, each of the chapters explores these main taphonomy areas: 1. Fossildiagenesis and biostratinomy of the foregut of Orthoptera from the Crato Formation; 2. Fossildiagenesis and biostratinomy of Ephemeroptera from the Crato and Solnhofen limestones; 3. Actuotaphonomy and biostratinomy of Ephemeroptera and Odonata from the Crato Formation.

This thesis presents the results of the taphonomic study of a total of 459 fossil insects of the orders Orthoptera (*chapters 1* and *2*), Ephemeroptera (*chapters 2* and *3*), and Odonata (*chapter 3*), as well as the study of 489 specimens of extant Ephemeroptera and Odonata (*chapter 3*). The fossil material comes from the laminated limestones of the Crato Formation (Lower Cretaceous), Brazil (*chapters 1, 2*, and *3*) and of the Solnhofen beds (Upper Jurassic), Germany (*chapter 2*). The first chapter of this thesis its a published paper which deals with the preservation and description of fossilized proventriculi from nine Grylloidea (Orthoptera: Ensifera) specimens of the Crato Formation of Brazil, the oldest preservation of this internal organ in orthopterans. The second chapter is a manuscript which contains a study of the exceptional preservation of insects from two renowned limestone Lagerstätten, the Crato Formation and the Solnhofen beds of Germany. Finally, the third chapter is a manuscript presenting actualistic data of extant aquatic insects to compare with and better understand the taphonomic processes responsible for the mode of preservation of the fossil insects that occur in the Crato Formation.

References

Allison PA. 1988. Konservat-Lagerstätten: Cause and classification. Paleobiology. 14: 331-344.

Anderson EP, Smith DM. 2017. The same picture through different lenses: Quantifying the effects of two preservation pathways on Green River Formation insects. Paleobiology. 43(2): 224-247.

Araújo-Júnior HI, Bissaro-Júnior MC. 2017. Tafonomia de vertebrados em ambientes continentais. In: Horodyski RS, Erthal F. (eds). Tafonomia: Métodos, processos e aplicação. Curitiba: Ed. CRV. 175-236.

Archibald SB, Makarkin VN. 2006. Tertiary giant lacewings (Neuroptera: Polystoechotidae): Revision and description of new taxa from western North America and Denmark. Journal of Systematic Paleontology. 4: 119-155.

Assine M, Perinotto A, Custódio MA, Neumann V, Varejão FG, Mescolotti PC. 2014. Sequências Deposicionais do Andar Alagoas (Aptiano superior) da Bacia do Araripe, Nordeste do Brasil. Boletim de Geociências Petrobras. 22(1): 3-28.

Barling N, Martill DM, Heads SW, Gallien F. 2015. High fidelity preservation of fossil insects from the Crato Formation (lower Cretaceous) of Brazil. Cretaceous Research. 52: 602-622.

Barling N, Martill DM, Heads SW. 2020. A geochemical model for the preservation of insects in the Crato Formation (Lower Cretaceous) of Brazil. Cretaceous Research. 116: 104608.

Barthel KW, Swinburne NHM, Conway-Morris S. 1990. Solnhofen: a study in Mesozoic palaeontology. Cambridge University Press, Cambridge. 236 pp.

Bechly G. 2007. Insects of the Crato Formation. In: Martill DM, Bechly G and Loveridge RF. (Eds), The Crato Fossil Beds of Brazil. Cambridge University Press, Cambridge. 142-149

Behrensmeyer AK, Boaz DD. 1980. The Recent bones of Amboseli National Park, Kenya, in relation to East African paleoecology. In: Behrensmeyer AK, Hill AP (eds). Fossils in the making; vertebrate taphonomy and paleoecology. Univ. Chicago Press. Chicago, Ill., United States. 72-92.

Bezerra F, Da Silva J, Agressot E, Freire P, Viana B, Mendes M. 2023. Effects of chemical weathering on the exceptional preservation of mineralized insects from the Crato Formation, Cretaceous of Brazil: Implications for late diagenesis of fine-grained Lagerstätten deposits. Geological Magazine. 160(5): 911-926.

Bezerra FI, Da Silva JH, De Paula AJ, Oliveira NC, Paschoal AR, Freire PTC, et al. 2018. Throwing light on an uncommon preservation of Blattodea from the Crato Formation (Araripe Basin, Cretaceous), Brazil. Revista Brasileira de Paleontologia. 21(3): 245-254.

Bezerra FI, Da Silva JH, Miguel EC, Paschoal AR, Nascimento Jr DR, Freire PTC, et al. 2020a. Chemical and mineral comparison of fossil insect cuticles from Crato Konservat Lagerstätte, Lower Cretaceous of Brazil. Journal of Iberian Geology. 46: 61-76.

Bezerra FI, Solórzano-Kraemer MM, Mendes M. 2020b. Distinct preservational pathways of insects from the Crato Formation, Lower Cretaceous of the Araripe Basin, Brazil. Cretaceous Research. 118: 104631.

Briggs DEG. 1995. Experimental Taphonomy. Palaios. 10: 539-550.

Carvalho MSS, Santos MECM. 2005. Histórico das Pesquisas Paleontológicas na Bacia do Araripe, Nordeste do Brasil. Anuário do Instituto de Geociências – UFRJ. 28(1): 15-34.

Datta D, Mukherjee D, Ray S. 2020. Taphonomic signatures of a new Upper Triassic phytosaur (Diapsida, Archosauria) bonebed from India: aggregation of a juvenile-dominated paleocommunity. Journal of Vertebrate Paleontology. 39(6): e1726361.

Dias JJ, Carvalho IS, Buscalioni AD, Umamaheswaran R, López-Archilla AN, Prado G, et al. 2023. Mayfly larvae preservation from the Early Cretaceous of Brazilian Gondwana: Analogies with modern mats and other Lagerstätten. Gondwana Research. 124: 188-205.

Dias JJ, Carvalho IS. 2020. Remarkable fossil crickets preservation from Crato Formation (Aptian, Araripe Basin), a Lagerstätten from Brazil. Journal of South American Earth Sciences. 98: 102443.

Dias JJ, Carvalho IS. 2022. The role of microbial mats in the exquisite preservation of Aptian insect fossils from the Crato Lagerstätte, Brazil. Cretaceous Research. 130: 105068.

Downen MR, Selden PA, Hasiotis ST. 2016. Spider leg flexure as an indicator for estimating salinity in lacustrine paleoenvironments. Palaeogeography, Palaeoclimatology, Palaeoecology. 445: 115-123.

Duncan IJ, Titchener F, Briggs DEG. 2003. Decay and disarticulation of the cockroach: Implications for preservation of the blattoids of Writhlington (upper Carboniferous), UK. Palaios. 18: 256-265.

Efremov JA. 1940. Taphonomy: new branch of paleontology. Pan-American Geologist. 74: 81-93.

Erthal F, Ritter MN, Kotzian CB. 2016. Assinaturas tafonômicas em moluscos recentes e seu significado paleoambiental. Terrae Didatica. 13(1): 5-30.

Fairchild TR, Sanchez EAM, Pacheco MLAF, Leme JM. 2012. Evolution of Precambrian life in the Brazilian geological record. International Journal of Astrobiology. 11(4): 309-323.

Fielding S, Martill DM, Naish D. 2005. Solnhofen-style soft-tissue preservation in a new species of turtle from the Crato Formation (Early Cretaceous, Aptian) of north-east Brazil. Palaeontology. 48(6): 1301-1310.

Greenwalt DE, Rose TR, Siljestrm SM, Goreva YS, Constenius KN, Wingerath JG. 2015. Taphonomy of the fossil insects of the middle Eocene Kishenehn Formation. Acta Palaeontologica Polonica. 60(4): 931-947.

Grimaldi D. 2009. Fossil Record. In: Resh VH, Cardé RT (Eds). Encyclopedia of Insects. 2nd ed. Academic Press, Elsevier, UK. 396-403.

Grimaldi D and Engel MS. 2005. Evolution of the insects. Hong Kong, Cambridge University. 407-467.

Ilger JM. 2011. Young bivalves on insect wings: A new taphonomic model of the Konservat-Lagerstätte Hagen-Vorhalle (early late Carboniferous; Germany). Palaeogeography, Palaeoclimatology, Palaeoecology. 310: 315-323.

Iniesto M, Buscalioni AD, Carmen-Guerrero M, Benzerara K, Moreira D, López-Archilla AI. 2016. Involvement of microbial mats in early fossilization by decay delay and formation of impressions and replicas of vertebrates and invertebrates. Scientific Reports. 6: 25716.

Iniesto M, Gutiérrez-Silva P, Dias JJ, Carvalho IS, Buscalioni AD, López-Archilla AI. 2021. Soft tissue histology of insect larvae decayed in laboratory experiments using microbial mats: Taphonomic comparison with Cretaceous fossil insects from the exceptionally preserved biota of Araripe, Brazil. Palaeogeography Palaeoclimatology Palaeoecology. 564: 110156.

Jarzembowski EA, Ross AJ. 1996. Insect origination and extinction in the Phanerozoic. Geological Society, London, Special Publications.

Lancaster JB, Briers R. (Eds). 2008. Aquatic insects: challenges to populations. CABI. pp. 23, 270, 284.

Larsson SG. 1975. Palaeobiology and mode of burial of the insects of the lower Eocene Mo-clay of Denmark. Bulletin of the Geological Society of Denmark. 24: 193-209.

Lutz H. 1984. Parallelophoridae: Isolierte Analfelder eozäner Schaben (Insecta: Blattodea). Paläontologische Zeitschrift. 58: 145-147.

Lutz H. 1997. Taphozönosen terrestrischer Insekten in aquatischen Sedimenten: Ein Beitrag zur Rekonstruktion des Paläoenvironments: Neues Jahrbuch für Geologie und Paläontologie. Abhandlungen. 203: 173-210.

Mabesoone JM and Tinoco IM. 1973. Palaeoecology of the Aptian Santana Formation (northeastern Brazil). Palaeogeography, Palaeoclimatology, Palaeoecology. 14: 97-118.

Mancuso AC, Gallego O, Martins-Neto RG. 2007. The Triassic insect fauna from the Los Rastros Formation (Bermejo Basin), La Rioja Province (Argentina): Their context, taphonomy and paleobiology. Ameghiniana. 44: 337-348.

Martínez-Delclòs X, Briggs DEG, Peñalver E. 2004. Taphonomy of insects in carbonates and amber. Palaeogeography, Palaeoclimatology, Palaeoecology. 203: 19-64.

Martínez-Delclòs X, Martinell J. 1993. Insect taphonomy experiments: Their application to the Cretaceous outcrops of lithographic limestones from Spain. Kaupia. 2: 133-144.

McCobb LME, Duncan IJ, Jarzembowski EA, Stankiewicz BA, Wills MA, Briggs DEG. 1998. Taphonomy of the insects from the Insect Bed (Bembridge Marls), late Eocene, Isle of Wight, England. Geological Magazine. 135: 553-563.

McNamara ME, Briggs DEG, Orr PJ, Noh H, Cao H. 2012. The original colours of fossil Beetles. Proceedings of the Royal Society B. 279: 1114-1121.

Medeiros MA. 2004. Fossildiagênese. In: Carvalho IS (ed). Paleontologia. 2a edição (vol 1). Rio de Janeiro: Interciência. 47-59.

Mendes M, Bezerra FI, Adami K. 2020. Ecosystem Structure and Trophic Network in the Late Early Cretaceous Crato Biome. In: Iannuzzi R, Rößler R, Kunzmann L (Eds). Brazilian Paleofloras. Springer, Cham. 1-19.

Moura-Júnior DA, Scheffler SM, Fernandes ACS. 2018. A Paleoentomofauna Brasileira: Cenário Atual. Anuário do Instituto de Geociências. 41(1): 142-166.

Osés GL, Petri S, Becker-Kerber B, Romero GR, Rizzutto MA, Rodrigues F, Galante D, da Silva TF, Curado JF, Rangel EC, Ribeiro RP, Pacheco MLAF. 2016. Deciphering the preservation of fossil insects: a case study from the Crato Member, Early Cretaceous of Brazil. PeerJ. 4: e2756.

Pan Y, Sha J, Fürsich FT. 2014. A model for organic fossilization of the early Cretaceous Jehol lagerstätte based on the taphonomy of "*Ephemeropsis trisetalis*". Palaios. 29: 363-377.

Ponciano LCMO. 2015. Tafonomia de invertebrados: O enigma das conchas. In: Soares MB (org). A paleontologia na sala de aula. Ribeirão Preto: Sociedade Brasileira de Paleontologia. 273-294.

Ribeiro AC, Ribeiro GC, Varejão FG, Battirola LD, Pessoa EM, Simões MG, Warren LV, Riccomini C, Poyato-Ariza FJ. 2021. Towards an actualistic view of the Crato Konservat-

Lagerstätte paleoenvironment: A new hypothesis as an Early Cretaceous (Aptian) equatorial and semi-arid wetland. Earth-Science Review. 216.

Rust J. 1998. Biostratinomie von Insekten aus der Fur-Formation von Dänemark (Moler, oberes Paleozän/unteres Eozän). Paläontologische Zeitschrift. 72: 41-58.

Saraiva AAF, Lima FJ, Barros AO, Bantim RAM (eds). 2021. Guia de fósseis da Bacia do Araripe. Governo do Estado do Ceará, 378 p.

Schäfer W. 1972. Ecology and palaeoecology of marine environments. The University of Chicago Press, 568 p.

Schopf JW, Kudryavtsev AB. 2009. Confocal laser scanning microscopy and Raman imagery of ancient microscopic fossils. Precambrian Research. 173: 39-49.

Smith DM. 2012. Exceptional preservation of insects in lacustrine environments. Palaios. 27: 346-353.

Smith DM, Moe-Hoffman AP. 2007. Taphonomy of Diptera in lacustrine environments: A case study from Florissant Fossil Beds, Colorado. Palaios. 22: 623-629.

Storari AP, Osés GL, Almeida-Lima DSD, Rizzutto MA, Bantim RAM, Lima FJ, Rodrigues T, Sayão JM. 2024. Exceptionally Well-preserved Orthopteran Proventriculi from the Cretaceous Crato Formation of Brazil. Journal of South American Earth Science. 133.

Storari AP, Rodrigues T, Bantim RAM, Lima FJ, Saraiva AAF. 2021. Mass mortality events of autochthonous faunas in a Lower Cretaceous Gondwanan Lagerstätte. Scientific Reports. 11: 6976.

Tischlinger H. 2001. Bemerkungen zur Insekten-Taphonomie der Solnhofener Plattenkalke. Archaeopteryx. 19: 29-44.

Thoene-Henning J, Smith DM, Nufio CR, Meyer HW. 2012. Depositional setting and fossil insect preservation: A study of the late Eocene Florissant Formation, Colorado. Palaios. 27: 481-488.

Vega CS, Dias EV, Rodrigues SC. 2015. Fósseis e processos de fossilização. In: Soares MB (org). A paleontologia na sala de aula. Ribeirão Preto: Sociedade Brasileira de Paleontologia. 90-101.

Wang B, Zhao FC, Zhang HC, Fang Y, Zheng DR. 2012. Widespread pyritization of insects from the Early Cretaceous Jehol biota. Palaios. 27: 707-711.

Wilson MVH. 1988. Reconstruction of ancient lake environments using both autochthonous and allochthonous fossils. Palaeogeography, Palaeoclimatology, Palaeoecology. 62: 609-623.

Zhehikhin VV. 2002. Pattern of insect burial and conservation. In: Rasnitsyn AP, Quicke DL (eds). History of insects. Dordrecht: Kluwer Academic Publisher. 17-63.

Zuschin M, Stachowitsch M, Stanton RJ. 2003. Patterns and processes of shell fragmentation in modern and ancient marine environments. Earth-Science Reviews. 63: 33-82.

Chapter I - Exceptionally well-preserved orthopteran proventriculi from the Cretaceous Crato Formation of Brazil

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Abstract

The Crato Formation (Santana Group, Araripe Basin, Brazil) bears a high abundance of fossils in exceptional state of preservation, with insects from the order Orthoptera standing out. However, so far, few studies have explored their preserved inner organs in detail. Here, we provide the first detailed description of fossilized proventriculi from nine Grylloidea (Orthoptera: Ensifera) specimens of the Crato Formation. In all analyzed specimens, the external cuticle of the abdomen is cracked exposing the proventriculi, which are preserved as a tridimensional organ with a globular body and a tubular neck, similar to that of modern crickets. However, in the globular region of all fossils analyzed there are 9–12 rows of parallel divisions, differing from the modern crickets which have, more frequently, six. SEM images of two specimens revealed the exceptional preservation of internal median teeth, folds, and microvilli texture preserved in the organ.

Keywords. Proventriculus; fossil insect; Orthoptera; Ensifera; Araripe Basin; soft tissue preservation.

1. Introduction

The digestive tract of insects is morphologically divided into three regions: foregut (or stomodeum), midgut (ventriculus, or stomach), and hindgut (or proctodeum) (Chapman et al., 2013). The foregut, which composes the anterior portion of the digestive tract from the oral cavity to the proventriculus, is lined by exoskeleton consisting of chitin and cuticular glycoproteins (Engel and Moran, 2013). The proventriculus is the last organ through which food passes in the first stage of digestion (Judd, 1948) and is responsible for functions such as storage, grinding, and transport of food (Isely, 1949; Liu and Hua, 2009). As the rest of the foregut, it is a sclerotized structure and is composed of several plaques (Chapman et al., 2013). It possesses the same basic structure among insects, but it has diverse modifications associated with adaptations for different feeding modes (Bland and Rentz, 1991; Engel and Moran, 2013).

The exceptional preservation of the digestive system organs, including proventriculi of fossils of the flea *Saurophthirus longipes* (order Siphonaptera), recovered from the Lower Cretaceous of the Zaza Formation, Baissa locality, Transbaikalia, East Siberia, Russia, have been documented in detail by the researchers Strelnikova and Rasnitsyn (Strelnikova and Rasnitsyn, 2016; Rasnitsyn and Strelnikova, 2018). Recently, Zharkov and Dubovikoff (2023) also presented

the first fossil record of ant proventriculi, from Eocene Baltic amber, accessed under Micro-CT scanning. For the Crato Formation of northeastern Brazil, the first mention of fossilized proventriculi was made by Grimaldi et al. (2008) on termites (Isoptera).

The Crato Formation was deposited during the Upper Aptian, Lower Cretaceous (Assine et al., 2014; Neumann and Assine, 2015), and is of worldwide importance due to its outstanding fossil record. Several studies have already explored the taphonomy of Crato insects (Menon and Martill, 2007; Barling et al., 2015; Osés et al., 2016; Bezerra et al., 2018; Barling et al., 2020; Bezerra et al., 2020a,b; Dias and Carvalho 2020; Iniesto et al., 2021; Storari et al., 2021; Dias and Carvalho, 2022; Bezerra et al., 2023; Dias et al., 2023), with orthopterans figuring as the most studied insect clade from that unit in respect to exceptional preservation (Barling et al., 2015; Osés et al., 2016; Bezerra et al., 2020a; Dias and Carvalho 2020; Mendes et al., 2020; Ribeiro et al., 2021). They constitute one of the most expressive groups of arthropods found in this lithostratigraphic unit due to their abundancy and preservation, with several fossils belonging to the suborders Ensifera (commonly known as crickets and katydids) and Caelifera (grasshoppers and locusts) (Menon and Martill, 2007). From those, grylloids (Ensifera) are the most diverse and are often well-preserved (Heads and Martins-Neto, 2007).

Recent orthopterans figure as one of the most prominent groups in terms of morphological variety of the proventriculus, presenting diverse patterns among its clades (Judd, 1948; Bland and Rentz, 1991; Szinwelski et al., 2009), but this structure has yet to be morphologically described in detail in their fossils. Here, we provide a detailed description of the oldest orthopteran proventriculi known so far, recovered from several fossil crickets (Orthoptera: Ensifera: Grylloidea) of the Crato Formation.

2. Material and Methods

2.1 Geological setting

The Araripe Basin crops out in Brazil's northeast region, covering areas of the states of Piauí, Pernambuco and mostly Ceará (Saraiva et al., 2007; Saraiva et al., 2021). While its units range from the Paleozoic through the Mesozoic eras (Assine et al., 2014), the Aptian deposits were formed by post-rift units associated with the separation of South America and Africa, and are known as the Santana Group, being subdivided into the Barbalha, Crato, Ipubi and Romualdo Formations, from bottom to top (Neumann and Cabrera, 1999; Assine et al., 2014).

From those units, the Crato Formation, a ca. 90 m thick succession, is formed by meter-scale horizontal strata of limestone that are interbedded with shales, siltstones and sandstones (Neumann and Cabrera, 1999; Castro et al., 2007; Assine et al., 2014). Most of its outcrops are exposed in commercial quarries or river margins, particularly between the Nova Olinda and Santana do Cariri municipalities, as well as on the Batateiras River banks, all located in the state of Ceará (Viana and Neumann, 2002).

Due to the absence of true marine fossils, there is strong indication that the Crato Formation strata were deposited under lacustrine conditions (Neumann et al., 2003; Heimhofer et al., 2020; Varejão et al., 2021), though the presence of halite pseudomorphs in some sections suggests that the basin experienced salinity variations with increased arid and evaporitic conditions (Martill et al., 2007; Warren et al., 2017; Heimhofer et al., 2010; Storari et al., 2021).

The layers of the Crato Formation have received several stratigraphic classifications to date. The most recent work addressing Nova Olinda's stratigraphy is from Corecco et al. (2022), in which the authors report the most common layer where insects are found with the informal name "veio do besouro", commonly used by local mine workers. For formal classifications, the most recent names the level with the exceptionally preserved fossils as facies association 4 (FA-4 - sensu Varejão et al., 2021), with insects usually found in its upper beds (Varejão et al., 2021). A previous classification by Varejão et al. (2019) pointed out that the stratigraphic position of most of insects is a layer called Interval III, consisting the upper 2-meter part of the Lagerstätte succession and characterized by finely laminated, rhythmic, laminites. Finally, the Crato Formation has also been divided in six carbonate intervals, designated C1 to C6 by Neumann and Cabrera (1999), with C6 encompassing the Lagerstätte succession (Neumann and Cabrera, 1999; Storari et al., 2021). However, since the C6 is not further subdivided, no specific profile has been associated with the presence of well-preserved insects.

2.2 Material

We analyzed nine specimens from the Crato Formation: CAV 0012-I (from the Paleontological collection of the Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão, Brazil) (Fig 1); LPU P1–P4, and LPU P6 (from the Paleontological collection of the Universidade Regional do Cariri, Crato, Brazil) (Figs 2,3); and

GP/1E 7268, 8691, and 8910 (from the Scientific Paleontological Collection of the Institute of Geosciences of the Universidade de São Paulo, São Paulo, Brazil) (Fig 4).

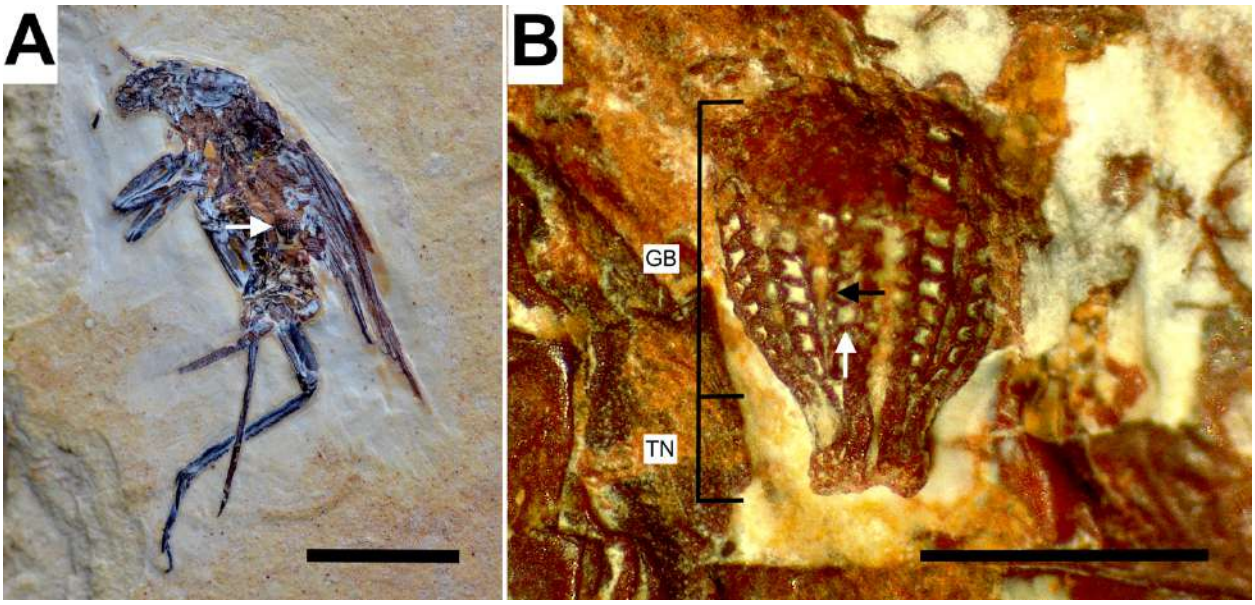

Fig 1. *Araripegryllus* cf. *femininus*, adult female CAV 0012-I. A) Overview. White arrow points to proventriculus. Scale bar 5 mm; B) Detail of proventriculus. Black arrow points to one longitudinal fold, and the white arrow points to one parallel division. GB – Globular body; TN – Tubular neck. Scale bar 0.5 mm.

CAV 0012-I was collected in 2009 during a field trip of biology students of the Centro Acadêmico de Vitória (Universidade Federal de Pernambuco) in the renowned limestone mine, Mina do Demar, on the road that connects Nova Olinda and Santana do Cariri municipalities, Ceará state. The LPU specimens lack exact locality information as they have been collected by mine workers and donated to that collection. The GP/1E specimens also lack exact locality information because they were recovered by the Brazilian federal police during an operation against fossil smuggling. Although those specimens lack exact locality details they were probably collected from upper portions of the FA-4 (or C6) layer.

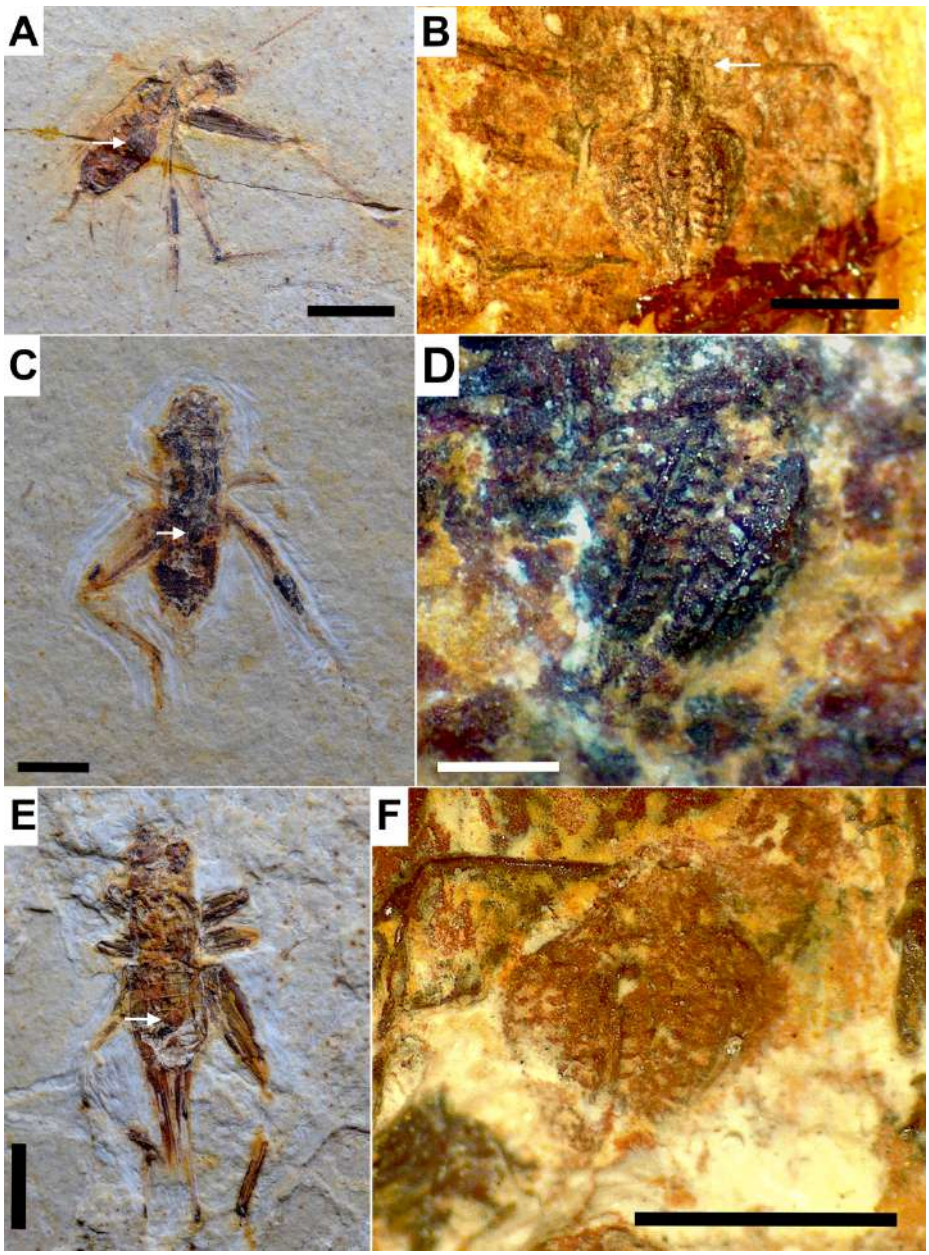

Fig 2. Grylloidea specimens. A) Specimen LPU P4 overview, adult female, Grylloidea indet., white arrow points to proventriculus. Scale bar 5 mm; B) Detail of proventriculus of specimen LPU P4; white arrow points to long, curved tube, that connects the proventriculus to the gastric crop. Scale bar 0.5 mm; C) Specimen LPU P1 overview, Grylloidea indet., white arrow points to proventriculus. Scale bar 3 mm; D) Detail of proventriculus of specimen LPU P1. Scale bar 0.2 mm; E) Specimen LPU P2 overview, Gryllidae indet., white arrow points to proventriculus. Scale bar 4 mm; F) Detail of proventriculus of the specimen LPU P2. Scale bar 0.5 mm.

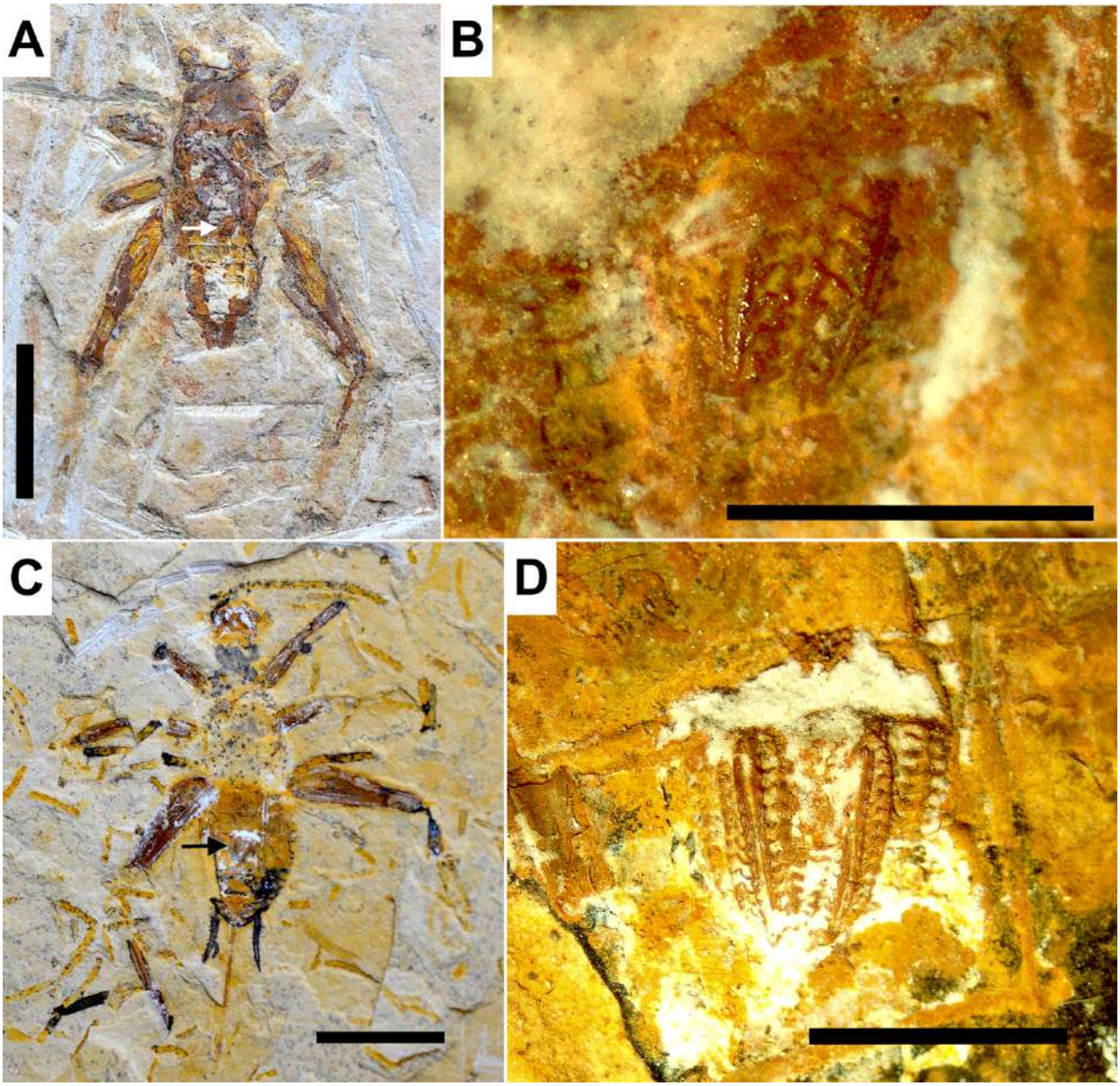

Fig 3. Grylloidea specimens. A) Specimen LPU P3 overview, Grylloidea indet., white arrow points to proventriculus. Scale bar 5 mm; B) Detail of proventriculus of specimen LPU P3. Scale bar 0.5 mm; C) Specimen LPU P6 overview, *Cearagryllus* sp. indet., adult female, black arrow points to proventriculus. Scale bar 10 mm; D) Detail of proventriculus of specimen LPU P6. Scale bar 1 mm.

2.3 Methods

The specimens were prepared mechanically under a stereomicroscope, and the sediment was removed with ultrafine needles and a brush. All figures were made with the software Autodesk Version 8.6.1, and the photos were taken with a Nikon D800 digital camera or an Olympus DSX110 microscope (Laboratório de Estudos Paleobiológicos, Instituto de Geociências, Universidade de São Paulo, São Paulo, Brazil). The descriptive morphological terminology follows Judd (1948), Bland and Rentz (1991), and Wang et al. (2012). The specimens LPU P5, GP/1E 7409, and GP/1E 8827 were analyzed only for biostratinomic features since their proventriculi are not well preserved. Micro morphological analyses were conducted on specimens CAV 0012-I and LPU P6 using the scanning electron microscope (SEM) FEI Quanta 650 FEG at the Instituto de Geociências, Universidade de São Paulo (São Paulo, Brazil), after a gentle bath of alcohol 70%. The specimens were kept uncoated and analyzed under low vacuum (environmental scanning electron microscope), with images taken at 20 kV of acceleration voltage, spot size 6, and variable working distances (as shown in each SEM image). The micrographs were taken with the microscope's secondary electron detector (LFD) and backscattered electron detector (CBS).

3. Results

Most analyzed specimens could be identified just as Grylloidea indet., given the poor preservation of diagnostic structures (such as the wings) at less inclusive levels (Table 1). Most of them are preserved flattened, dorso-ventrally, with the legs spread out and wings absent. CAV 0012-I is preserved in lateral aspect with the wings folded over the abdomen in a characteristic 'rest position' (Martins-Neto, 1992) (Fig 1), while LPU P4 is also preserved in lateral view but its wings are absent (Fig 2A). GP/1E 8910 has its wings open laterally, as typical of individuals who died in the depositional environment, possibly owing to asphyxiation and drowning after exhaustion (Martins-Neto, 1992; Martínez-Delclòs et al., 2004) (Fig 4E). The posterior limbs are preserved in all specimens. For their general morphological description, see Supplementary Table 1.

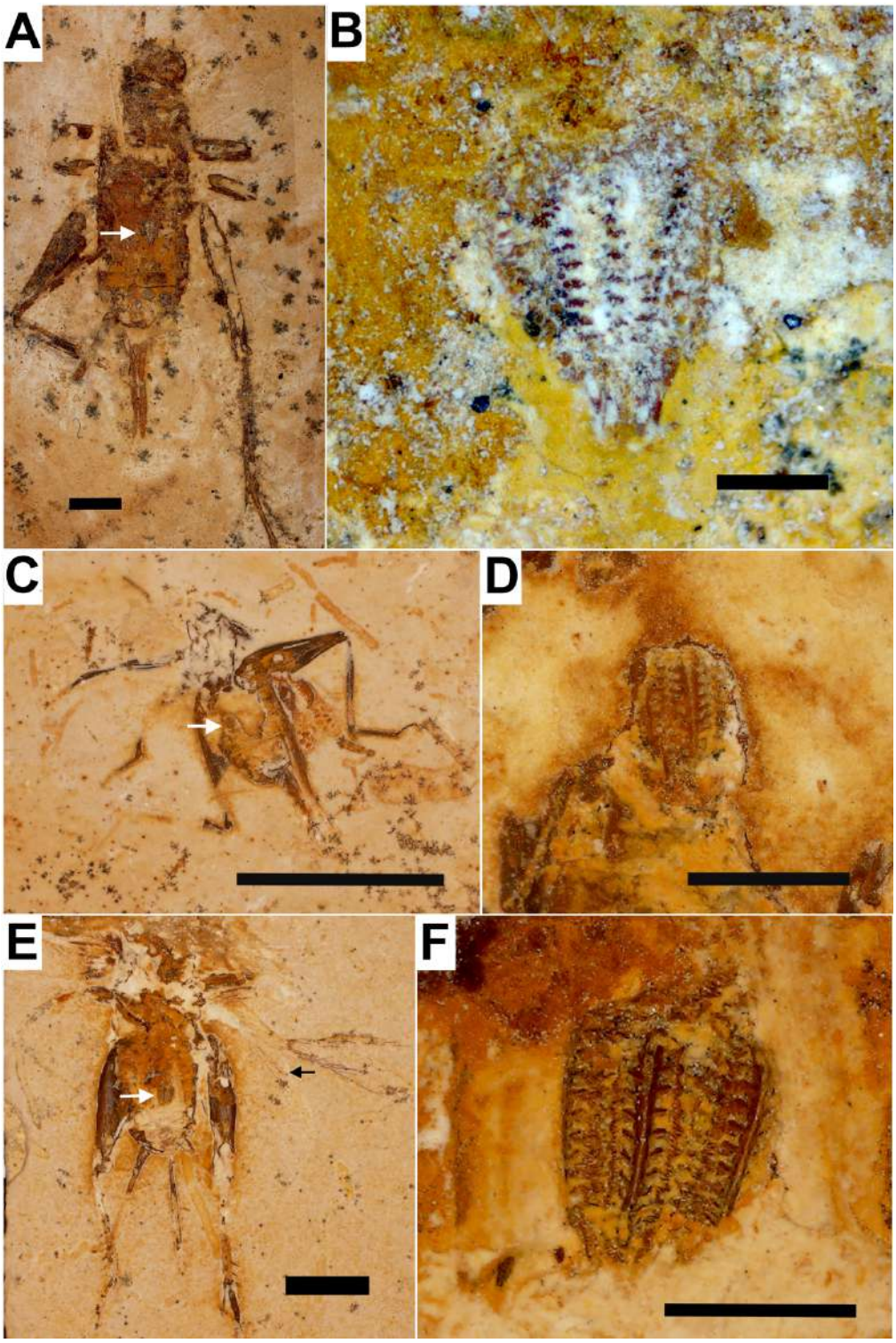

Fig 4. Grylloidea specimens. A) Specimen GP/1E 7268 overview, Grylloidea indet., white arrow points to proventriculus. Scale bar 5 mm; B) Detail of proventriculus of specimen GP/1E 7268. Scale bar 0.2 mm; C) Specimen GP/1E 8691 overview, Grylloidea indet., white arrow points to

proventriculus. Scale bar 10 mm; D) Detail of proventriculus of specimen GP/1E 8691. Scale bar 1 mm; E) Specimen GP/1E 8910 overview, *Araripegryllus* sp. indet., adult female, white arrow points to proventriculus; and black arrow pointing to antennae. Scale bar 5 mm; F) Detail of proventriculus of specimen GP/1E 8910. Scale bar 1 mm.

Table 1 here. Summary of the main characters of the analyzed grylloids. '?' unknown.

The analyzed specimens are compressed with their cuticles cracked in their abdominal plates, fully exposing the inner cavity and proventriculi, except in GP/1E 8691, which presents the proventriculus partially covered by external cuticle (Fig 4C,D).

The proventriculi are three-dimensionally preserved and, as in modern crickets, consist of an anterior globular body (GB) and a posterior tubular neck (TN), a structure that connects with the gastric caeca of the midgut (Bland and Rentz, 1991; Li et al., 2011) (Fig 1). At the globular region, there are rows formed by groups of sclerotized appendices (parallel divisions) united to each other by sclerotized partitions (longitudinal folds). The specimens LPU P4 and GP/1E 8910 also present, anteriorly to the GB, a long, curved tube, similar to the TN in morphology, that connected to the gastric crop (Figs 2B and 4D).

SEM images of CAV 0012-I revealed internal median teeth, as well as microvilli texture throughout the GB and TN (Fig 5). In that specimen, the upper part of the proventriculus has a flat external wall, unlike other areas, which are preserved in three dimensions. SEM images of LPU P6 show some preserved remains of the inner cavity of the foregut, which consist of several small denticles that could be associated with the crop cavity (Fig 6B,C). Finally, as in specimen CAV 0012-I, LPU P6 also preserves some of the folds that form internally the median and lateral teeth of the proventriculus (Fig 6D–F).

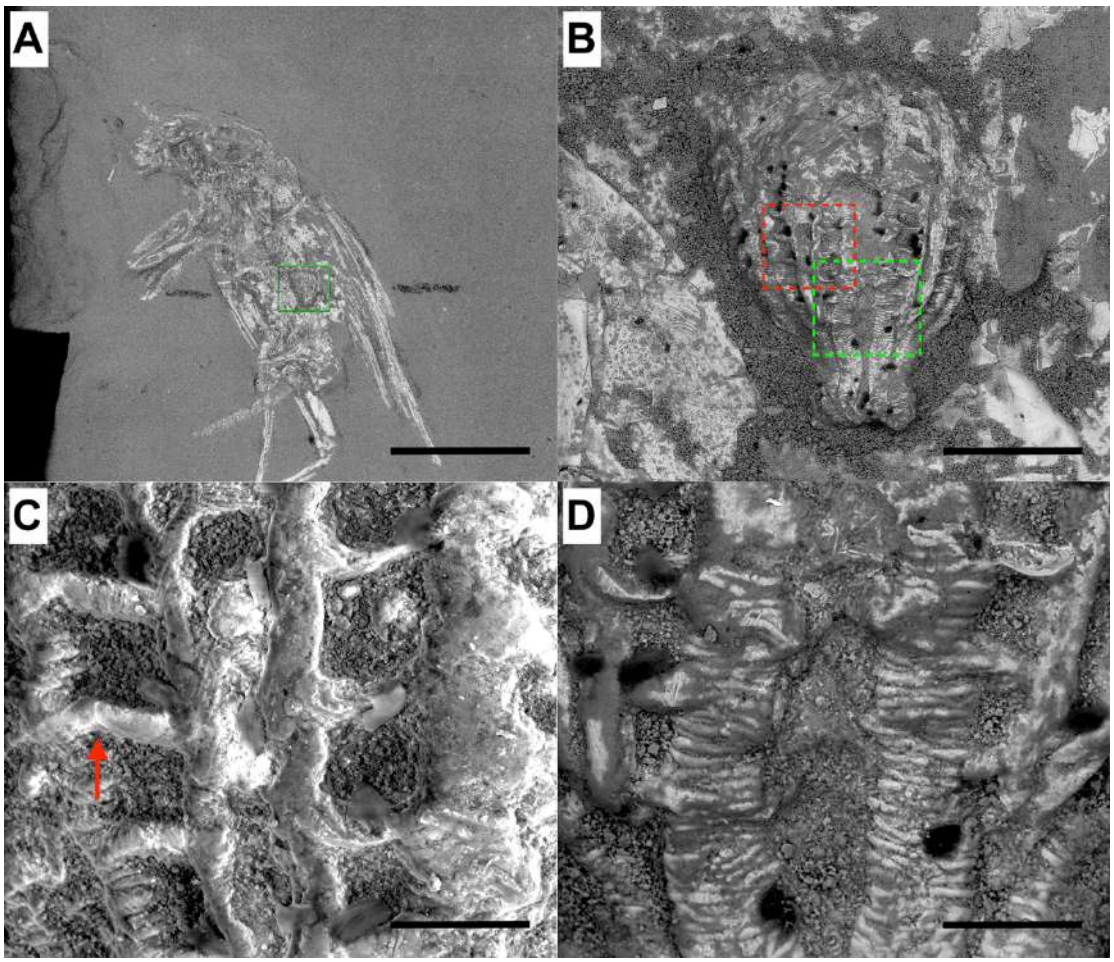

Fig 5. *Araripegryllus* cf. *femininus*, adult female CAV 0012-I. Morpho-anatomical features of proventriculus visualized on scanning electron microscopy; A) Overview, CBS detector. Green square delimits proventriculus showed in detail in figure B. Magnification 7x. Scale bar 5 mm; B) Detail of proventriculus, CBS detector. Red square evidencing region magnified in figure C, and green square delimiting part of proventriculus under magnification in D. Magnification 80x. Scale bar 500 µm; C) Part of the GB, LFD detector. Red arrow points to fold, indicating an internal median tooth. Magnification 450x. Scale bar 100 µm; D) Part of the GB and TN with preserved microvilli texture. Magnification 400x. Scale bar 100 µm.

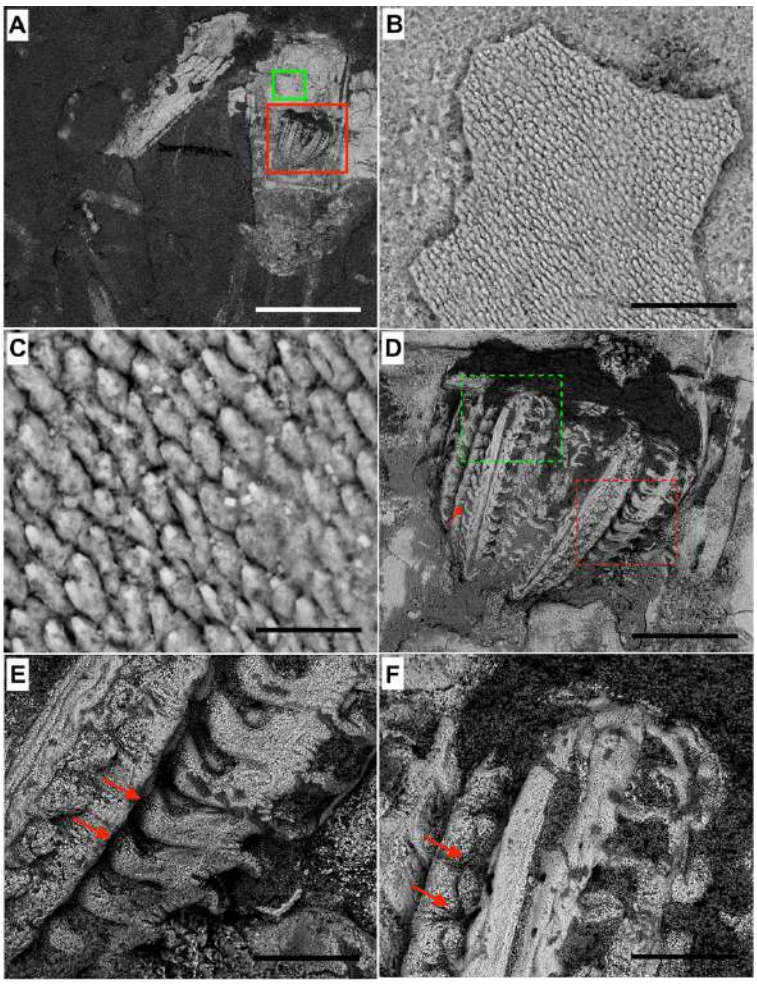

Fig 6. *Cearagryllus* sp. indet., adult female LPU P6. Morpho-anatomical features of inner cavity of foregut visualized on scanning electron microscopy, CBS detector; A) Overview of abdomen. Green square evidencing region detailed in figures B and C, and red square evidencing region detailed in figure D. Magnification 8x. Scale bar 5 mm; B,C) Detail of remains of inner cavity of foregut under 450x and 2000x magnifications, with surface consisting of several denticles. Scale bars 100 µm and 30 µm; D) Detail of proventriculus. Red square shows part of proventriculus under magnification in figure E, and green square delimits part of proventriculus under magnification in F. Red arrow points to fold indicating internal median tooth. Magnification 40x. Scale bar 1 mm; E) Part of GB. Red arrows point to fold indicating internal lateral teeth. Magnification 150x. Scale bar 400 µm; F) Part of GB. Red arrows point to fold indicating internal median teeth. Magnification 150x. Scale bar 400 µm.

4. Discussion

Grylloids from the Crato Formation can show three types of fossilization processes: pyritization, phosphatization, and kerogenization (Bezerra et al., 2020a,b; Dias and Carvalho 2020). The fossils we analyzed have iron-oxide preservational microfabrics, which suggest oxidation after pyritization (Osés et al. 2016; 2017; Bezerra et al., 2023). Pyritization depends on the concentration of sulphate and iron ions within the decaying carcass, and on their subsequent reduction and pyrite precipitation, that are controlled by microbial mats (Osés et al., 2016; 2017). If fissures are present in the insect cuticle, solutes can penetrate and facilitate the mineralization of both external cuticle and inner soft tissues (Osés et al., 2016; 2017).

Although the insect fore and hindguts are highly sclerotized, as they are of ectodermic origin (Fantazzini et al., 1998; Douglas, 2013), their organs are rarely preserved in the fossil record and to date were only mentioned for Orthoptera by Rust (1999) and Dias and Carvalho (2020). Rust (1999) revealed the presence of a proventricular structure in a fossil specimen of Gryllidae from the Paleogene of the Ølst Formation in Denmark, but it was not well-preserved, and Dias and Carvalho (2020) reported anatomical microstructures such as cutaneous plaques and denticles as associated with the proventriculus of a female Baissogryllidae from the Crato Formation. However, their location in the specimen was not described nor it was possible to observe in the image provided the whole globular organ preserved, perhaps due to taphonomic imprints, or simply because they recovered only fragments of the putative proventriculus associated with the foregut region. Our SEM results show similar cutaneous plaques and denticles as those found by Dias and Carvalho (2020) preserved in LPU P6 but associated with a different body area than that of the proventriculus that, based on their location and seemingly sclerotized appearance, we interpret as remains of the foregut's internal surface, most possibly of the crop, which is situated anteriorly to the proventriculus (Chapman et al., 2013) (see Fig 6). Therefore, to our knowledge, the orthopteran proventriculi we present here are the oldest known, and the first ones described in detail.

In most cricket fossils from the Crato Formation, the proventriculi are not preserved; for instance, they were preserved in only seven out of the 208 orthopteran specimens in the Scientific Paleontological Collection of the Institute of Geosciences of the Universidade de São Paulo, although only three were investigated in details in the present work since the remaining four were badly preserved. Interestingly, even in specimens with well-preserved proventriculi, their bodies

are frequently not well-preserved at hand-scale. To explain the exceptional preservation of this internal organ but not of the remaining of the carcass, we hypothesize that despite the proventriculus being an extremely hard organ, it can only be observed in the fossils if exposed after the decomposition and/or cracking of the overlying body exoskeleton. This cuticle cracking could be related to decaying processes due to longer exposure time of carcasses after death in the waterbody, following a short period of decay on land (Martínez-Delclòs et al., 2004). Alternatively, the proventriculi could simply be observed because the outer cuticle was cracked open during sampling and/or preparation of the material; unfortunately, counterparts of the analyzed material are not available. In any case, since insects with well-developed wings such as grylloids most often die, decompose, disarticulate, and/or fragment/crack while floating on the lake surface, but seldom sink and are buried (Martínez-Delclòs and Martinell, 1993), cases as the present ones are rare in the fossil record.

All proventriculi analyzed are tridimensionally preserved structures (albeit to different degrees), even though internal organs are easily affected by compression by the overlying sediments. However, in most specimens we could only recognize proventricular external features: their length and diameter, the general number of sclerotized appendices preserved on each longitudinal fold, and the number and width of preserved sclerotized partitions between adjacent rows. Other characters useful for taxonomical attributions among extant groups, such as anatomical features of the lateral and median teeth (Li et al., 2011; Wang et al., 2012), could not be evaluated in detail since they are only visible on the internal surface of the proventriculus, although we could identify in two of the analyzed specimens the folds that form internally the median and lateral teeth of the proventriculus.

Regarding the rows of parallel divisions, in the fossils we analyzed there are 9–12, while in all the recent 102 orthopteran species whose proventriculi have been studied so far (belonging to the Ensifera families Trigonidiidae, Gryllidae, and Tettigonidae; and also the Caelifera family Acrididae) there are six (Judd, 1948; Bland and Rentz, 1991; Szinwelski et al., 2009; Li et al., 2011; Wang et al., 2012). These data, although limited, suggests a possible trend of decreasing number of parallel divisions in modern taxa, while the overall size of the proventriculus increased (e.g., body/proventriculus proportions between 1/8 to 1/12 in extant taxa—see Wang et al., 2012). This could represent an evolutionary tendency to lose parallel divisions while increasing the

overall size of the organ, but more data on fossil and recent grylloids are needed to test this hypothesis.

The specimens LPU P2 and LPU P6 presented their proventriculi located more posteriorly in the body than other specimens. Wang et al.'s (2012) results showed that recent Phaneropterinae (Tettigonidae) with a short foregut and a longer midgut and hindgut are carnivorous or omnivorous since the main place for grinding food is the foregut, so herbivorous species have a larger one. One could thus hypothesize that specimens LPU P2 and LPU P6 were phytophagous because they have a longer foregut. However, considering the possibility that the proventriculi as well as the entire intestine may be displaced due to decomposition [as similarly noted for termites of Crato Fm. by Grimaldi et al. (2008)], we cannot attest that this character is related to a specific feeding habit, though Crato orthopterans are frequently associated to phytophagy (Grimaldi, 1990; Martins-Neto, 2005; Santos Filho et al., 2017). On the other hand, carnivores and omnivores present high degree of chitinization in their proventriculi (as all the specimens analyzed by us), and big and numerous teeth on their proventriculi (as specimen LPU P6), due to processing hard parts of food (Gibbs, 1967; Muralirangan, 1979; Wang et al., 2012). Based on the latter we could hypothesize that the fossils analyzed here are carnivorous or omnivorous. But for that, it would be important to consider many additional characters (e.g. structure of mouthparts, forelegs, degree of chitinization of foregut), which are, unfortunately, unavailable in our specimens.

5. Conclusion

The proventriculi of Crato Formation grylloid specimens described above are the oldest known (Lower Cretaceous) for this group, and the first characterized in detail. They show that these extinct orthopterans had an overall similar proventricular morphology compared to recent taxa, with a globular body and a posterior tubular neck. However, in the globular region of all the fossils analyzed, there are between 9 and 12 rows of parallel divisions, differing from all the modern crickets studied so far, which usually have only six. Since here we only worked with nine specimens, it is not possible to provide a statistical analysis to test for evolutionary hypotheses that could explain the observed differences among extant and extinct taxa, though it remains open to debate.

The cuticle of all the grylloids analyzed here are cracked in their abdominal plates, exposing the inner cavity and proventriculi, which are highly sclerotized and robust. The exposure

and 3-D preservation of the proventriculi is, probably, related to a short preservational window, in which the carcasses needed to decay before exposition to favorable environment for the exceptional preservation. Additionally, two of the specimens analyzed with SEM revealed preserved internal median teeth and folds, as well as microvilli texture, revealing astonishing internal preservation.

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References

Assine M, Perinotto A, Custódio MA, Neumann V, Varejão FG, Mescolotti PC. Sequências Deposicionais do Andar Alagoas (Aptiano superior) da Bacia do Araripe, Nordeste do Brasil. Boletim de Geociências Petrobras. 2014;22(1):3-28.

Barling N, Martill DM, Heads SW, Gallien F. High fidelity preservation of fossil insects from the Crato Formation (lower Cretaceous) of Brazil. Cretac Res. 2015;52:602-622. doi: 10.1016/j.cretres.2014.05.007

Barling N, Martill DM, Heads SW. A geochemical model for the preservation of insects in the Crato Formation (Lower Cretaceous) of Brazil. Cretac Res. 2020;116:104608. doi: 10.1016/j.cretres.2020.104608

Bezerra F, Da Silva J, Agressot E, Freire P, Viana B, Mendes M. Effects of chemical weathering on the exceptional preservation of mineralized insects from the Crato Formation, Cretaceous of Brazil: Implications for late diagenesis of fine-grained Lagerstätten deposits. Geol Magaz. 2023;160(5):911-926. doi: 10.1017/S0016756823000043

Bezerra FI, Da Silva JH, De Paula AJ, Oliveira NC, Paschoal AR, Freire PTC, et al. Throwing light on an uncommon preservation of Blattodea from the Crato Formation (Araripe Basin, Cretaceous), Brazil. Rev Bras Paleo. 2018;21(3):245-254. doi: 10.4072/rbp.2018.3.05

Bezerra FI, Da Silva JH, Miguel EC, Paschoal AR, Nascimento Jr DR, Freire PTC, et al. Chemical and mineral comparison of fossil insect cuticles from Crato Konservat Lagerstätte, Lower Cretaceous of Brazil. J Iber Geol. 2020a;46:61-76. doi: 10.1007/s41513-020-00119-y

Bezerra FI, Solórzano-Kraemer MM, Mendes M. Distinct preservational pathways of insects from the Crato Formation, Lower Cretaceous of the Araripe Basin, Brazil. Cretac Res. 2020b;118:104631. doi: 10.1016/j.cretres.2020.104631

Bland R, Rentz D. Studies in Australian Gryllacrididae: the proventriculus as a taxonomic character. Invertebr Syst. 1991;5:443-455.

Castro JC, Valença LMM, Neumann VHL. Ciclos e sequências deposicionais das formações rio da Batateira e Santana (Andar Alagoas), Bacia do Araripe, Brasil. Geociências (São Paulo). 2007;25:289-296.

Chapman RF, Simpson SJ, Douglas AE. The Insects: Structure and Function. 5th ed. Cambridge University Press; 2013.

Corecco L, Bezerra FI, Filho WFS, Júnior DRN, Da Silva JH, Felix JL. Petrological meaning of ethnostratigraphic units: Laminated Limestone of the Crato Formation, Araripe Basin, NE Brazil. Pesquisas em Geociências. 2022;49(1):e121139. doi: 10.22456/1807-9806.121139

Dias JJ, Carvalho IS. Remarkable fossil crickets preservation from Crato Formation (Aptian, Araripe Basin), a Lagerstätten from Brazil. J S Am Earth Sci. 2020;98:102443. doi: 10.1016/j.jsames.2019.102443

Dias JJ, Carvalho IS. The role of microbial mats in the exquisite preservation of Aptian insect fossils from the Crato Lagerstätte, Brazil. Cretac Res. 2022;130:105068. doi: 10.1016/j.cretres.2021.105068

Dias JJ, Carvalho IS, Buscalioni AD, Umamaheswaran R, López-Archilla AN, Prado G, et al. Mayfly larvae preservation from the Early Cretaceous of Brazilian Gondwana: Analogies with modern mats and other Lagerstätten. Gond Res. 2023;124:188-205. doi: 10.1016/j.gr.2023.07.007

Douglas AE. Alimentary canal, digestion and absorption. In: Simpson SJ, Douglas AE, editors. The Insects: Structure and Function. Cambridge University Press, New York; 2013. pp. 46-80.

Engel P, Moran NA. The gut microbiota of insects–diversity in structure and function. Microbiol Rev. 2013;37:699-735. doi: 10.1111/1574-6976.12025

Fantazzini ER, Fontanetti CS, Camargo-Matias MI. Anatomy of the digestive tube, histology and bistochemistry of tbe foregut and salivary glands of Rhinocricus padbergi Verhocff (1938) (Diplopoda: Spirobolida: Rhinocricidae). Arthropoda Sel. 1998;7(4):257-264.

Gibbs DG. The proventriculus of some trichopterous larvae. J Zool. 1967;152:245-256.

Grimaldi DA, Engel MS, Krishna K. The Species of Isoptera (Insecta) from The Early Cretaceous Crato Formation: A Revision. Am Mus Nov. 2008;3626:1-30. doi: 10.1206/616.1

Grimaldi DA. Insects from the Santana Formation, Lower Cretaceous, of Brazil. B Am Mus Nat Hist. 1990;195:1-191.

Heads S, Martins-Neto R. Orthopterida: grasshoppers, crickets and stick insects. In: Martill DM, Bechly G, Loveridge RF, editors. The Crato Fossil Beds of Brazil: Window into an ancient world. Cambridge University Press; 2007. p. 265-283.

Heimhofer U, Ariztegui D, Lenniger M, Hesselbo SP, Martill DM, Rios-Netto AM. Deciphering the depositional environment of the laminated Crato fossil beds (early Cretaceous, Araripe Basin, north-eastern Brazil). Sedimentology. 2010;57:677-694. doi: 10.1111/j.1365-3091.2009.01114.x

Iniesto M, Gutiérrez-Silva P, Dias JJ, Carvalho IS, Buscalioni AD, López-Archilla AI. Soft tissue histology of insect larvae decayed in laboratory experiments using microbial mats:

Taphonomic comparison with Cretaceous fossil insects from the exceptionally preserved biota of Araripe, Brazil. Palaeo Palaeo Palaeo. 2021;564:110156. doi: 10.1016/j.palaeo.2020.110156

Isely FB, Alexander G. Analysis of insect food habits by crop examination. Science. 1949;109:115-116.

Judd WW. A comparative study of the proventriculus of orthopteroid insects with reference to its use in taxonomy. Can J Res. 1948;26:93-161. doi: 10.1139/cjr48d-011

Li X, Ren B, Zou Y, Zhang J, Wang Y. The study of proventricular micromorphological characterization of ten Grylloidea species (Orthoptera: Grylloidea) from China. Zootaxa. 2011;2906:52-60.

Liu S, Hua B. Ultramorphology of the proventriculus in Panorpidae and Bittacidae (Mecoptera). Micron. 2009;40:899-905. doi: 10.1016/j.micron.2009.05.009

Martill DM. The geology of the Crato Formation. In: Martill DM, Bechly G, Loveridge RF, editors. The Crato Fossil Beds of Brazil: Window into an ancient world. Cambridge University Press; 2007. pp. 8-24.

Martins-Neto RG. Nova ocorrência, variabilidade morfológica e relações filogenéticas do gênero Cratoelcana Martins-Neto, 1991 (Insecta, Ensifera, Elcanidae), da Formação Santana, Bacia do Araripe, Brasil. Rev Bras Entomol. 1992;36(4):817-830.

Martins-Neto RG. Estágio atual da paleoartropodologia Brasileira: hexápodes, miriápodes, crustáceos (Isopoda, Decapoda, Eucrustacea e Copepoda) e quelicerados. Arq M Nac. 2005;63(3):471–494.

Martínez-Delclòs X, Briggs DEG, Peñalver E. Taphonomy of insects in carbonates and amber. Palaeo Palaeo Palaeo. 2004;203:19-64. doi: 10.1016/S0031-0182(03)00643-6

Martínez-Delclòs X, Martinell J. Insect taphonomy experiments: their application to the Cretaceous outcrops of lithographic limestones from Spain. Kaupia. 1993;2:133-144.

Mendes M, Bezerra FI, Adami K. Ecosystem Structure and Trophic Network in the Late Early Cretaceous Crato Biome. In: Iannuzzi R, Rößler R, Kunzmann L, editors. Brazilian Paleofloras. Springer, Cham; 2020. pp. 1-19. doi: 10.1007/978-3-319-90913-4_33-1

Menon F, Martill DM. Taphonomy and preservation of Crato Formation arthropods. In: Martill DM, Bechly G, Loveridge RF, editors. The Crato Fossil Beds of Brazil: Window into an ancient world. Cambridge University Press; 2007. p. 79-96.

Muralirangan M. On the food preference and the morphological adaptations of the gut of some species of Orthoptera. Curr Sci. 1979;49:240-241.

Neumann VH, Assine ML. Stratigraphic proposal to Post-Rift I Tectonic- Sedimentary Sequence of Araripe Basin, Northeastern Brazil. Proceedings of the 2nd International Congress on Stratigraphy, Graz, Austria; 2015.

Neumann VH, Borrego AG, Cabrera L, Dino R. Organic matter composition and distribution through the Aptian–Albian lacustrine sequences of the Araripe Basin, northeastern Brazil. Int J Coal Geol. 2003;54:21-40.

Neumann VH, Cabrera L. Una nueva propuesta estratigráfica para la tectonosecuencia post-rifte de la cuenca de Araripe, nordeste de Brasil. Proceedings of the Boletim do 5° Simpósio sobre o Cretáceo do Brasil. 279-85;1999.

Osés GL, Petri S, Becker-Kerber B, Romero GR, Rizzutto MA, Rodrigues F, et al. Deciphering the preservation of fossil insects: a case study from the Crato Member, Early Cretaceous of Brazil. PeerJ. 2016;4:e2756. doi: 10.7717/peerj.2756

Osés GL, Petri S, Voltani CG, Prado GMEM, Galante D, Rizzutto MA, et al. Deciphering pyritization-kerogenization gradiente for fish soft-tissue preservation. Sci Rep. 2017;7:1-15. doi: 10.1038/s41598-017-01563-0

Rasnitsyn AP, Strelnikova OD. Digestive System of the Early Cretaceous Insect *Saurophthirus longipes* Ponomarenko (Insecta, ?Aphaniptera, Saurophthiroidea). Paleontol J. 2018;52:146-154. doi: 10.1134/S0031030118020119

Ribeiro AC, Ribeiro GC, Varejão FG, Battirola LD, Pessoa EM, Simões MG, Warren LV, Riccomini C, Poyato-Ariza FJ. Towards an actualistic view of the Crato Konservat-Lagerstätte paleoenvironment: A new hypothesis as an Early Cretaceous (Aptian) equatorial and semi-arid wetland. Earth Sci Rev. 2021;216. doi: 10.1016/j.earscirev.2021.103573

Rust J. Oldest known pteroplistine cricket and other Gryllidae (Orthoptera) from the Paleogene Fur and Ølst Formations of Denmark. Insect Syst Evol. 1999;30:35-45. doi: 10.1163/187631200X00192

Santos Filho EB, Adami-Rodrigues K, Lima FJ, Bantim RAM, Wappler T, Saraiva AAF. Evidence of plant–insect interaction in the Early Cretaceous Flora from the Crato Formation, Araripe Basin, Northeast Brazil. Hist Biol. 2017;31(7):926-937. doi: 10.1080/08912963.2017.1408611

Saraiva AAF, Hessel MH, Guerra NC, Fara E. Concreções Calcárias da Formação Santana, Bacia do Araripe: uma proposta de classificação. Estud Geol. 2007;17(1):40-58

Saraiva AAF, Lima FJ, Barros OA, Bantim RAM. Guia de fósseis da Bacia do Araripe. 2nd ed. 378 pp. Crato: Ceará; 2021.

Storari AP, Rodrigues T, Bantim RAM, Lima FJ, Saraiva AAF. Mass mortality events of autochthonous faunas in a Lower Cretaceous Gondwanan Lagerstätte. Sci Rep. 2021;11:6976. doi: 10.1038/s41598-021-85953-5

Storari AP, Rodrigues T, Saraiva AAF, Salles FF. Unmasking a gap: a new oligoneuriid fossil (Ephemeroptera: insecta) from the Crato Formation (upper Aptian), Araripe Basin, NE Brazil, with comments on *Colocrus* McCafferty. PLoS One. 2020;15(10):e0240365. doi: 10.1038/s41598-021-85953-5

Strelnikova OD, Rasnitsyn AP. Preliminary results of study of the breathing and alimentary systems in the Early Cretaceous "flea" *Saurophthirus longipes* Ponomarenko, 1976 (Insecta?, Aphaniptera, Saurophthiridae). Far East Entomol. 2016;327:1-7.

Szinwelski N, Rodrigues MS, Pereira MR, Serrão JE, Sperber CF. Proventriculus of Three Nemobiinae Crickets (Orthoptera: Grylloidea: Trigonidiidae). J Orthoptera Res. 2009;18(1):59-63. doi: 10.1665/034.018.0104

Varejão FG, Warren LV, Simões MG, Buatois LA, Angano GM, Bahniuk Rumbelsperger AN, et al. Mixed siliciclastic–carbonate sedimentation in an evolving epicontinental sea: Aptian record of marginal marine settings in the interior basins of north-eastern Brazil. Sedimentology. 2021;68(5):2125-2164. doi: 10.1111/sed.12846

Varejão FG, Warren LV, Simões MG, Fürsich FT, Matos SA, Assine ML. Exceptional preservation of soft tissues by microbial entombment: insights into the taphonomy of the Crato Konservat-Lagerstätte. Palaios. 2019;34:331-348. doi: 10.2110/palo.2019.041

Viana MS, Neumann VHL. Membro Crato da Formação Santana, Chapada do Araripe, CE - Riquíssimo registro de fauna e flora do Cretáceo. In: Schobbenhaus C, Campos DA, Queiroz ET, Winge M, Berbert-Born MLC, editors. Sítios Geológicos e Paleontológicos do Brasil. DNPM/CPRM - Comissão Brasileira de Sítios Geológicos e Paleobiológicos; 2002. p. 113-120.

Wang Y, Su Y, Zhang X, Li N, Ren BA comparative study of the proventricular structure in twenty Chinese Tettigoniidae (Orthoptera) species. Entomol Fenn. 2012;23:140-148. doi: 10.33338/ef.6898

Warren LV, Varejão FG, Quaglio F, Simões MG, Fürsich FT, Poiré DG, et al. Stromatolites from the Aptian Crato Formation, a hypersaline lake system in the Araripe Basin, northeastern Brazil. Facies. 2017;63:1-19. doi: 10.1007/s10347-016-0484-6

Zharkov D, Dubovikoff D. A fossil "Social Stomach": Micro-CT imaging unearths first fossil record of ant proventriculi and comparative analysis with recent species. J Morphol. 2023;284(11):e21645. doi: 10.1002/jmor.21645.PMID:37856279.

Chapter 2 - Palaeometric approaches reveal striking differences in the insect fossilization of two Mesozoic Konservat-Lagerstätten

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Abstract

The Crato Formation (Lower Cretaceous, Brazil) is a well-known Konservat-Lagerstätte that preserves a great number of various exceptionally well-preserved insects both at micro and macro scales. Here, we sought to explore the preservational modes of two abundant aquatic and terrestrial groups of this unit, mayflies (Ephemeroptera) and crickets (Orthoptera). To better understand how exceptional the preservation in the Crato Formation is, we also present detailed data on the modes of preservation of mayflies from the renowned Solnhofen limestones (Upper Jurassic, Germany). For the Crato Formation, from 234 fossil mayflies and crickets studied, six mayflies and four

crickets were additionally analyzed using scanning electron microscopy coupled to energy dispersive X-ray spectroscopy (SEM-EDS), energy dispersive X-ray fluorescence (EDXRF), micro-energy dispersive X-ray fluorescence (μEDXRF), and μRaman spectroscopy. A total of 85 adult mayflies from the Solnhofen limestones were analyzed, and five of them were subjected to SEM-EDS and μEDXRF analyses. The majority of Crato specimens have several conserved external and internal microfeatures. The areas with the highest fidelity of preservation present smaller, and more closely-packed crystals when compared to the less-preserved parts. We also recovered microscopic features that suggest the presence of microbial mats during the fossilization process. The studied Crato specimens are preserved by the replacement of the cuticle and of internal organs (e.g., proventriculi) by iron oxides after pyritization. Sulphur occurs scattered in some regions of the proventriculi, but associated with low iron counts, that may indicate the presence of sulphates after pyrite oxidation at the Crato insects. Additionally, the cricket proventriculi have calcium phosphate preserving some of its areas. In contrast with Crato insects, the Solnhofen fossils are preserved as imprints. In most examined fossils, their micron-scale morphology is obliterated by coarse mineral growth, whereas tissues are obliterated by calcite crystals alone or in combination with globular material. In the rare cases of handscale well-preserved fossil mayflies, there is a marked influence of Si, K, Ca, Ti, Mn, and Fe, in comparison to the host rock, that may be related to a yet unknown mineral phase(s). Solnhofen fossils are, in general, complete and fully articulated, but with no submicron-scale replication of both external and internal morphology. Although the palaeoenvironmental settings of the Crato Formation and the Solnhofen beds are different, there are many known similarities in the style of preservation of their vertebrates and similarities in some palaeoenvironmental conditions such as anoxic hypersaline bottom-water conditions and deposition of laminated limestones. However, we show that the same does not apply to the preservation of insects, specifically mayflies, which are considerably poorly preserved in the Solnhofen limestones.

Keywords. Paleoentomology, insect taphonomy, Araripe Basin, Altmühltal Formation, Ephemeroptera, Orthoptera.

Introduction

Most of the exceptionally-preserved insect biotas are restricted to the Cenozoic, with a variety of preservational modes occurring, like calcification, phosphatisation, silicification, and

specimens preserved as gypsum crystals (Leakey, 1952; Palmer, 1957; Duncan and Briggs, 1996; Duncan et al., 1998; McCobb et al., 1998; Schlüter et al., 2002; Parker and Mckenzie, 2003; Wappler and Ben-Dov, 2008; Schwermann et al., 2016). However, athough less frequent than in the Cenozoic, there are many important insect biotas in the Mesozoic. An example is the renowned Crato Formation of Brazil which, in most of its reported cases, preserves insects by phosphatization or pyritization (Dias and Carvalho, 2022). Calcium phosphate is commonly known to replicate soft-tissues since Precambrian times, and it is often associated with Lagerstätten (Wilby and Briggs, 1997; Martínez-Delclòs et al., 2004; Eriksson et al., 2012; Chen et al., 2014). The preservational fidelity in phosphatized fossils is achieved by calcium phosphate impregnation of soft tissues, attributed to bacterial precipitation, although it has been observed that arthropod carcasses can supply enough phosphate for mineralisation of their own tissues (Briggs et al., 1993; Martínez-Delclòs et al., 2004; Maas et al., 2006). Pyrite, however, is less frequent in comparison to other types of mineral replacements, and in most of the cases do not replicate soft tissues with the same fidely as calcium phosphate, with one of the exceptions being the Crato Formation fossils (Osés et al., 2016).

The Lower Cretaceous (Upper Aptian) Crato Formation is regarded as one of the most important Mesozoic Lagerstätten. It is widely accepted that this unit, known for its laminated limestones locally called "pedra cariri", represents a lacustrine past ecosystem. However, in regards of salinity of its past waters, there are contrasting hypotheses, either supporting a brackish or more saline paleolake (episodically and/or in certain strata of the water body), or an exclusive freshwater setting (Grimaldi, 1990; Martill and Wilby, 1993; Neumann et al., 2003; Martill et al., 2007; Martill et al., 2008; Heimhofer et al., 2010; Barling et al., 2015; 2020; 2021; Oliveira and Kellner, 2017; Warren et al., 2017; Varejão et al., 2019; Moura-Júnior et al., 2021; Ribeiro et al., 2021; Storari et al., 2021a).

This lithostratigraphic unit is of worldwide importance due to its outstanding fossil record, particularly preserving a great number of various insect taxa (Martins-Neto, 2005; Assine et al., 2014; Neumann and Assine, 2015). Several publications already explored the taphonomy of the entomofauna from the Crato Formation, with studies varying from general remarks (Martínez-Delclòs et al., 2004), to detailed preservational analyses, including studies with certain processes affecting the preservation of insects like the action of microbial mats or weathering (Menon and Martill, 2007; Barling et al., 2015; 2020; Osés et al., 2016; Prado et al., 2020; Bezerra et al. 2021;

Iniesto et al., 2021; Ribeiro et al., 2021; Dias and Carvalho, 2022; Bezerra et al., 2023). Detailed analyses including specific groups of insects are also available, as with cockroaches (Bezerra et al. al., 2018), crickets (Bezerra et al., 2020; Dias and Carvalho, 2020, Storari et al., 2024), odonatans (Nel and Pouillon, 2020; Pouillon and Nel, 2020; Barling et al., 2021), hemipterans (Moura-Júnior et al., 2021), beetles (Santos et al., 2021), and mayflies (Staniczek et al., 2022; Storari et al., 2021a; Dias et al., 2023).

Like the Crato Formation, many other fossiliferous units with finely laminated carbonates also preserve a diverse fossil insect assemblage, examples are the Solnhofen limestones of Germany, the Las Hoyas and Montsec localities of Spain, and the Green River Formation of the USA (Bezerra et al., 2020). In particular, the Solnhofen limestones *sensu stricto* of the Upper Jurassic of southern Germany, state of Bavaria, is arguably one of the most famous fossil Lagerstätten due to the iconic theropod *Archaeopteryx* and represents a past marine environment (Barthel et al., 1990). Of the terrestrial fossils recovered there, insects are among the most diverse groups (Bechly, 2015), with eleven orders described so far, although some taxa still await further investigation and interpretation (Barthel et al., 1990). While excellent preservation of fossils is commonly reported for vertebrates, with soft parts such as the skin or feathers preserved (Frey et al., 2003; Tischlinger and Unwin, 2004), preservation of their insects is not well-studied, being discussed only in general bibliographies, or briefly cited either as examples of exceptional preservation or as poorly-preserved specimens (Ponomarenko, 1985; Barthel et al., 1990; Frickhinger, 1994; 1999; Martínez-Delclòs et al., 2004; Wang et al., 2010; Bechly, 2015).

The Crato Formation and the Solnhofen limestones are well-known deposits that, although differing in their paleoenvironmental settings, include exceptionally preserved fossils in laminated limestones, and interestingly vertebrates of both units share phosphatization as the main preservation type of their soft tissues (Martínez-Delclòs et al. 2004; Fielding et al., 2005; Gobbo and Bertini, 2013; Warren et al., 2017; Varejão et al., 2019). Thus, we sought to analyze if the invertebrates from these deposits also share similar modes of preservation. We studied primarily insects of the Crato Formation, focusing on two abundant aquatic and terrestrial groups of this unit, Ephemeroptera and Orthoptera. For the comparison, we studied in detail the modes of preservation of mayflies from the Solnhofen limestones.

Material and Methods

1. Geological setting

1.1. Crato Formation

The Araripe Basin is an intracratonic basin located in northeast Brazil, covering the states of Piauí, Pernambuco, and Ceará (Saraiva et al., 2021) (Figure 1). The base of this area is of Precambrian age, which were affected over time by extensive rifting processes resulting from the split between Africa and South America during the break-up of Pangaea (Matos, 1992; Assine, 2007). The Araripe Basin is composed of several formations, the oldest is the Cariri Formation, from Late Ordovician/Early Devonian (*sensu* Beurlen, 1962). Followed by the Mesozoic strata of the basin, Brejo Santo and Missão Velha Formations from the Upper Jurassic (pre-rift sequence), Abaiara Formation from Lower Cretaceous (rift sequence) (Assine et al., 2014). The next Lower Cretaceous deposits of the Basin are collectively known as the Santana Group (post-rift I sequence) (Figure 1). Finally, the basin cycle ends with its post-rift II sequence of Araripina and Exu Formations (Assine et al., 2014).

The Santana Group is subdivided, from bottom to top, into the Barbalha (pelites and sandstones), Crato (pelites and carbonates), Ipubi (evaporites), and Romualdo (carbonate concretions) Formations (Neumann and Cabrera, 1999; Assine et al., 2014). The Crato Formation is interpreted as Late Aptian based on biostratigraphic data (Arai and Assine, 2020; Melo et al., 2020; Coimbra and Freire, 2021; Varejão et al., 2021). It is a ca. 90 m thick succession formed by horizontal strata of micritic limestone that are interbedded with shales, siltstones, and sandstones (Neumann and Cabrera, 1999; Castro et al., 2007; Assine et al., 2014; Catto et al., 2016). Most of its outcrops are exposed in commercial quarries, particularly between the Nova Olinda and Santana do Cariri municipalities, as well as on the Batateiras River banks, all located in the state of Ceará (Viana and Neumann, 2002) (Figure 1). Due to the absence of bioturbation and true marine fossils, there is strong indication that the Crato Formation strata were deposited under calm lacustrine conditions (Neumann et al., 2003; Heimhofer et al., 2010; Varejão et al., 2021; Ribeiro et al., 2021), though the presence of halite pseudomorphs and stromatolites in some sections suggests that the basin experienced salinity variations with increased arid and evaporitic conditions (Martill et al., 2007; Heimhofer et al., 2010; Warren et al., 2017; Storari et al., 2021a; Varejão et al., 2021).

The Crato Formation is divided into four Members, namely, Nova Olinda, Caldas, Jamacaru and Casa de Pedra, from bottom to top. The Nova Olinda Member holds the well-preserved fossils of the Formation (Assine, 2007), and is where the fossil insects from this study were found. Additionally to this traditional grouping in Members, the strata of the Crato Formation have received several classifications. Neumann et al. (2003) divided its carbonate facies into two sub-facies: clay-carbonate rhythmites and laminated limestones. Often, the first yields dark-gray-colored layers, while the latter corresponds to yellow-colored layers. More recently, Varejão et al. (2021) discussed that the Crato Formation encompasses six facies associations (FA-3 to FA-8), in which the exceptionally preserved fossils occur at the facies association 4 (FA-4), with insects usually found in its upper layers (Varejão et al., 2021). A previous classification by Varejão et al. (2019) pointed out that the stratigraphic position of most insects is a layer called Interval III, consisting of the upper 2-meter part of the Lagerstätte succession (Nova Olinda Member). Finally, the Crato Formation has also been divided into six carbonate intervals, designated C1 to C6 by Neumann and Cabrera (1999), with C6 encompassing the Lagerstätte succession (Neumann and Cabrera, 1999; Storari et al., 2021a).

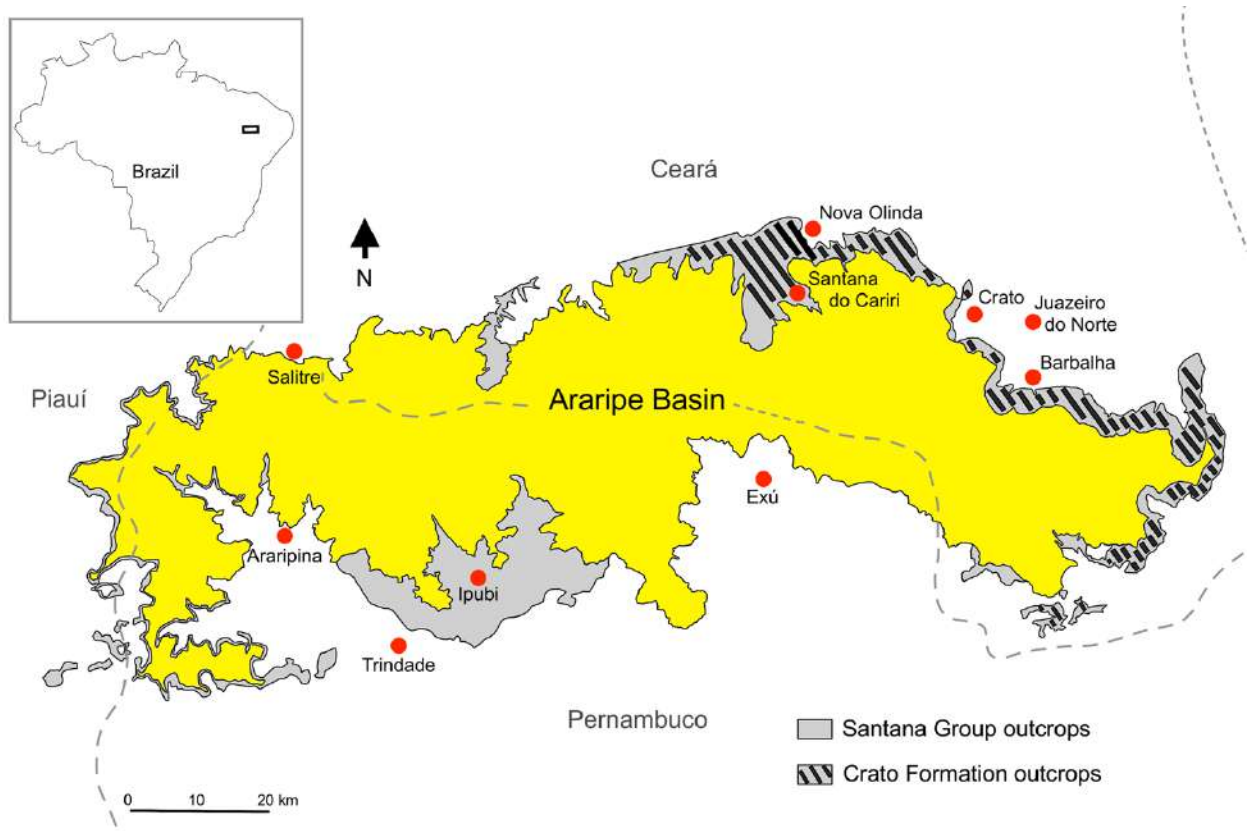

Figure 1. Crato Formation locality map. Locality map of the Araripe Basin and outcrops of the Santana Group and the Crato Formation within Brazil (modified from Storari et al., 2020; Lima et al., 2021).

1.2. Solnhofen limestones

The Franconian Alb (Baviera state) and Swabian Alb (Baden-Württemberg state) of Germany form a low mountain range extending from southwest to northeast in southern Germany and is composed of Lower to Upper Jurassic marine sedimentary rocks (Viohl, 2015) (Figure 2). The fossil sites of the Franconian Alb belong to five different Formations, namely, the Torleite, Geisental, Painten, Altmühltal and Mörnsheim, from oldest to youngest, ranging from the late Kimmeridgian to the early Tithonian (Schweigert, 2015). These Formation compose the Weißjura Group, a package of mainly calcareous marine sediments (Röper, 2005). Most fossils historically reported from these rocks come from the Altmühltal Formation (*sensu* Niebuhr and Pürner, 2014), however, fossils have also been recovered from the underlying Torleite and the overlying Mörnsheim formations (e.g., Tischlinger, 2001). They are often collectively called 'Solnhofen

limestones', but the Solnhofen limestones *sensu stricto* are included only in the Altmühltal Formation and are restricted to the area northwest of the municipality of Ingolstadt, more commonly from areas of the municipalities of Solnhofen and Eichstätt, deposited during the Tithonian (Niebuhr and Pürner, 2014) (Figure 2). The Altmühltal Formation is biostratigraphically part of the Hybonotum Zone (Riedense and Rueppelianus subzones) (Barthel, 1978; Schweigert, 2007; Niebuhr and Pürner, 2014; Schweigert, 2015). To date, most insects were recovered from the areas around Eichstätt, and more rarely from the Solnhofen area or the rest of the Franconian Alb (Bechly, 2015). From now on, every time we mention Solnhofen limestones in the text, we refer to the Solnhofen limestones *sensu stricto*, i.e., the Altmühltal Formation.

The Solnhofen limestones are divided into two types of rock, called Flinz and Fäule, that intercalate somehow cyclically (Munnecke et al., 2008). The fine-grained, hard limestone and putatively micritic Flinz beds show carbonate contents of more than 96% (Bausch et al., 1994; Bausch, 2004; Munnecke et al., 2008). They are intercalated by softer interlayers (Fäule) that present a foliaceous appearance and are slightly less rich in calcium carbonate (usually under 85%; Bausch 2004). The Fäule beds are also laminated, although the laminae are thinner compared with the Flinz beds (Park and Fürsich, 2002).

All sedimentary models proposed for the Solnhofen limestones agree that they were deposited in individual marine basins at the northern margin of the Tethys Sea, with the depositional setting composed of relatively shallow waters isolated from the open ocean by coral reefs (Keupp, 1977; Viohl, 1990). It has been suggested that the water column of the depositional setting was stratified, with hypersaline bottom waters and an anoxic sea floor (Barthel, 1978; Barthel et al., 1990). According to the latest depositional model published, proposed by Viohl (2015), tropical storms led to episodic mixing of these hypersaline, anoxic bottom waters with the oxygenated surface waters, which caused the death of nektonic and planktonic organisms.

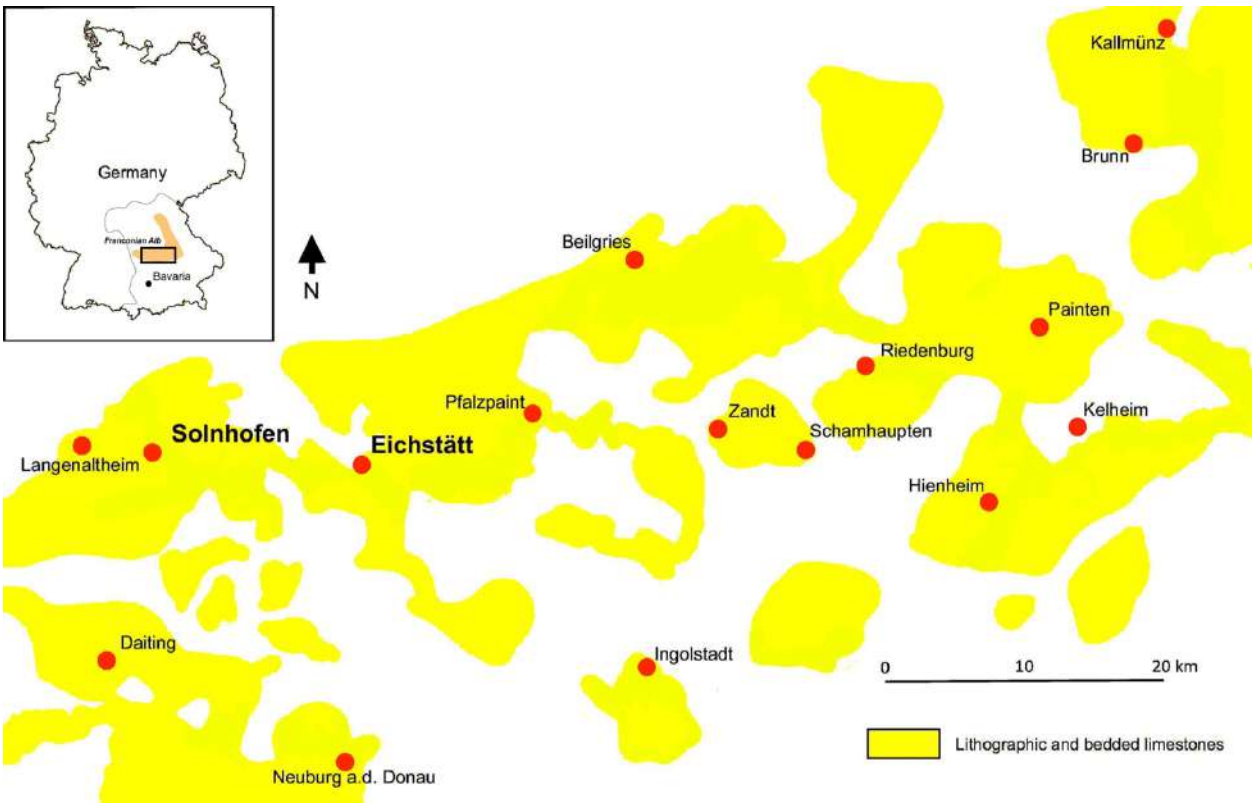

Figure 2. Solnhofen limestones locality map. Map of the southern Franconian Alb with localities of the Upper Kimmeridgian and Lower Tithonian limestones deposits from the Solnhofen Archipelago (modified after Röper and Rothgaenger, 1995; Hess, 1999; and Heyng et al., 2015).

2. Material

2.1. Crato Formation

We analyzed a total of 222 fossil mayflies (192 larvae and 30 adults of Hexagenitidae: Ephemeroptera) and 12 Grylloidea (Ensifera: Orthoptera) from the Crato Formation. The specimens analyzed are housed in the following Brazilian institutions: Museu de Paleontologia Plácido Cidade Nuvens, Universidade Regional do Cariri, Santana do Cariri (MPPCN); Paleontological collection of the Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão (CAV); Paleontological collection of the Universidade Regional do Cariri, Crato (LPU); Scientific Paleontological Collection of the Instituto de Geociências of the Universidade de São Paulo, São Paulo (GP) (Table 1).

From the 222 fossil mayflies, 124 unnumbered larval specimens were collected during controlled excavations at the Mine Antônio Finelon (S 07° 07' 22,5" e W 39° 42' 01"), Nova Olinda,

Ceará State, Brazil (Table 1). The orthopteran CAV 0012 was collected in 2009 during a field trip of biology students of the Centro Acadêmico de Vitória (Universidade Federal de Pernambuco) in a well-known limestone mine, Mina do Demar, on the road that connects Nova Olinda and Santana do Cariri municipalities, Ceará state. The LPU specimens lack exact locality information as they have been collected by mine workers and donated to that collection. The GP specimens also lack exact locality information because they were recovered by the Brazilian federal police during an operation against fossil smuggling. Although those specimens lack exact locality details, all of them were probably collected from upper portions of the FA-4 layer (*sensu* Varejão et al., 2021) or layer C6 (*sensu* Neumann and Cabrera, 1999) from the Lagerstätte portion of the Nova Olinda Member.

Table 1. Specimens from the Crato Formation analyzed. 'n/a' means that the exact locality of the specimen sampling within the Crato Formation is unknown. Gray cells represent the orthopteran specimens studied.

Identification	Ontogeny	Locality	Specimen(s)	Reference
Araripegryllus cf. *femininus*	Adult	Mina do Demar, Nova Olinda	CAV 0012	Analyzed in person at LPU
Cearagryllus sp. Indet	Adult	n/a	LPU P6	Analyzed in person at LPU
Costalimella zucchii	Adult	n/a	LP/UFC CRT 1276 (holotype)	Brandão et al. (2021)
Cratohexagenites longicercus	Adult	n/a	MSF O46	Staniczek (2007)
Cratohexagenites longicercus	Larva	n/a	MURJ 447 (holotype)	Staniczek (2007)
Cratohexagenites minor	Larva	n/a	MB.I.2026 (holotype)	Staniczek (2007)
Gryllidae *indet.*	Adult	n/a	LPU P2	Analyzed in person at LPU

Grylloidea *indet.*	Adult	n/a	GP1E 7268; GP1E 7409; GP1E 8691; GP1E 8827; GP1E 8910; LPU 1196; LPU P1; LPU P3; LPU P4	Analyzed in person at the IG-USP and LPU
Hexagenitidae *incertae sedis*	Adult	n/a	LPU 1144, MPPCN I 763, MPPCN I 1559	Analyzed in person at the MPPCN
Protoligoneuria heloisae	Adult	n/a	AMNH 43499 (holotype)	Storari et al. (2021b)
Protoligoneuria limai	Adult	n/a	GP/1E 6763, GP/1E 6764, GP/1E 6766, GP/1E 6884, GP/1E 7221, GP/1E 8754, GP/1E 9034, GP/1E 9562, LPU 1696, MPPCN I 1559, MPPCN I 1631, MPPCN I 409, MPPCN I 4286cp, MPPCN I 4286p, MPPCN I 4287, MPPCN I 4313, MPPCN I 4422, MPPCN I 456, MPPCN I 469, MPPCN I 472	Analyzed in person at the IG-USP, and MPPCN
Protoligoneuria limai	Adult	n/a	RGMN T002, RGMN T004, RGMN T005, SMNS 66635, SMNS 70312	Martins-Neto (1996); Staniczek (2007)
Protoligoneuria limai	Larva	Mina Antonio Finelon, Nova Olinda	124 unnumbered specimens	Analyzed in person at the MPPCN
Protoligoneuria limai	Larva	n/a	AMNH 43404, AMNH 43415, AMNH 43418, AMNH 43435, AMNH 43452, AMNH 43455, AMNH 43469, DGM 6255, DGM 6256, DGM 6277, LPRP/USP 0583, RGMN T001, RGMN T003, RGMN T006, SMNS 66537	Brito (1987); McCafferty (1990); Martins-Neto (1996); Staniczek (2007); Brandão et al. (2021)

Protoligoneuria limai	Larva	n/a	GP/1T 2583, LPU 1141a, LPU 1141b, LPU 1647, LPU 1698, LPU-EC03, MPPCN I 1336, MPPCN I 1354, MPPCN I 1368, MPPCN I 1439, MPPCN I 2309, MPPCN I 2503, MPPCN I 2504, MPPCN I 2505, MPPCN I 2506, MPPCN I 2507, MPPCN I 2508, MPPCN I 2509, MPPCN I 2510, MPPCN I 2511, MPPCN I 2512, MPPCN I 2513, MPPCN I 2514, MPPCN I 2515, MPPCN I 2516, MPPCN I 2519, MPPCN I 2521, MPPCN I 2522, MPPCN I 2524, MPPCN I 2525, MPPCN I 2526, MPPCN I 2528, MPPCN I 2529, MPPCN I 253, MPPCN I 2531, MPPCN I 2532, MPPCN I 2533, MPPCN I 2536, MPPCN I 2537, MPPCN I 289, MPPCN I 293, MPPCN I 300, MPPCN I 301, MPPCN I 310, MPPCN I 770, MPPCN I 946, MPPCN I 5227, MPPCN I 5229, MPPCN I 5230	Analyzed in person at the IG-USP, and MPPCN

2.2. Solnhofen limestones

Most German collections that house mayflies from the Solnhofen limestones were visited to reduce bias in data collection, including already published material. The following institutions were visited: Bayerische Staatssammlung für Paläontologie und historische Geologie, Munich (SNSB-BSPG); Bürgermeister-Müller-Museum, Solnhofen (BMMS); Jura Museum, Eichstätt (JME); Museum Bergér, Eichstätt (MB); Seckenberg Research Institute and Museum, Frankfurt (SMF); Staatliches Museum für Naturkunde, Karlsruhe (SMNK); and Staatliches Museum für Naturkunde, Stuttgart (SMNS).

The localities of the fossils analyzed here (when available) are quarries in Eichstätt, but most lack stratigraphic information. All the 20 fossils from the Bérger Museum come from a quarry in Blumenberg, Eichstätt (stratigraphically from the Riedense Subzone; *eigeltingense* Horizon, Schweigert, 2015) (Table 2). We infer that the analyzed specimens come from Flinz layers based on the characteristics of their limestone slabs (see Results section), but more precise stratigraphic placement is unavailable as original sampling information is lost. We analyzed only winged individuals since mayfly larvae were never reported for the Solnhofen limestones.

Table 2. Winged mayfly specimens from the Solnhofen limestones biostratinomically analyzed and their available locality information.

Identification	**Locality**	**Specimen(s)**
Ephemeroptera inc. sed.	Eichstätt	JME 1748, JME 4675, JME 2054, JME 3668a, JME 3668b
Ephemeroptera inc. sed.	n/a	JME 1747, JME 3703a, JME 3703b, SMNK 2123, SMNK 2850, SMNK 2851, SMNK-PAL 45775
Hexagenites cellulosus	Eichstätt	BSPG AS I 748 (holotype)
Hexagenites sp.	Eichstätt	SMNS 70310
Hexagenites sp.	Eichstätt, Blumenberg quarry of the Bérger family	MB2021.10.151, MB2021.10.152a, MB2021.10.152b, MB2021.10.153, MB2021.10.154, MB2021.10.155, MB2021.10.156, MB2021.10.157a, MB2021.10.157b, MB2021.10.158, MB2021.10.159, MB2021.10.160, MB2021.10.161, MB2021.10.162, MB2021.10.163, MB2021.10.164a, MB2021.10.164b, MB2021.10.165, MB2021.10.166
Hexagenites sp.	n/a	BMMS 369, BMMS 53/72a, BMMS 53/72b, BMMS A, BMMS B, BMMS 2020, JME 2327, SMF VI 1362, SMNS 70311A, SMNS 70311B
Hexagenites multinervosa	n/a	BSPG 1964 XXIII 589 a, BSPG 1964 XXIII 589 b

Identification	**Locality**	**Specimen(s)**
Ephemeroptera inc. sed.	Eichstätt	JME 1748, JME 4675, JME 2054, JME 3668a, JME 3668b
Ephemeroptera inc. sed.	n/a	JME 1747, JME 3703a, JME 3703b, SMNK 2123, SMNK 2850, SMNK 2851, SMNK-PAL 45775
Hexagenites cellulosus	Eichstätt	BSPG AS I 748 (holotype)
Mesephemera procera	Eichstätt	BSPG AS I 1027 (holotype), JME 1728, JME 1736a, JME 1736b, JME 1738, JME 1742, JME 1743, JME 3691a, JME 3691b, JME 4672
Mesephemera procera	n/a	BSPG 1964 XXIII 3a, BSPG 1964 XXIII 3b
Mesephemera lithophila	Eichstätt	BSPG AS VII 497 (holotype)
Mesephemera speciosa	Eichstätt	BSPG 1882 XVI 30 (holotype), BSPG AS I 808, BSPG AS I 809, JME 1745
Mesephemera sp.	Eichstätt	BSPG 1965 III 20, JME 4673, JME 4674
Mesephemera sp.	n/a	BSPG 55229
Mesephemera ? prisca	Eichstätt	BSPG AS V 37
Olgisca mortua	Eichstätt	JME 1744
Olgisca schwertschlageri	n/a	JME 1746
Pedephemera multinervosa	Eichstätt	BSPG 1961 III 54
Pedephemera multinervosa	n/a	BSPG 1961 I 467a, BSPG 1961 I 467b, BSPG 1964 XXIII 1, BSPG 1964 XXIII 2a, BSPG 1964 XXIII 2b, BSPG 1964 XXIII 3, BSPG 1972 XX 12, BSPG 1972 XX 13
Paedephemera schwertschlageri	Eichstätt	JME 1750a, JME 1750b, JME 1756
Paedephemera speciosa	Eichstätt	JME 2056

Identification	**Locality**	**Specimen(s)**
Ephemeroptera inc. sed.	Eichstätt	JME 1748, JME 4675, JME 2054, JME 3668a, JME 3668b
Ephemeroptera inc. sed.	n/a	JME 1747, JME 3703a, JME 3703b, SMNK 2123, SMNK 2850, SMNK 2851, SMNK-PAL 45775
Hexagenites cellulosus	Eichstätt	BSPG AS I 748 (holotype)
Paedephemera sp.	n/a	BSPG 1964 XXIIIa, BSPG 1964 XXIIIb

3. Methods

We analyzed 222 mayflies from the Crato Formation and 110 winged mayfly specimens from the Solnhofen limestones. Ten specimens from the Crato Formation were subjected to analytical analyses using scanning electron microscopy coupled to energy-dispersive X-ray spectroscopy (SEM-EDS), microenergy-dispersive X-ray fluorescence (μEDXRF), and μRaman spectroscopy (six mayflies: MPSC I 763, MPSC I 2533, MPSC I 5224, MPSC I 5227, MPSC I 5229, MPSC I 5230; and three crickets: CAV 0012, LPU P6, GP1E 8910) (Table 1, Figure 3). Five specimens from the Solnhofen limestones were also taken for SEM-EDS and μEDXRF analyses under the following inventory numbers: SMNS 70310; SMNS 70311a; SMNS 70311b; MB2021.10.155; MB2021.10.163; and MB2021.10.164a (Figure 4).

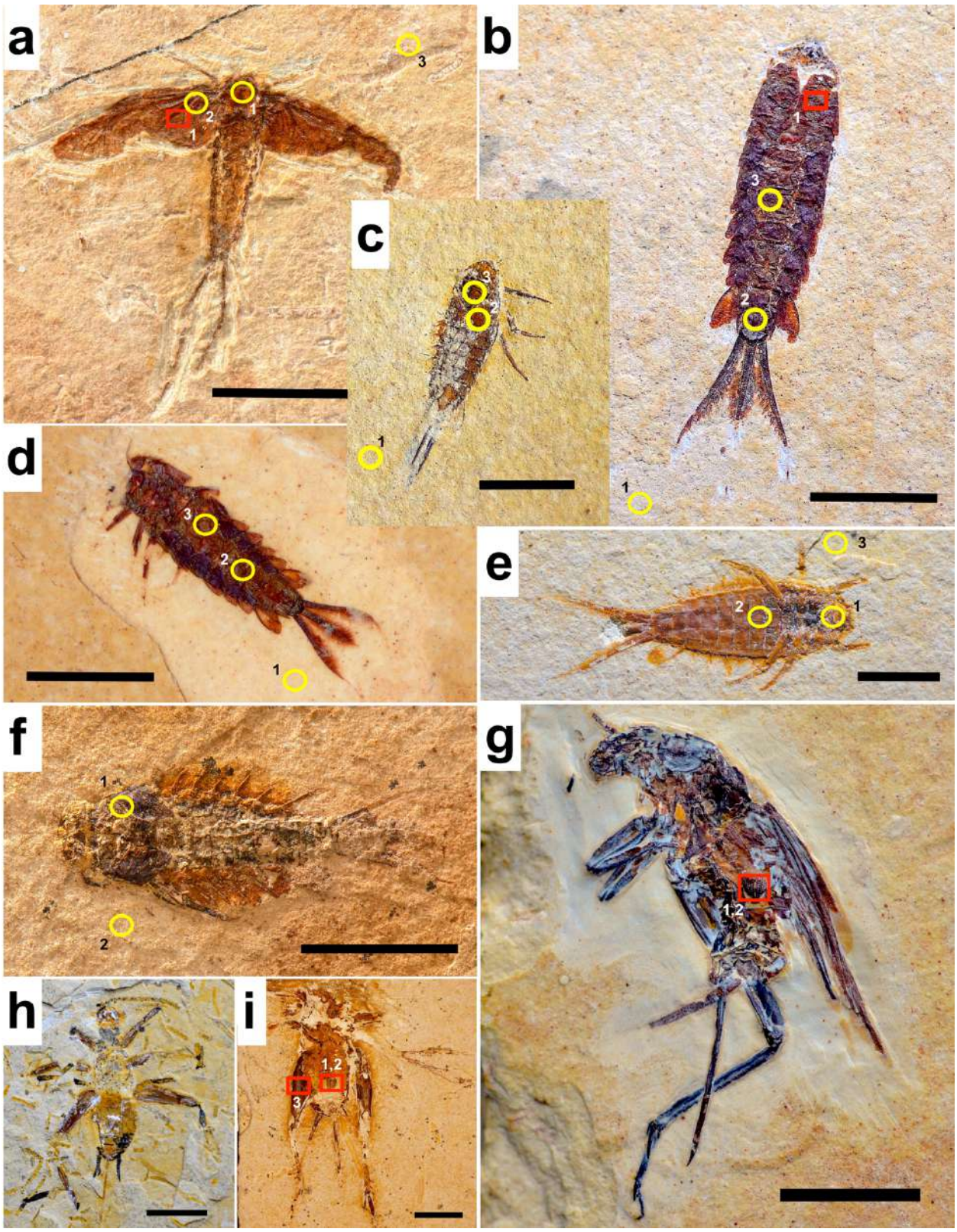
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Figure 3. Crato insects examined under analytical techniques. Yellow circles refer to the locality of EDXRF points analyzed shown in Figures 13 and SMb. Red rectangles refer to the locality of the points analyzed under Raman spectroscopy shown in Figures 14 and SMc. a) Winged Hexagenitidae mayfly MPSC I 763. Scale bar 10 mm. b) Larval *Protoligoneuria limai* MPSC I 5229. Scale bar 5 mm. c) Larval *P. limai* MPSC I 5224. Scale bar 5 mm. d) Larval *P. limai* MPSC I 5227. Point 3 = P13 of Fig 13. Scale bar 5 mm. e) Larval *P. limai* MPSC I 5230. Point 1 = P14 of Fig 13. Scale bar 5 mm. f) Larval *P. limai* MPSC I 2533. Scale bar 5 mm. g) Adult *Araripegryllus femininus* CAV 0012. Scale bar 5 mm. h) Adult *Cearagryllus* LPU P6. Scale bar 10 mm. i) Adult Grylloidea GP1E 8910. Scale bar 5 mm.

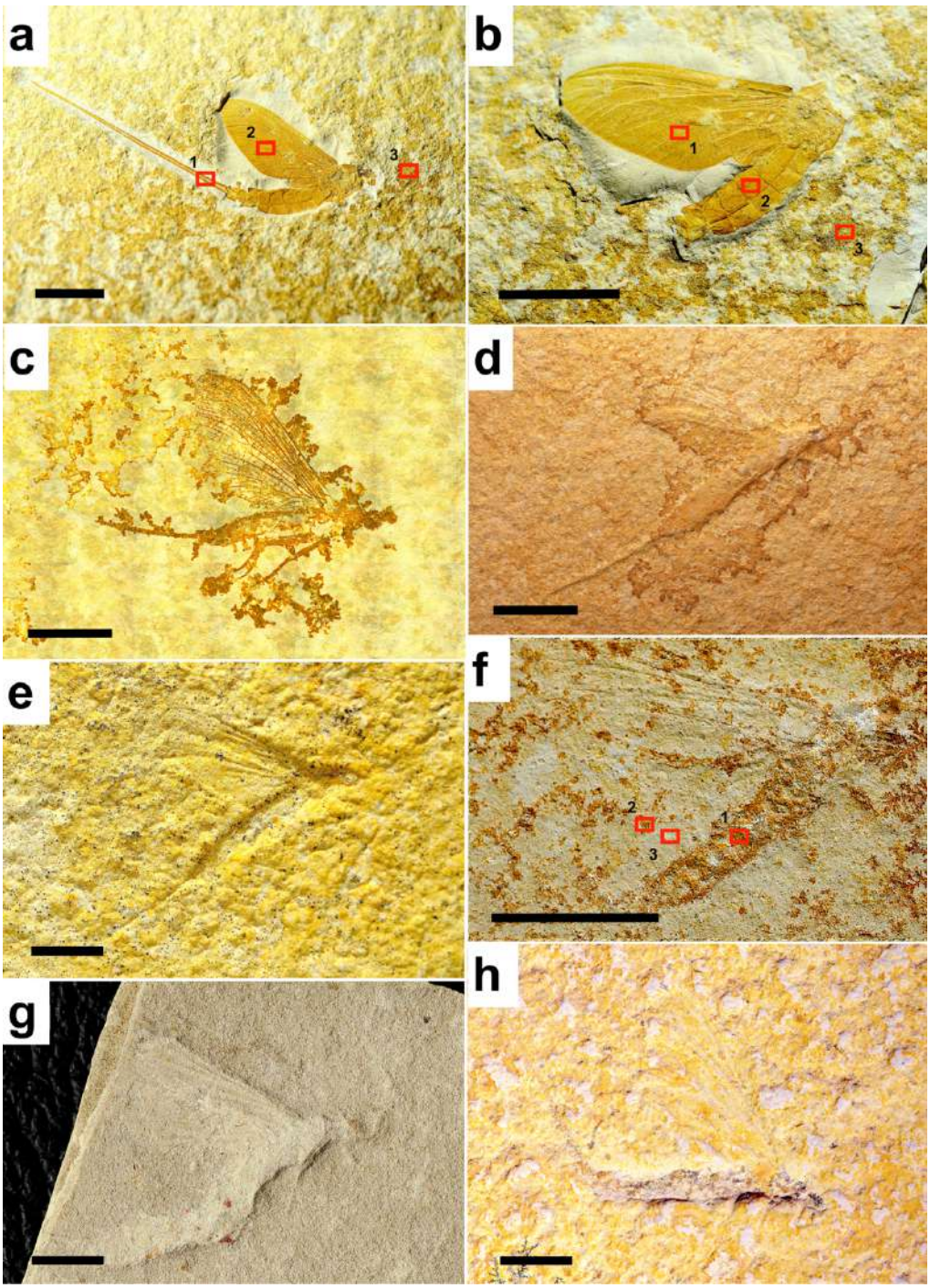


Figure 4. Solnhofen mayflies examined under analytical and biostratinomic analyses. a) *Hexagenites* SMNS 70311a; b) *Hexagenites* SMNS 70311b; c) *Hexagenites* SMNS 70310; d) *Hexagenites* BMMS 369; e) *Paedephemera* SNSB 1964 XXIII; f) *Hexagenites* MB2021.10.155; g) *Hexagenites* SMF VI 1362; h) Ephemeroptera *inc. sed.* JME 2054. Scale bars 10 mm. Red rectangles refer to the locality of the EDS point spectra shown in Figure 16.

Micro morphological and elemental analyses of the fossils of the Crato Formation were conducted using a scanning electron microscope (SEM) FEI Quanta 250 with an Oxford Si(Li) energy-dispersive X-ray spectroscopy (EDS) detector coupled to the Oxford AZTec software at the Instituto de Geociências, Universidade de São Paulo, Brazil. Micro morphological and elemental analyses of the fossils of the Solnhofen limestones were conducted using a scanning electron microscope JEOL JSM-6500F equipped with an Oxford INCA Energy 200 EDS system with a crystal type 300 (energy resolution 133eV), at the Center for Light-Matter Interaction, Sensors & Analytics (LISA+), at the University of Tübingen, Germany. EDS point and mapping spectra were employed to highlight qualitative elemental heterogeneities in different regions (mostly due to topography differences, as specimens are presented as a flat surface with topographic irregularities), thus results obtained with EDS were considered in a qualitative approach only (Osés et al., 2016).

Energy-dispersive X-ray fluorescence (EDXRF) analyses were performed for rapid characterization of heavier and trace elements, to complement EDS measurements, since EDS detects better light elements (Pan et al., 2019). EDXRF analyses of the Crato Formation specimens were performed at the Instituto de Física, Universidade de São Paulo, Brazil. The portable equipment used consists of a mini Amptek X-ray tube of Ag anode and an Amptek fast Silicon Drift Detector (SDD) of 125 eV FWHM for the 5.9 keV line of Mn. Measurements were carried out with 30 kV voltage and 30 μA of tube current and with an excitation/detection time of 200 s (Barling et al., 2015; Osés et al., 2016; Osés et al., 2017). Data was processed in the softwares WinQxas, Spectragryph (Menges, 2022), Excel, and Inkscape. Solnhofen limestone specimens were analyzed in a different equipment at the Mineralogical and Geochemical Micro-Analytical Laboratory (MAGMA Lab), Department of Applied Geochemistry, Technische Universität Berlin, Germany. μEDXRF analyses were made using the "area" mode of a Bruker Tornado M4 micro-XRF. Acceleration voltage was 50 kV using a beam current of 600 μA. Since Solnhofen fossils are more challenging for analyzing, after several tests with specimens, we choose to work with a higher voltage. The measuring point distance was 20μm at 20μm beam diameter. The measuring time was 30 ms per analysis spot. To obtain more precise data from a stronger signal, the analyses were run with two simultaneously operating spectrometers.

The mineralogical composition of the Crato Formation fossils was analyzed by Raman spectroscopy at the Instituto de Química, Universidade de São Paulo, Brazil, using a micro-Raman inVia Renishaw, coupled to a confocal light microscope. The excitation laser used for the measurements was of 785 nm wavelength. It was used a x50 lens, 10s and 20s of exposure time, laser power of 1% and 5%, and 5-40 accumulations. The calibration was performed with the Si band at 520.7 cm-1 (ASTM International, 2002). The data processing of the Raman spectra was made using the softwares Spectragryph (Menges, 2022), and Inkscape. The obtained spectra were identified by comparison to the RRUFF project database (Lafuente et al., 2015).

Results

1. Crato Formation

1.1. Exceptional preservation of the Crato Formation insects

All the 124 mayfly specimens recovered from controlled excavations are preserved in yellowish limestones, with the fossils themselves displaying orange/rusty coloration (e.g., Figure 3). From those, the majority are preserved by replication (substitution) of the cuticle and tissues (86%; n=107), and only 14% of specimens are preserved as imprints (n=17).

SEM imaging of selected specimens reveals the preservation of fine details not discernible by the naked eye. External features include different microtextures of body segments (mayflies and crickets - specimens MPSC I 5227, MPSC I 5230, GP1E 8910), setation (mayflies - MPSC I 5227, MPSC I 5230), spines (mayflies - MPSC I 5227, MPSC I 5230), and spiracles (mayflies and crickets - MPSC I 5227, MPSC I 5230, GP1E 8910, LPU P6) (Figure 5). The observed internal features are interpreted as muscle fibers (mayfly MPSC I 5227), parts of larval mouthparts (mayfly MPSC I 5230), and parts of the inner gut, such as proventriculus (crickets - CAV 0012, GP1E 8910, LPU P6) (see also Storari et al., 2024) and crop (cricket specimen LPU P6) (Figure 5); as well as putative tracheal channels, and the morphology of gills (mayfly MPSC I 5227) (Figure 6a).

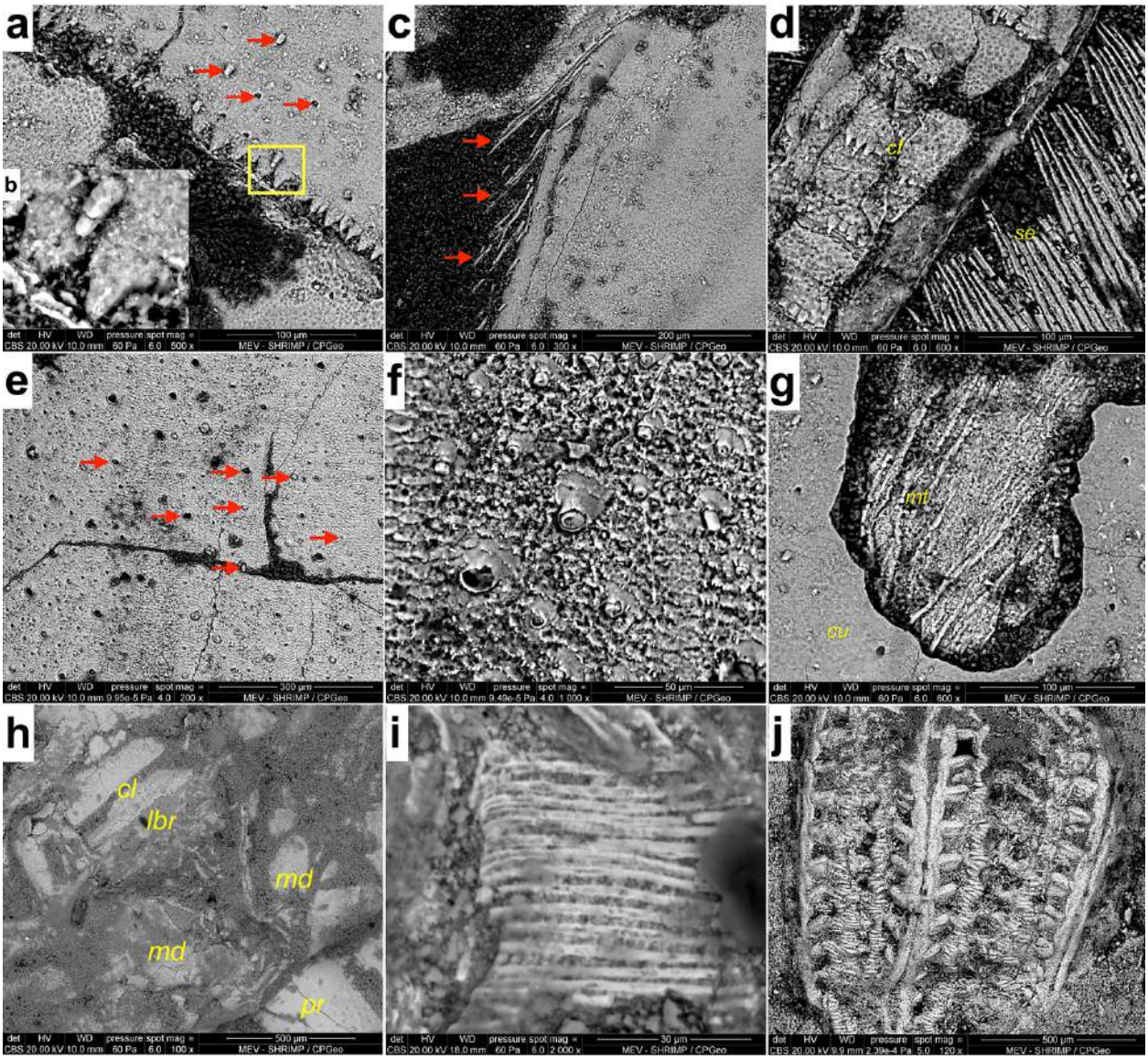


Figure 5. Exceptional preservation of morphological structures from the Crato Formation mayfly larva and crickets. a) Microtexture of the cuticle and serrated border of abdominal segments of specimen MPSC I 5227 (*Protoligoneuria limai* larva); arrows point to spiracles as well as delicate setae. b) Detail at higher magnification of delicate seta base delimited by the rectangle in 'a'. c) Setation on the last abdominal segments of the larva MPSC I 5230 (*P. limai*). d) Microtexture of the cuticle and serrated border of caudal filaments [cf] of the specimen in c; extensive setation [se] of caudal filaments laterally. e) Scale-like texture of cuticle from specimen GP1E 8910 (Grylloidea) showing several spiracles as well as base of sensilla (arrows). f) Detail of structures from 'e'. g) Internal muscle fibers [mf] evidenced after cracking of outer cuticle [cu] at the last segment of the abdomen of the mayfly larva MPSC I 5227. h) Mouth parts of larval mayfly

specimen MPSC I 5230; cl - clypeus; lbr labrum; md - mandible; pr - prosternum. i) Muscle fibers in the inner gut of *Araripegryllus* specimen CAV 0012. j) Proventriculus of Grylloidea specimen GP1E 8910.

We also observed wrinkle-like texture of the cuticle (Figure 6a). There is a clear differentiation between the fossil and its matrix (Figure 6b), as they are replicas of the original organisms. We also visualized a superficial thin film at some parts of the well-preserved tissues of some fossils (only in the SEM secondary mode – Figure 6c) that we interpret as an extracellular polymeric substance (EPS) of biofilm, mineralized (due to its carbon composition and morphology) coating over the fossil, obscuring the fabrics below (Barling et al., 2020), which in this case are scattered anhedral to subhedral crystals between rhombohedral crystals infilling cavities. While secondary electrons emit from a few nm in depth, backscattered electrons originate deeper in the sample, thus accounting for imaging of the calcite crystals underneath the shallow film. Some authors also call this surface, which occurs consistently along the specimen body, 'amorphous material', however, in some cases, it is only a thin layer (as seen here), and under which there is a carbonate matrix with abundant euhedral to subhedral calcite crystallites (Barling et al., 2020; Dias and Carvalho, 2020).

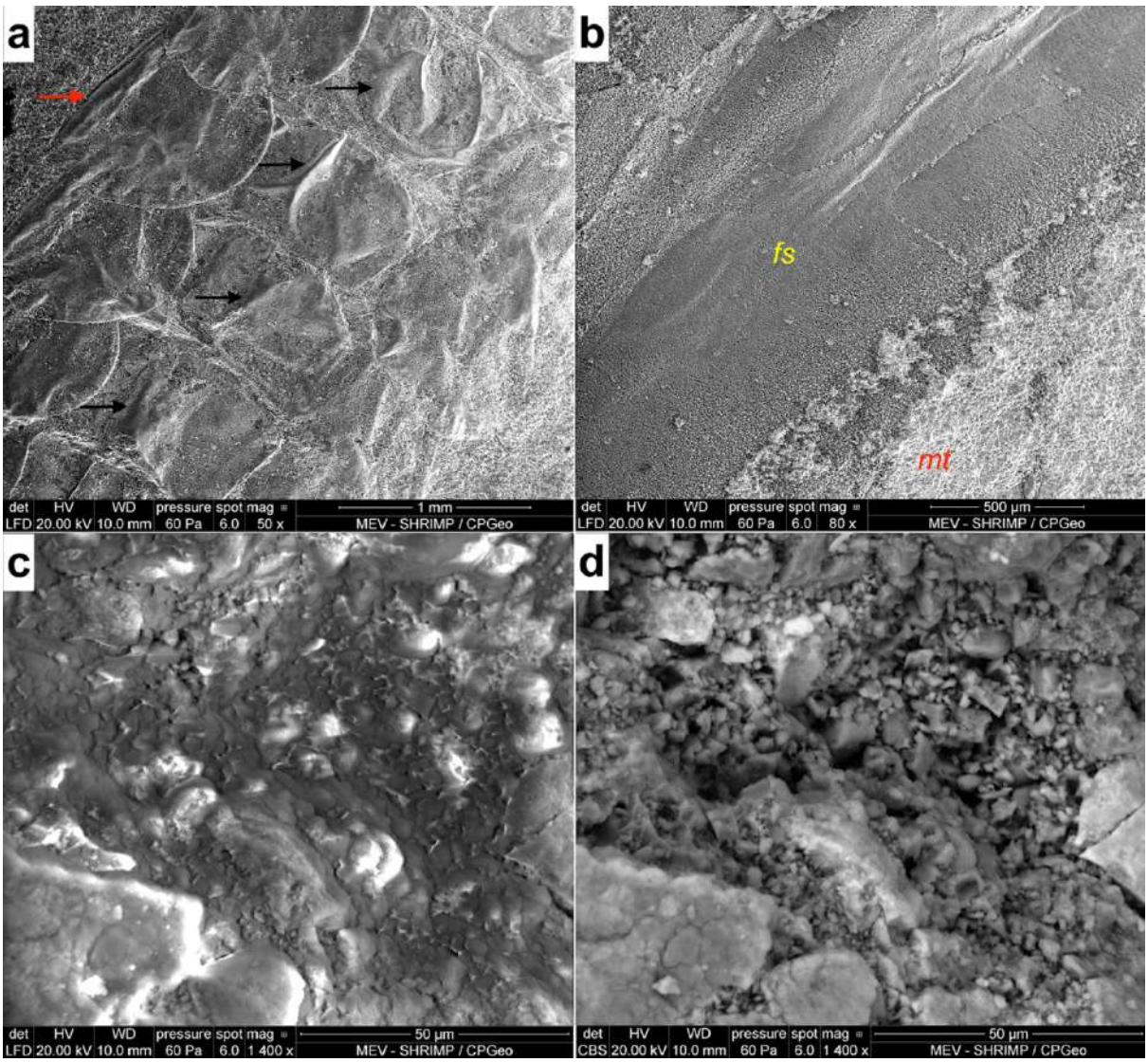
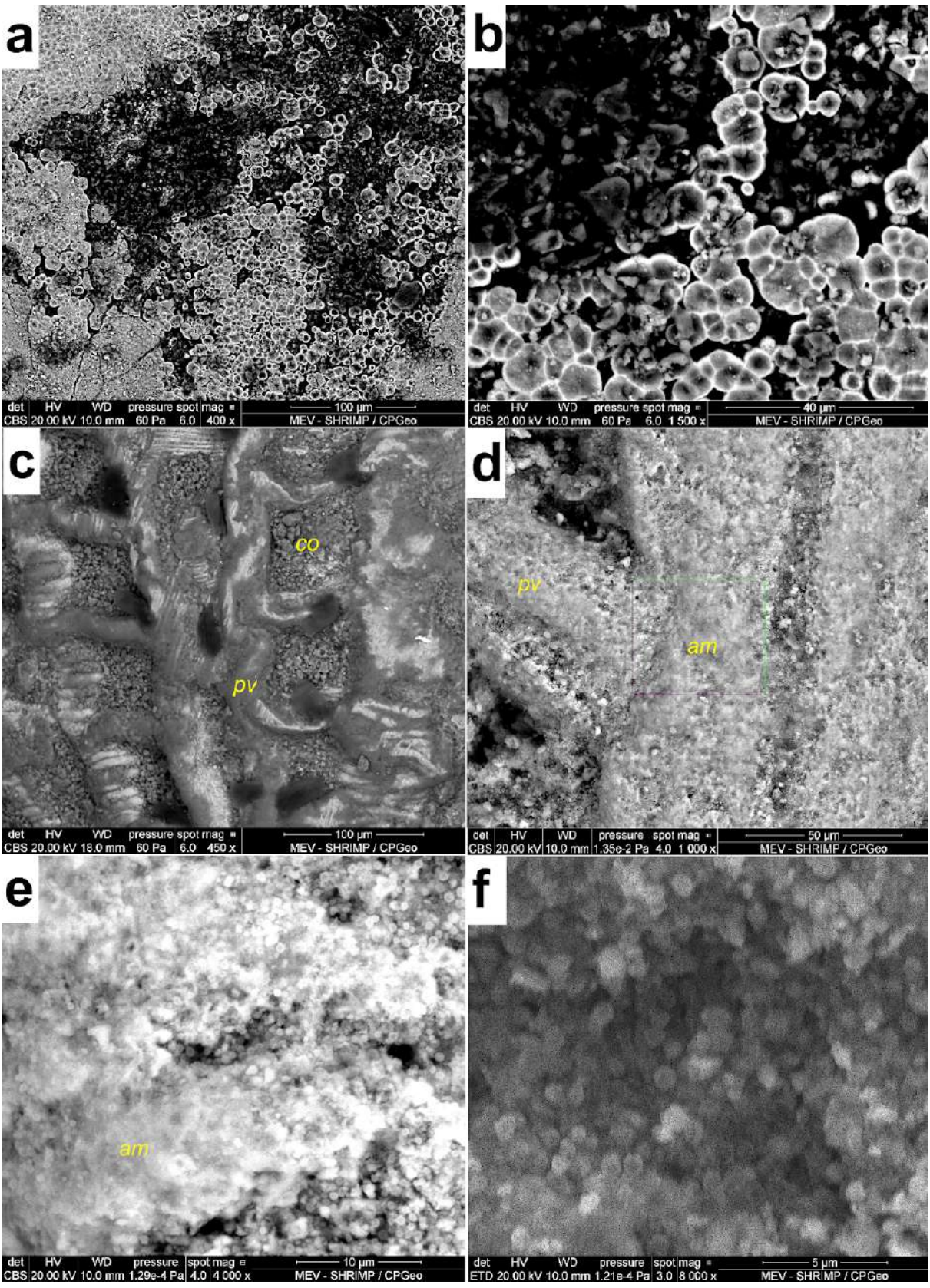


Figure 6. External structural features of preservation in mayfly larvae and orthopterans of the Crato Formation. a) larval *Protoligoneuria limai*, specimen MPSC I 5227 preserving wrinkle-like external texture of the cuticle in the abdominal terga (wrinkles indicated by black arrows); laterally the border of thin layer of gills and hard costal rib (red arrow) are also visible. b) Clear differentiation between fossil [fs] and matrix [mt], grylloid specimen LPU P6 (*Cearagryllus)*. c) Superficial film preserved at mouthparts of the mayfly larva MPSC I 5230 (*P. limai*). d) The same area of Figure 'c' showing scattered anhedral to subhedral crystals between rhombohedral crystals, underneath the film in 'c', which usually infill body cavities in the specimens analyzed.

1.2. Microfabrics of external cuticle and internal tissues

SEM analysis revealed that the exceptionally well-preserved parts of fossils are, often, preserved by sub-spherical to spherical closely-packed grains, which we identify here as pyrite framboids or pseudoframboids (Figures 7a,b; 8, based on elemental composition and morphology of grains - following Grimes et al., 2002; Barling et al., 2015; Osés et al., 2016). The size of the framboids differs between the external cuticle and internal tissues (Figures 7e,f), with the external cuticle ones presenting diameters in the range of 5.0–10.0 μm, while in inner tissues they range from 0.4 to 1.0 μm. There is a clear differentiation among the microfabrics of the external cuticle and of the internal organs: large framboids compose the external cuticle (Figures 7a,b) (as already illustrated by Osés et al., 2016; Barling et al., 2020; Bezerra et al., 2020; Dias and Carvalho, 2020), while the more rarely preserved inner organs (e.g., proventriculus) are formed by much smaller and more packed framboids; Figures 7e,f illustrate how the crystals are visible only between 4.000–8.000x of magnification in the inner tissue, while in the cuticle they are visible in a smaller magnification. We additionally observed, associated with the well-preserved inner tissues, a cemented agglutinated mass (or amorphous matter as referred by Dias and Carvalho, 2022), probably related to EPS, following recent interpretations of Dias and Carvalho (2022) (Figures 7d,e).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100 µm
MEV - SHRIMP / CPGeo
b
det HV WD pressure spot mag
CBS 20.00 kV 10.0 mm 60 Pa 6.0 1 500 x
40 µm
MEV - SHRIMP / CPGeo
c
co
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CBS 20.00 kV 18.0 mm 60 Pa 6.0 450 x
100 µm
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det HV WD pressure spot mag
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5 µm
MEV - SHRIMP / CPGeo

Figure 7. Microfabrics of external cuticle and internal tissues from insects of the Crato Formation. a,b) Pseudoframboids from external cuticle in different magnifications. c,d,e,f) Parts of proventriculi under different magnifications. c) Coating [co] of proventriculus with a random coarse mix of disorganized anhedral and euhedral crystals, together with the 3D well-preserved part of the proventriculus [pv]. d) A close-up of [pv] from figure 'c' showing the external microfabric texture of the proventriculus. e) Microfabric of 3D well-preserved proventriculus showing the aggregate of microframboids immerse in a mass [am] of unstructured form. f) Close-up of figure 'e' showing sub-spherical to spherical microframboids.

We also notice a gradient pattern in the external cuticle. The parts with the best morphological fidelity consist of closely-packed and not framboid-shape (less circular) crystals, arranged like a scale; and the less preserved the surface is, the more dispersed and rounded-framboid-like the crystals are (Figure 8), as well-portrayed in the literature (Barling et al., 2015; Osés et al., 2016; Barling et al., 2020; Bezerra et al., 2020; Dias and Carvalho, 2020).

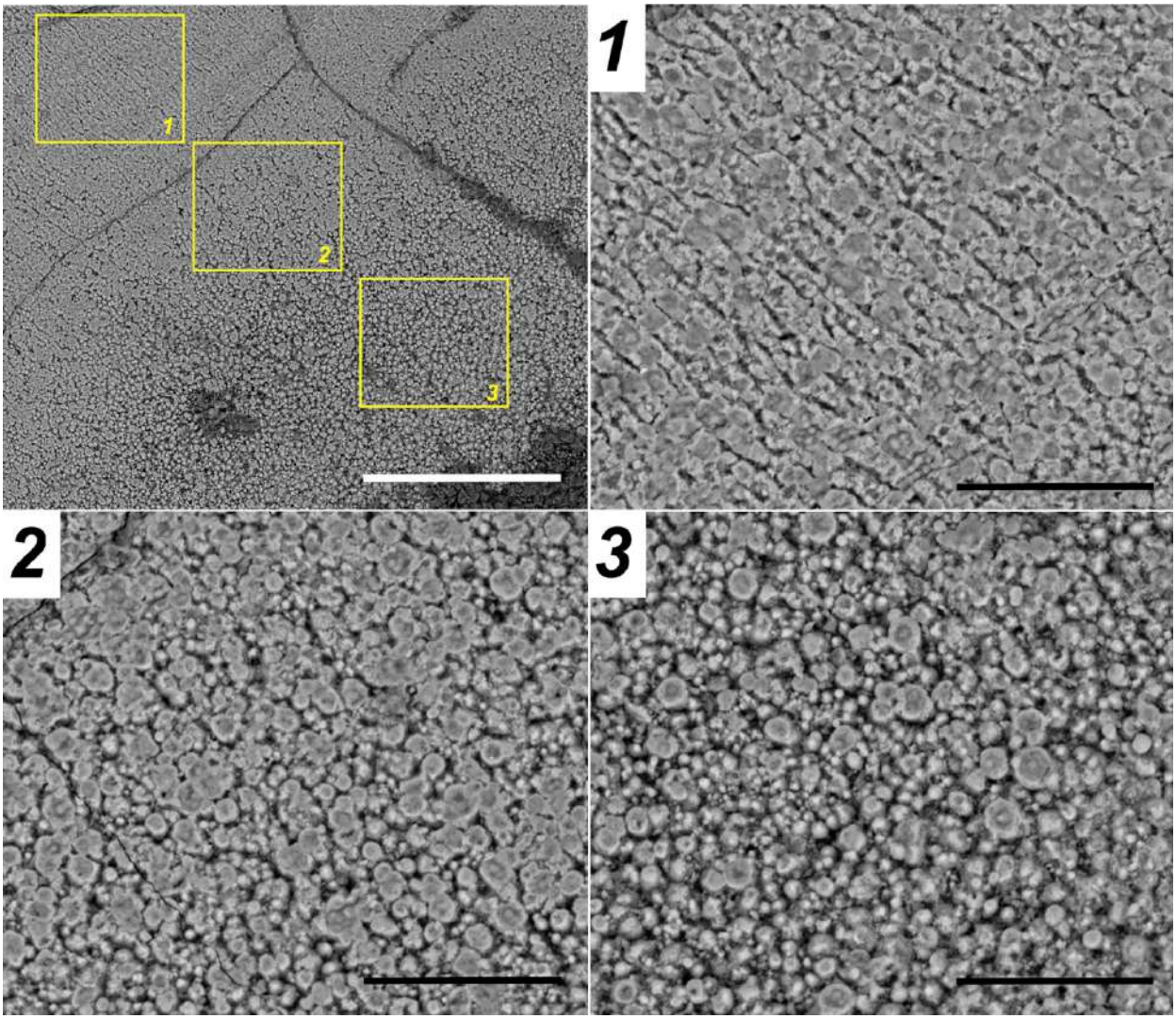


Figure 8. External cuticle gradient pattern from insects of the Crato Formation. External cuticle of the grylloid LPU P6 showing a gradient pattern related to the preservation of the cuticle. In the more preserved areas [1], the crystals are less spherical in shape, and are more scale-like and long, in a closely-packed arrangement. [2] shows an intermediate area and [3], a less well-preserved area. Images were recovered using the backscattered detector; magnifications are 250x in the first photo and 1000x in the subsequent ones; scale bars 200µm in the first photo and 50µm in the subsequent ones; spot size 6.0; working distance (WD) of 10 mm.

1.3. Elemental characterization of the Crato Formation fossils

EDS and EDXRF elemental mapping of the Ephemeroptera specimens revealed a marked preferential distribution of iron (Fe), oxygen (O), copper (Cu), zinc (Zn), and lead (Pb) in the fossil, and of calcium (Ca) in the host rock (Figures 9 and SMa).

In the orthopteran fossils, EDS point analyses of the proventriculi show mainly Ca, phosphorous (P), and O occurring associated with the coatings of anhedral to euhedral crystals, and mainly Ca, P, O, Fe, and silicon (Si) at the film-embedding crystals (Figure 7b), though Ca and P counts are lower than those of the Ca/P-rich coating. Peaks of Ca and P are present in several parts of proventriculi and indicate preservation by calcium phosphate (Figures 10, 11).

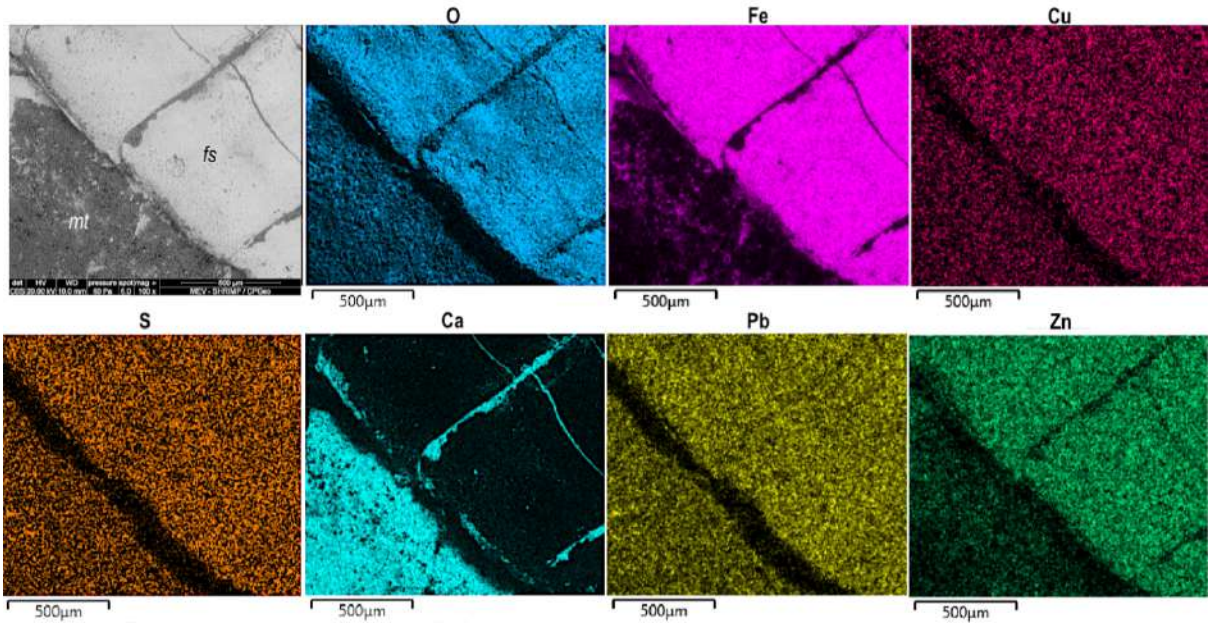


Figure 9. Elemental mapping of abdominal segments of a mayfly larva. Specimen MPSC I 5230. fs - fossil, mt -matrix, O - oxygen, Fe - iron, Cu - copper, S - sulfur, Ca - calcium, Pb - lead, Zn - zinc.

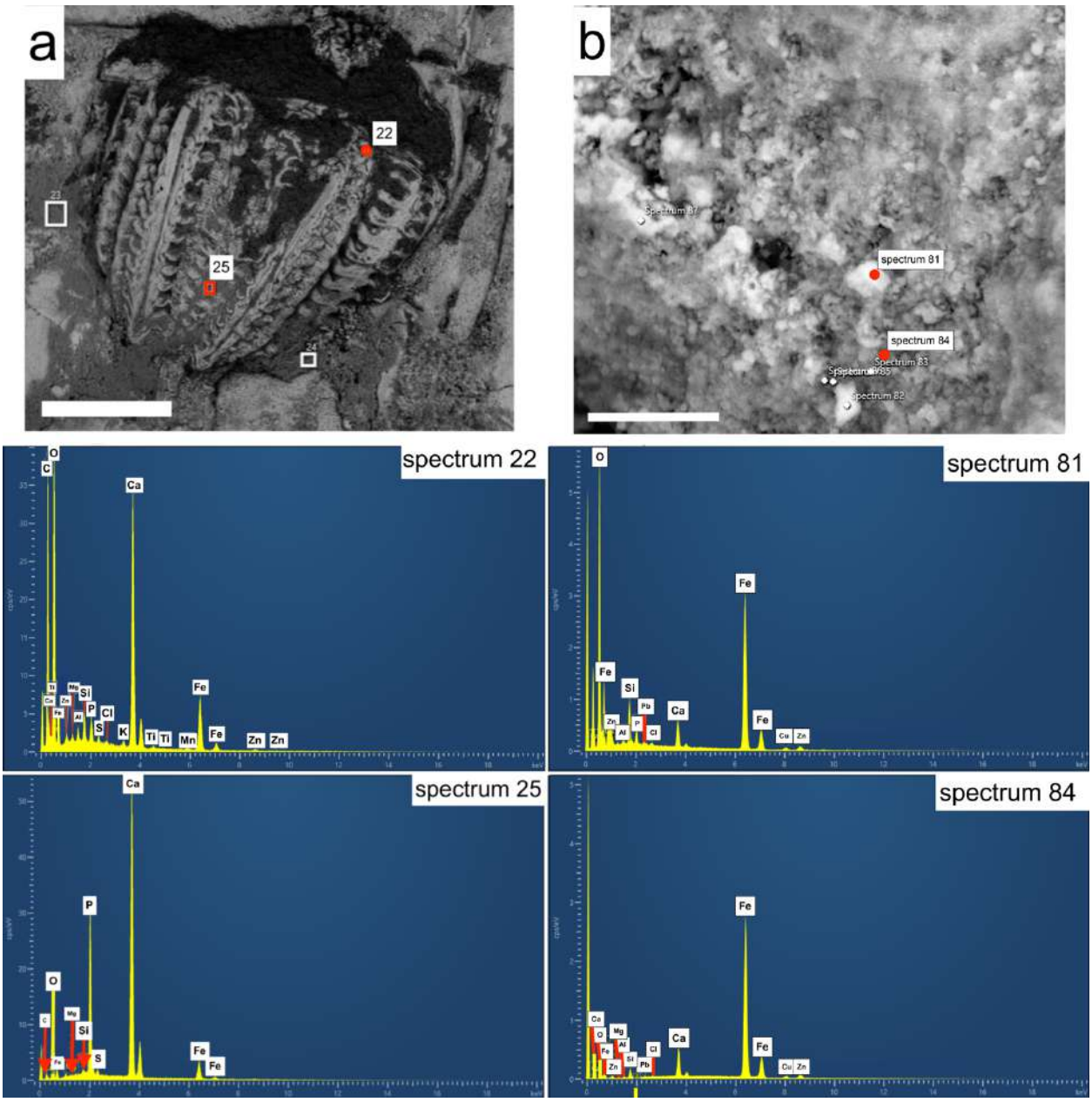


Figure 10. Energy dispersive X-ray spectroscopy (EDS) point spectra of the proventriculus of the orthopterans. a) Some point spectra of the proventriculus of specimen LPU P6. Scale bar 1 mm. b) EPS-like superficial foam covering and infilling parts of proventriculus of specimen GP/1E 8910. Scale bar 10 µm.

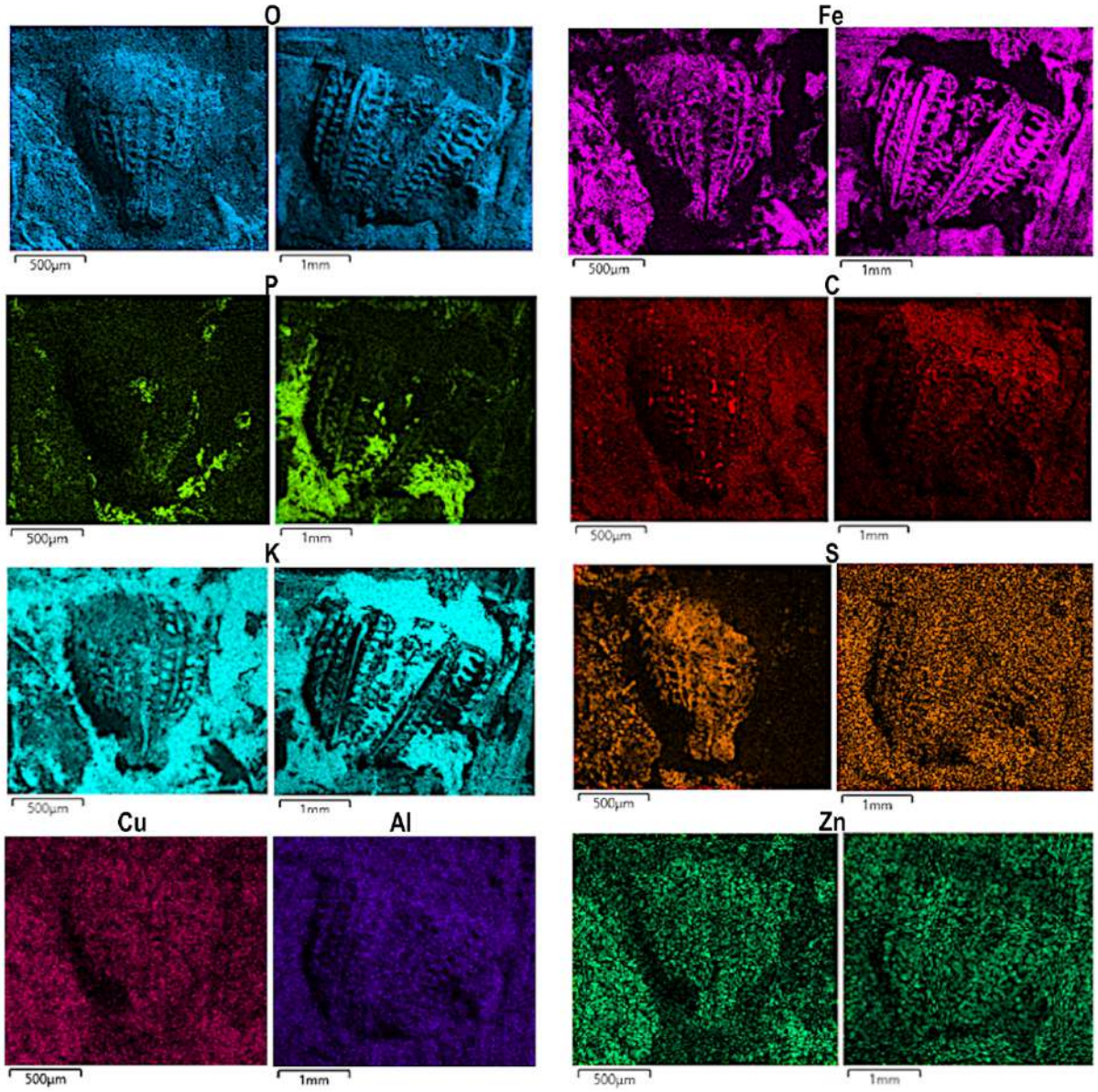


Figure 11. Elemental mapping of the proventriculi of the orthopteran specimens CAV 0012 (left) and LPU P6 (right). O - oxygen, Fe - iron, P - phosphorus, C - carbon, Ca - calcium, S - sulfur, Cu - copper, Al - aluminum, Zn - zinc. Cu was measured only for specimen CAV and Al only for specimen LPU. Images were recovered using the backscattered detector, with spot size 6.0. CAV 0012 - magnification 80x, WD 18 mm. LPU P6 - magnification 40x, WD 10 mm.

In the EDS maps of the proventriculi of specimens CAV 0012 and LPU P6 (Figure 11), we also observe sulfur (S) associated with Fe in some areas. Zn is associated with Fe in the fossil. As

observed in the EDS spectra, maps show the spatial correlation of Ca and P at the proventriculi coating.

In EDS point spectra of the proventriculus of specimen GP/1E 8910, the main elements are Ca, S, and O, followed by Fe and Si, while in some cases, Fe peaked more expressively than these latter elements (Figure 12). The inner gut tissue also has high counts, mainly of Si and O at the well-preserved denticle-like surface (Figure 17b). Finally, peaks of manganese (Mn) were recovered around the well-preserved inner gut, associated with peaks of both Ca and Fe (Figure 12).

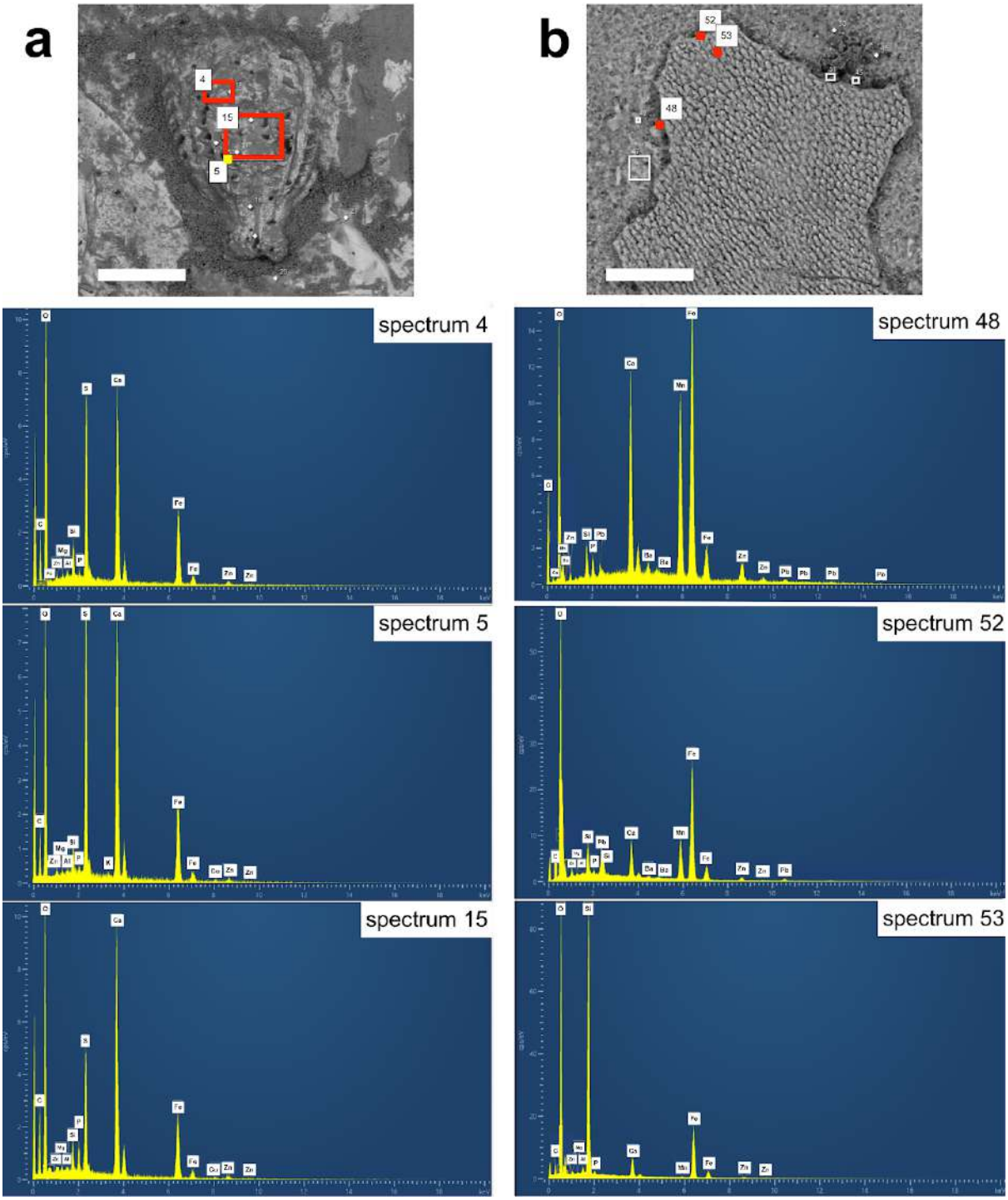


Figure 12. Energy dispersive X-ray spectroscopy (EDS) point spectra of orthopteran inner tissues. a) Elemental mapping of specimen CAV 0012 evidencing some point spectra from its proventriculus. The image was recovered using the backscattered detector (CBS), scale bar 500 µm, spot size 6.0, magnification 80x, WD 18 mm. b) Elemental mapping of specimen LPU P6

evidencing some point spectra from its well-preserved inner gut tissue. CBS detector, scale bar 100 μm, spot size 6.0, magnification 450x, WD 10 mm.

EDXRF analyses of Ephemeroptera larvae show that the fossils are composed of Ca, Cu, Zn, Fe, Si, S, potassium (K), Ti, chromium (Cr), Mn, strontium (Sr), Pb, S, and Cl (Figures 13 and SMb). The main element of the host rock is Ca, but it also has negligible counts of S, Cl, and Ti (Figures 13 and SMb). Si, Ca, K, Sr, Pb, and Mn are more abundant in the host rock, while the other elements do not show a clear correlation with either the host rock or the fossils, or are more significant in the latter (Figure 13b). The relative intensities of Ca and of Sr seem to be correlated, with more counts of these elements in the host rock (Figure 13b). When only a single fossil is considered, the relative intensities of some metals vary among different measurement points. For instance, in specimen MPSC I 763, Ti, Cr, Fe, Cu, Zn, and Pb have lower counts in P14 relative to P13 (Figure 13b).

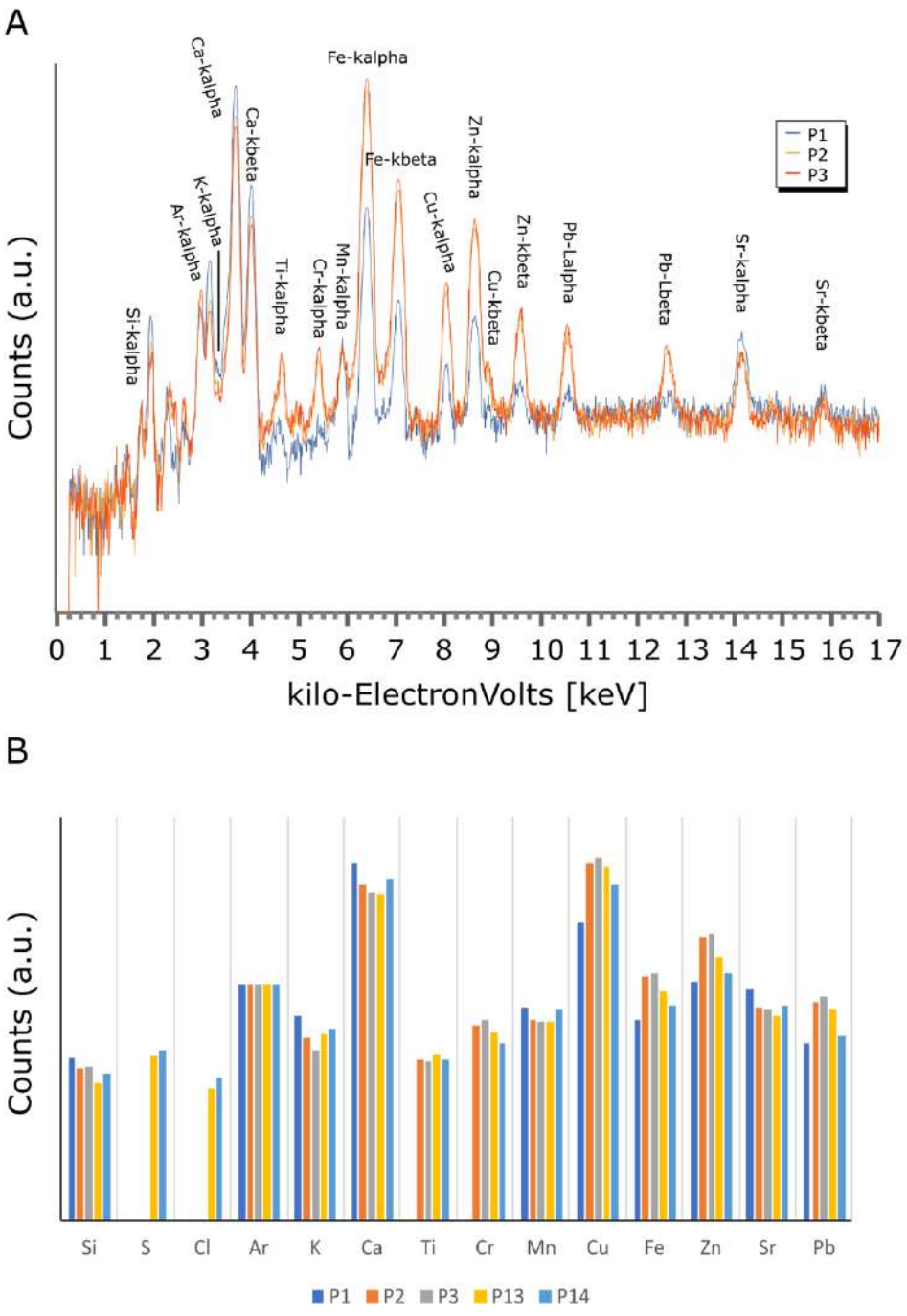
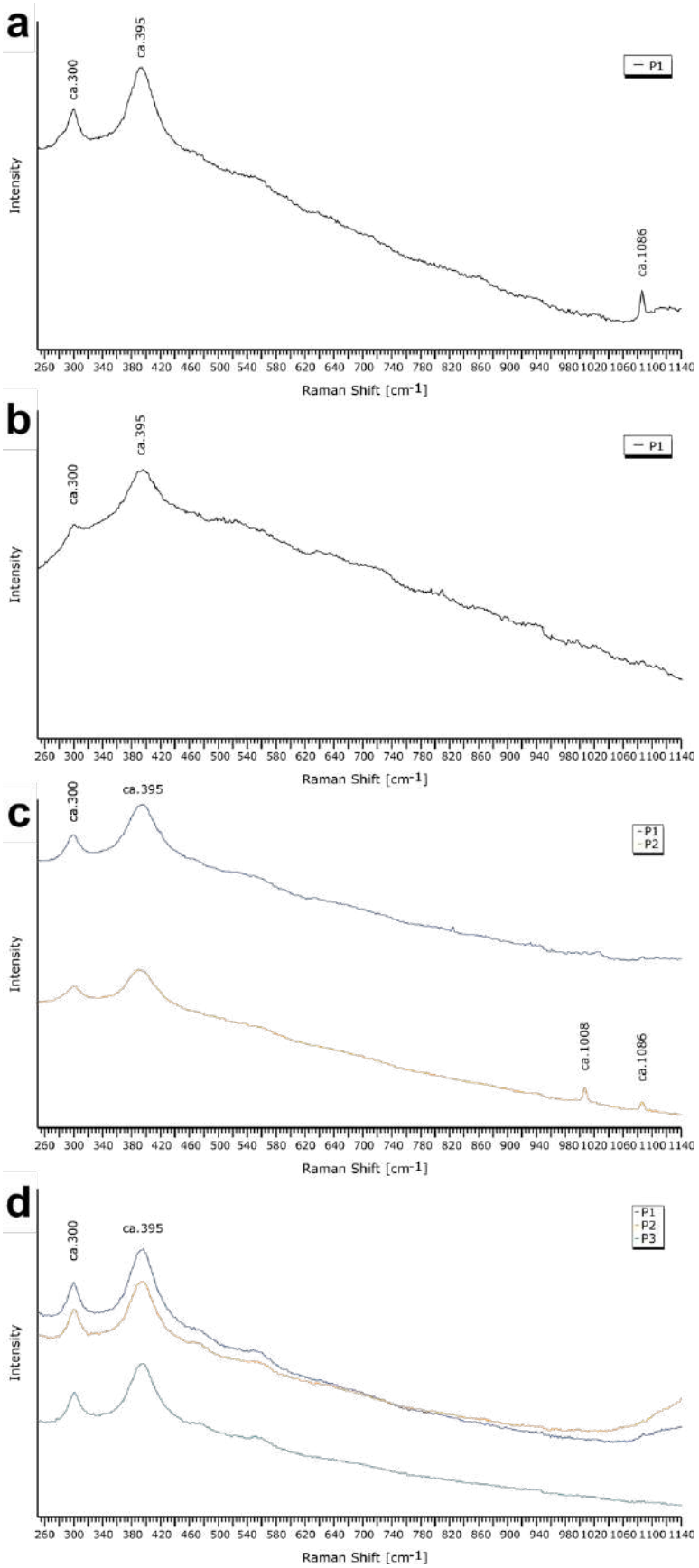


Figure 13. Energy dispersive X-ray spectroscopy analyses of Ephemeroptera larvae and host rock. a) EDXRF point spectra measured in specimen MPSC I 5224. P1-Rock, P2-Fossil and P3-Fossil. b) Plot of relative intensities (log scale) for measured elements in different points of host rock and fossils (specimens MPSC I 5224 - P1,2,3; MPSC I 5227 - P13; and MPSC I 5230 - P14).

P1-Host rock, P2-Fossil, P3-Fossil, P13-Fossil, and P14-Fossil. Points analyzed within specimens are marked in Figure 3.

Micro-Raman results are similar with the EDS results and indicate that iron oxides/hydroxides, particularly goethite, preserve the biological tissues of specimens. Strong bands defining goethite are 295 and 393 cm^{-1} (Perardi et al., 2000; Li and Hihara, 2015), and in our fossils, Raman spectra also have bands at ca. 300 and 395 cm^{-1}(Figure 14). Calcite was also found to be represented by a strong band in 1086 cm^{-1} (Figure 14a,c).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Raman Shift [cm-1]
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Intensity
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P3
Intensity
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Raman Shift [cm-1]

Figure 14. Raman spectra from larval mayflies and adult orthopterans. a) Specimen MPSC I 763. b) Specimen MPSC I 5229. c) Specimen CAV 0012. d) Specimen GP/1E 8910. Band of goethite (ca. 300 cm^{-1} and ca. 395 cm^{-1}) and of calcite (ca. 1086 cm^{-1}). Location of points analyzed in Figures 3 and SMc.

2. Solnhofen limestones

2.1. Preservation of mayflies from Solnhofen limestones

Almost all the specimens are fossilized in lateral position (n=82), except two isolated wings (BMMS 53/72a and BMMS 53/72b), and specimen BSPG 4672 preserved either in dorsal or ventral position (preservation is so low that not discernible). Also, almost half of the specimens are complete (n=38), considering the main body parts (head, thorax, abdomen, and caudal filaments). All fossils studied show a low degree of morphological fidelity, with body segments indistinguishable in most of the cases (e.g., lines that mark where is the end of the thorax and beginning of the abdomen; lines delimiting abdominal segments – Figures 4d-h).

Solnhofen mayflies are preserved only as faded imprints in the limestone slabs (Figure 4). The only exception is the specimens SMNS 70311a and b (the slab and counterslab of the same individual), which are preserved by a mineral film covering their imprints (visually similar to a mineral replacement), and are the only specimens macroscopically well-preserved (Figures 4a,b).

SEM imaging revealed that the preservation of the external morphology of the fossils is very low, and internal preservation is non-existent, with tissues obliterated by calcite crystals or in combination with globular material (possibly crystals of iron oxi/hydroxides) (Figure 15). Half of the specimens (n=43) present calcite crystals associated with and obliterating body parts, especially the eyes (Figures 4a,d,f,h). Only seven specimens present their abdominal segments delimited, although weakly (Figures 4e,g,h). Also, in a few cases (specimens BSPG 1882 XVI 30, JME 1728, JME 1745, JME 3703a/b, and SMNS 70311), the wing venation and/or body was traced by iron oxide dendrites in combination with ferrous solutions in a disorganized way (Figures 4c,d - see elemental results below).

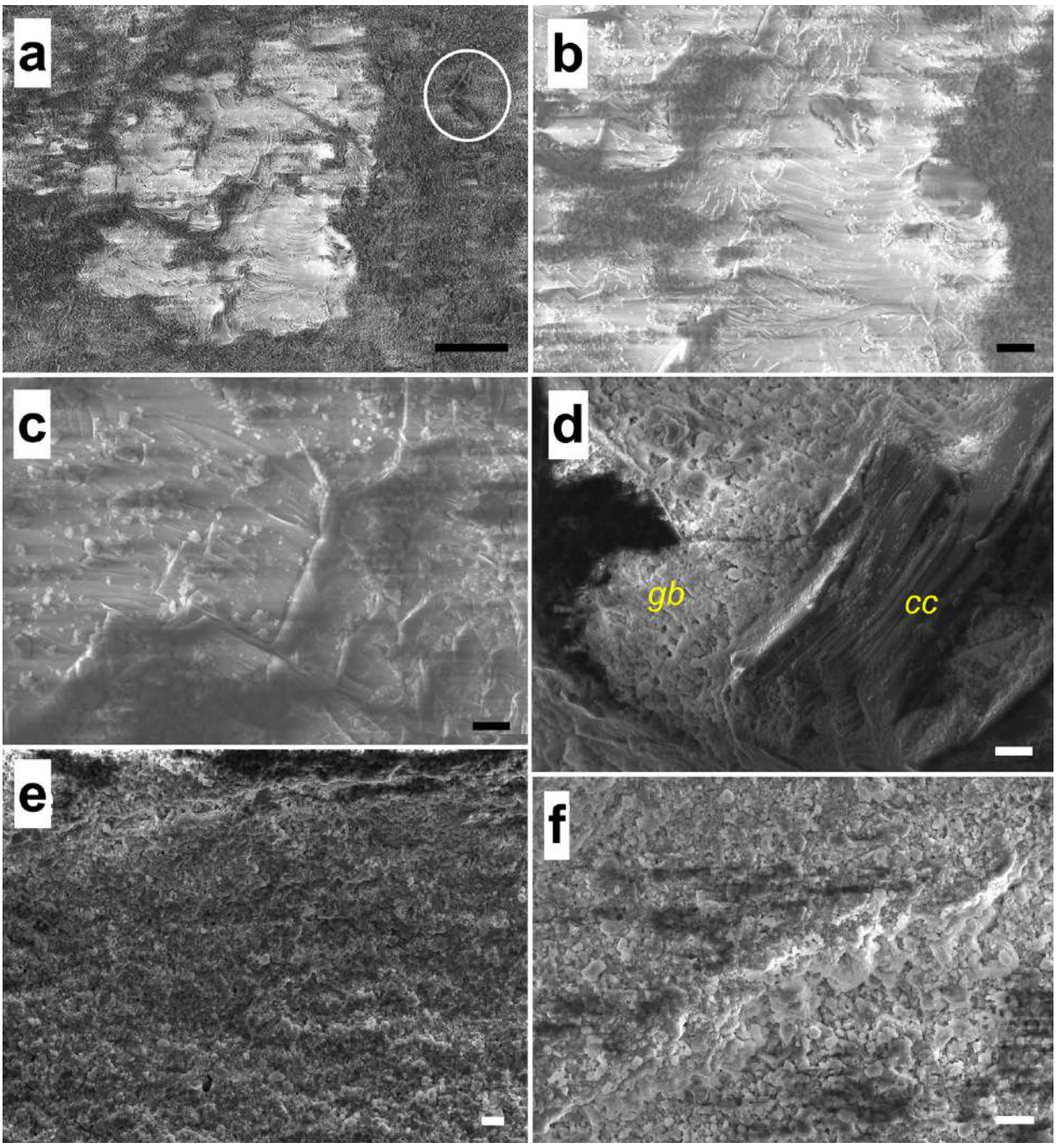


Figure 15. Micromorphological preservation of Solnhofen mayflies. SEM images from specimen SMNS 70311a show tissues obliterated by calcite crystals in combination with globular material, or alone. a,b,c) Calcite crystals between the area in which originally were the head cuticle plates, revealing many cleavage planes, in progressively larger magnifications (120x, 285x, 800x, respectively). Additionally, in 'a' the white circle highlights a possible cuticular element/putative piece of setae. Scale bars 100 µm, 30 µm, and 10 µm, respectively. Voltage 20 kV. WDs 35.98

mm, 36.88 mm, and 36.07 mm, respectively; d) Calcite crystals [cc] between globular material [gb] in the eye; e) Matrix showing globular material; f) Globular material in abdominal segments; d,e,f) Magnifications 800x, 430x and 850x, respectively. Scale bars 10 μm. Voltage 25kV. WDs 8.8 mm (d) and 11.09 mm (e,f).

2.2. Elemental characterization of the mayflies from Solnhofen limestones

EDS point analyses of mineral fabrics revealed similar chemical concentrations when comparing the fossil and the matrix, with a marked preferential concentration of Ca, with only slight differences. Although abundant Ca can also indicate the presence of aragonite, the fossils we analyzed here are clearly dominated by calcite, recognized due to the distinct orthorhombic shape of their crystals (see Figure 15; Bragg, 1924).

The typical pattern among all areas we analyzed is that Ca is the most abundant element, followed by O and C, except the matrix of specimen MB2021.10.155 in which Ca was most abundant, followed by Si, then aluminum (Al) (Figure 16). Phosphorus was noted in only one sampled point of the abdomen of specimen MB2021.10.155. Sulfur was also only recovered in small amounts in specimen MB2021.10.155 (both fossil and matrix).

Generally, apart from Ca, O, and C as the most abundant elements, in most analyzed areas, Si stands out, together with Fe, sulphur (S), magnesium (Mg), Al, K, barium (Ba), and Mn. Fe, Al, Mg, and Ba are found frequently, while S, K, and Mn are recovered occasionally (Figure 16).

Elemental analyses revealed that Fe is more concentrated in fossils than in the rock matrix, while Ca is slightly more concentrated in the rock, although overall signals are very similar. The preferential distribution of these elements is consistent with Fe compounds replacing the fossils and the calcitic composition of the rock matrix.

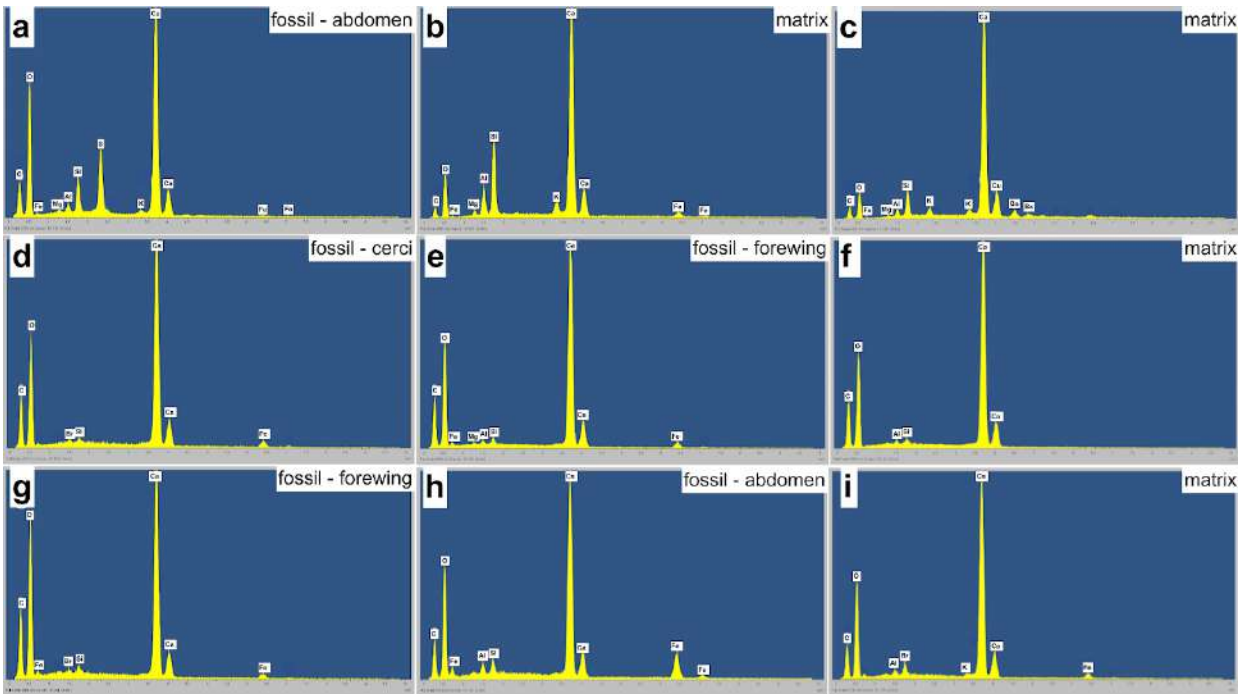
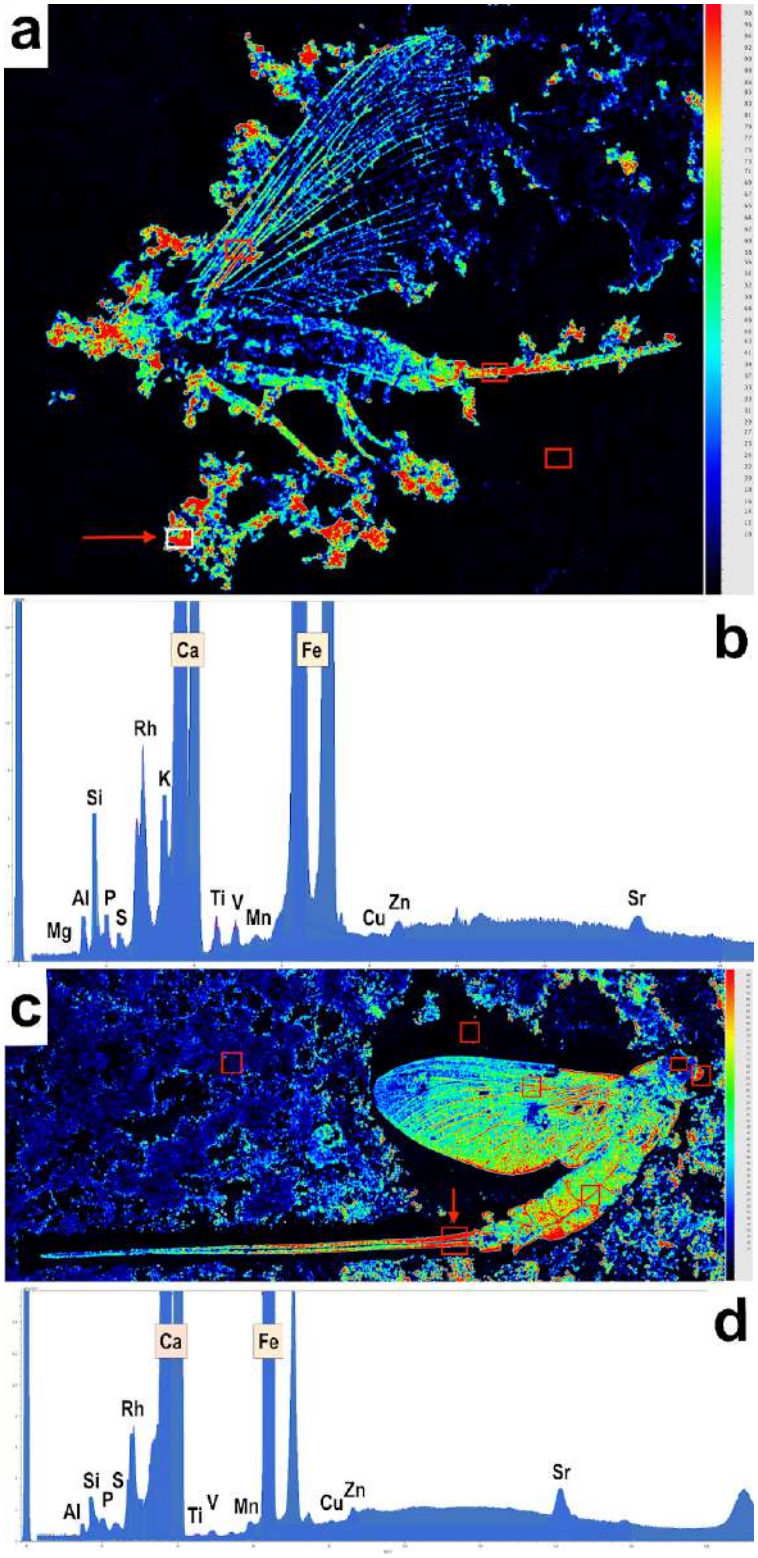


Figure 16. Microenergy dispersive X-ray spectroscopy point spectra of Solnhofen mayflies. MB2021.10.155, P1 - abdomen. b) MB2021.10.155, P2 - matrix. c) MB2021.10.155, P3 - matrix. d) SMNS 70311a, P1 - cerci. e) SMNS 70311a, P2 - forewing. f) SMNS 70311a, P3 - matrix. g) SMNS 70311b, P1 - forewing. h) SMNS 70311b, P2 - abdomen. i) SMNS 70311b, P3 - matrix. Location of points analyzed in the specimens is shown in Figure 4.

Microenergy dispersive X-ray fluorescence analyses (μEDXRF) agree with EDS, recovering Ca and O as the more prominent elements in all the specimens (Figure 17). Iron is relatively well represented but not as much as O. The remaining elements occurring in fossils are Mg, Sr, S, Mn, titanium (Ti), Zn, and Cu (Figure 17). Usually, better-preserved parts are those with high Fe content (Figures 17-19); parts that are massively taken by calcite crystals, as the eyes of specimen SMNS 70311a, have no Fe and the elemental composition of these crystals is very similar to that of the calcitic matrix, with also almost no Fe at all (Figures 18 and 19). The calcite crystals obliterating the eyes, for instance, are also associated with metals such as Sr and Zn (Figure 19). In the only case where the specimen is associated with iron/manganese oxide dendrites (SMNS 70310), we could identify that the Fe is in a combination with other metals such as Ti and Mn (Figure 18). Similarly, in the best-preserved specimen SMNS 70311a, Fe, Mn, Ti, and Zn occur preferentially in the fossil relative to the host rock (Figure 19).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Zn
Sr

Figure 17. µEDXRF intensity maps of Solnhofen mayflies. a) µEDXRF intensity map of iron (Fe) of specimen SMNS 70310, with analyzed spots marked by red rectangles. The intensity is higher towards the reddish colorations. b) Spectrum of the elements recovered at the point highlighted by the arrow in 'a'. c) Intensity map of iron (Fe) of specimen SMNS 70311a, with analyzed spots marked by red rectangles. d) Spectrum measured on the point highlighted by the arrow in 'c'. The intensity maps of the remaining analyzed points are shown in Figures SMd and SMe.

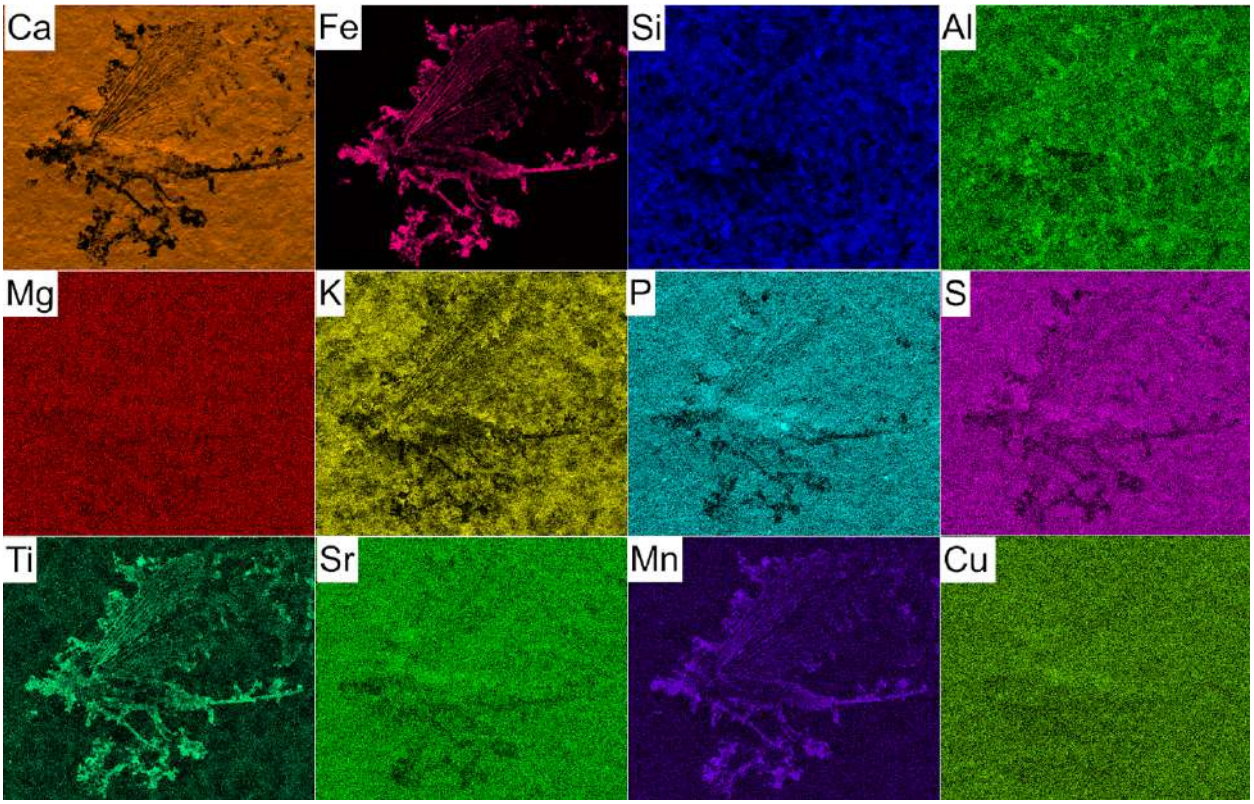

Figure 18. µEDXRF distribution maps of elements from the Solnhofen mayfly SMNS 70310. Ca - calcium, Fe - iron, Si - silicon, Al - aluminum, Mg - magnesium, K - potassium, P - phosphorus, S - sulfur, Ti - titanium, Sr - strontium, Mn - manganese, Cu - copper.

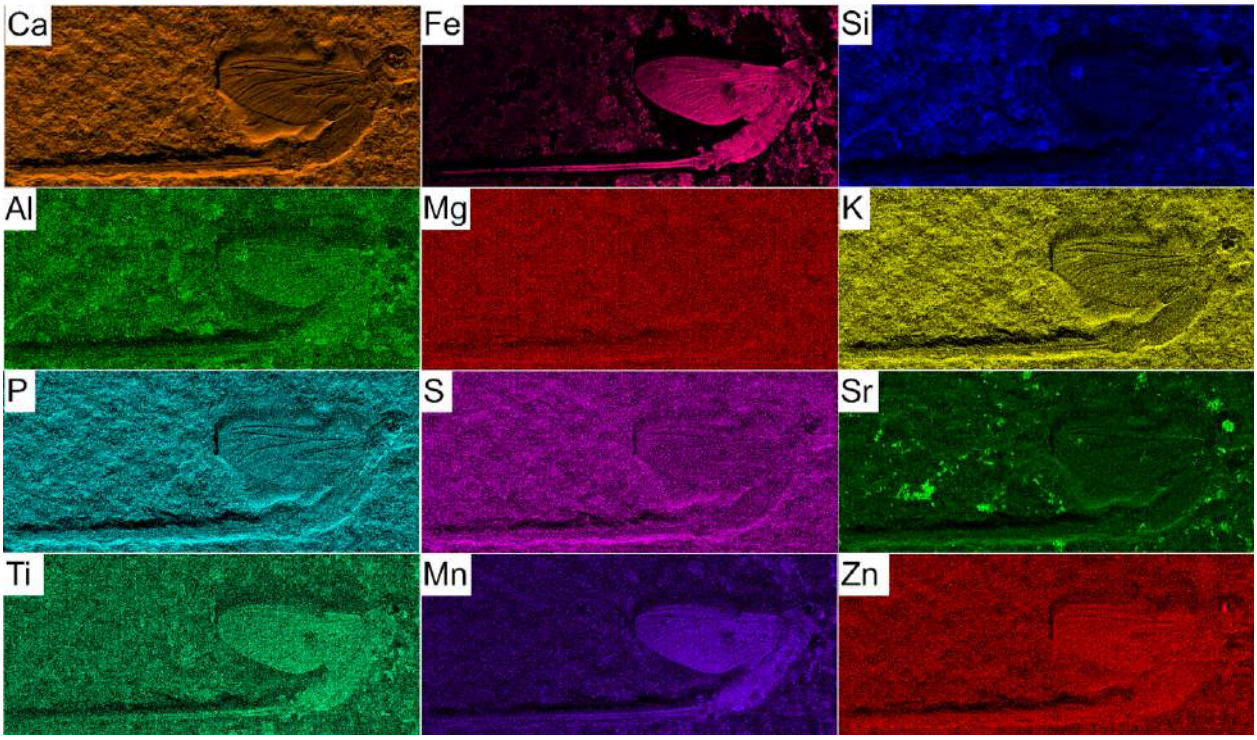

Figure 19. µEDXRF distribution maps of elements from the Solnhofen mayfly SMNS 70311a. Ca - calcium, Fe - iron, Si - silicon, Al - aluminum, Mg - magnesium, K - potassium, P - phosphorus, S - sulfur, Sr - strontium, Ti - titanium, Mn - manganese, Zn - zinc.

Discussion

All the Crato fossils we analyzed in the present work were initially pyritized specimens that were secondarily replaced by iron oxides/hydroxides, as indicated by the brown, yellow, and orange-brown colors of the fossils (Figure 3) (Osés et al., 2016; Osés et al., 2017), and by Raman data. An original pyrite mineralogy is additionally supported by both the microfabric morphology of pseudoframboids and the elemental composition. The regions of high preservational fidelity, like the proventriculi, are replaced by pseudoframboids that are always smaller when compared to the cuticle. Barling et al. (2020) showed similar results, but they discussed that the high-fidelity fabric (i.e., smaller crystals) only replaces the epicuticle, whereas the pseudomorphic framboid (i.e., bigger crystals) replaces internal tissues and subsurface cuticular layers, which is the opposite of what we show here. Probably, the smaller close-packed grains are not necessarily related to inner or outer parts, but to the well-preserved ones. According to Briggs et al. (1993), the smaller the calcium phosphate particle aggregates are, the higher the morphological fidelity of

preservation. A similar interpretation is supported for pyritization of Crato insects by Delgado et al. (2014), Osés et al. (2016), and Barling et al. (2020), suggesting that areas of high-fidelity cuticle replacement are a consequence of the close packing of the framboids.

Soft tissue phosphatization has been reported before in insects from the Crato Formation (Dias and Carvalho, 2020). In our observations, it is restricted to some areas of the orthopteran proventriculi and to regions surrounding them. μEDXRF data indicates negligible amounts of sulphur in the host rock relative to the Crato mayfly fossils, and EDS maps of orthopteran proventriculi indicate that this element is not associated with iron. This may imply that sulfur in the Crato fossils occurs in other mineral phases, like sulfates, after sulfide oxidation (Osés et al., 2016). This is supported by low sulfur counts when iron is abundant and high counts of sulfur and calcium in some areas, which relates to the remaining iron sulfides of the primary pyritization process (Osés et al., 2016). We noticed that when only a single fossil is considered, the relative intensities of some metals vary among different measurement points (e.g. specimen MPSC I 763, Fe, Cu, Zn and Pb have lower counts in P14 relative to P13). This observation may be related to the distinct preservation of the morphological structures in these two points, as P14 was measured in a wing and P13 in the head/thorax. Osés et al. (2016) hypothesized that these metals were originally associated with sulfides. It is possible that more available labile organic matter in the head/thorax region favored more decay and sulfide precipitation relative to the wings, thus explaining more abundance of these elements in the body.

Nevertheless, the preservation of Crato insects is diverse and includes further preservational pathways than those exemplified here (pyritization and phosphatization). For example, carcasses may also be kerogenized (see Barling et al., 2020; Bezerra et al., 2020; Dias and Carvalho, 2020; 2022). Pyritization and kerogenization are associated with different types of laminated limestones: pyritized specimens occur mainly in yellowish limestones, while kerogenized fossils are preserved in grayish limestones (Barling et al., 2020; Dias and Carvalho, 2022). As we report above, the dominant fabric of preservation of the pyritized fossils is goethite pseudomorphs of framboidal pyrite. In the kerogenized fossils, there is a massive carbonaceous film on the carcasses' surface (Barling et al., 2020), also mainly composed by goethite, but these fossil insects show worse preservation when compared to pyritized ones (Barling et al., 2020; Bezera et al., 2020; Dias and Carvalho, 2020; Bezerra and Mendes, 2024).

We report mayfly larvae from the Crato Formation with a wrinkle-like surface in the cuticle of their abdominal terga, which we hypothesize were imprinted after dehydration of the cuticle due to the action of microbial mats. Recently, Dias et al. (2023) showed similar results and hypothesized that the microbial mats created a sealing effect during the coating of the carcasses, causing dehydration, followed by later dehydration during diagenesis. We also show microscopic features that suggest the presence of mats in fossil orthopterans, such as the EPS-like amorphous material covering and filling parts of the preserved inner organs, agreeing with Dias and Carvalho (2022), who reported negative-relief impressions on the cuticle of orthopterans (like the wrinkle-like surfaces shown here), and web-like texture (as the amorphous matter of our specimens) which they also interpreted as EPS originally excreted by microorganisms. These microorganisms produce mucilage, a gelatinous material capable of mobilizing solutions and altering the biogeochemistry of sedimentary environments (Gerdes, 2003). The presence of pseudomorphs of framboidal pyrite replacing insects, in association with putative EPS, corroborates the hypothesis that microbial-mat-forming sulfate-reducing bacteria precipitated the pyrite responsible for the preservation of Crato fossil insects (Briggs, 2003; Peterson et al., 2010; Wang et al., 2012; Delgado et al., 2014; Barling et al., 2015; Osés et al., 2016; Dias and Carvalho, 2022).

Recently, several works assessed the role of microbial mats on the preservational mechanics of Crato Formation arthropods (Varejão et al., 2019; Iniesto et al., 2020; Dias and Carvalho, 2022). Based on these previous authors and our additional results, we assume that specialized benthic communities formed microbial mats that proliferated in the lake substrate of the Crato Formation. According to Prieto-Barajas et al. (2018), photosynthesis is the the main source of energy and nutrition for a microbial mat, which is a limiting factor for its occurrence and distribution across a hypersaline lacustrine environment. Therefore, the waters of the Crato paleolake should have been shallow enough for appropriate luminosity to reach the substrate, allowing photosynthesis of these benthic communities (Varejão et al., 2019).

In addition to pyrite, the occurrence of calcium phosphate, when associated with other microbial features, can also indicate the effect of microbial mats (Noffke and Awramik, 2013; Prieto-Barajas et al., 2018). Wang et al. (2012) reported pyrite halos around insect fossils suggesting the presence of reducing microenvironments facilitated by microbial mats; also, it is known that during the replication of cells of the microbial mats, pyrite is commonly formed (Noffke and Awramik, 2013). Dias and Carvalho (2022) inferred that the paleoenvironments of

yellowish (with pyritized insect fossils) and grayish limestones (with kerogenized insect fossils) of the Crato Formation were distinct. The yellowish limestones represent more arid conditions, which are favorable for the development of microbial mats, while the grayish limestones represent more humid conditions, which limits the growth of microbial mats (Dias and Carvalho, 2022).

Since the mayflies of Solnhofen limestones do not present any morphological or preservational signs of previous action of microbial mats during their diagenesis, we assume that microbial mats were not available or, if present, were more restricted in the Solnhofen substrate, similarly to what is hypothesized by Dias and Carvalho (2022) for the grayish layers of the Crato Formation (though those features could also be simply not preserved). However, since almost the entire Solnhofen vertebrate fauna is exceptionally preserved through phosphatization (Wilby and Briggs, 1997; Frey et al., 2003; Klug et al., 2015; Tałanda, 2018; Barlow et al., 2021; Delsett et al., 2022), those mats were likely prospering during probably more arid phases of the basin and thus preserving them.

Unlike the larval stages of mayflies, which are dominant in the Crato Formation (Martins-Neto 2006; Storari et al., 2021a), no larvae are present in the Solnhofen limestones, which could indicate that their adults were reproducing in stagnant waters in close, emergent lands, and that the carcasses of the adults were washed out directly to the marine depositional site during floodings (Bechly, 2015; Staniczek et al., 2022), or were carried out by the wind. This could also indicate that the marine paleoenvironment was isolated from any adjacent stream (Barthel et al., 1990), suggesting that, at least during the phases when the mayflies were deposited, the Solnhofen water setting was a partly or completely closed system, with restricted water flow (Barthel et al., 1990). This also differs from the Crato Formation, which had streams that flowed into the paleolake where the limestones were deposited (Ribeiro et al., 2021). The great majority of Solnhofen mayflies we studied here are preserved in lateral view, with wings closed at rest, which suggests that dead individuals were transported floating on the water surface (Martínez-Delclòs and Martinell, 1993). An interesting feature of the taphonomic history of Solnhofen mayflies is that, although their preservation is very poor, few specimens are disarticulated, likely due to the low disarticulation rates of specimens in the calm depositional environment (Viohl, 1994). Insects that arrived dead on the depositional site would remain on the surface of the water longer and undergo greater necrolysis until the water tension broke (e.g., through storms), while those that drowned would sink promptly, reaching the bottom faster (Martínez-Delclòs and Martinell, 1993; Martínez-

Delclòs et al., 2004). This taphonomic history is, for instance, different from the Solnhofen dragonflies, which are commonly preserved with open wings, a sign that has been interpreted as indicating drowning (Martínez-Delclòs and Martinell, 1993; Barling et al., 2021), and which might account for their good preservation at hand scale compared to the mayflies of this unit (Bechly, 2015).

Although the Solnhofen limestones are exceedingly fine-grained, enabling the preservation of fine details, for example, the feathers of *Archaeopteryx lithographica* (de Buisonjé, 1985), the preservation of mayflies is very modest, which suggests a different taphonomic history than well-preserved taxonomic groups, or could simply represent different temporal phases of deposition of the basin, with different conditions for fossil diagenesis. Fossil insects from the Solnhofen limestones have been the topic of many studies describing them as being mostly poor-quality (sometimes called 'rough') calcite and pyrolousite casts that retain some three-dimensionality (Ponomarenko, 1985; Viohl, 1990; Martínez-Delclòs et al., 2004; Grimaldi and Engel, 2005), similarly to our results. However, until now, arthropod groups from this unit have lacked any published geochemical characterization. Despite this, Martínez-Delclòs et al. (2004) describe Solnhofen as a typical site of phosphatization for insects, as commonly described for vertebrates of this unit (Frey et al., 2003), which we believe is unlikely, at least for the mayflies, since none of the analyzed specimens presented such type of preservation. Instead, we observe that the mineralogy of the Solnhofen mayflies is largely calcitic, with a chemical composition similar to that of the host rock, but with metals associated with the best-preserved parts and no phosphatic influence, differing largely from the pattern observed in the vertebrates (Kundrát et al., 2019; Li et al., 2021). In Solnhofen vertebrates, the rock where the fossil is embedded is usually rich in elements like Si, K, Ca, Ti, Mn, and Fe, with Fe, Mn and Ti being reported as low in the fossils themselves (Kundrát et al., 2019; Li et al., 2021), while Zn had contrasting reports (Bergmann et al., 2010; Li et al., 2021). The preferential association of Fe, Mn, Ti, and Zn with the mayfly fossils we describe here may imply early mineralization of a yet undetermined mineral phase that favored the preservation of some specimens.

Based on our data, we propose hypotheses about the preservation of mayflies in the frame of the Flinz and Fäule phases of the Solnhofen limestones. Fossil mayflies are preserved mainly in the Flinz layers, which are associated with episodes of more humid conditions (Munnecke et al., 2008), suggesting times with more habitats for these aquatic insects near the depositional site.

It is also possible that the mayflies are not well-preserved because microbial mats were absent or restricted in the depositional site, compared to Fäule phases, which are associated with drier periods that are ideal for the proliferation of microbial mats (Keupp, 1977; Seilacher et al., 1985; Iniesto et al., 2013; Iniesto et al., 2015; Viohl, 2015). Hess (1999) noticed that the preservation of *Saccocoma* crinoids is best in the Fäule beds, while in Flinz beds recrystallized calcite obscures their details, similarly to the preservation of mayflies. From the scarcity of fossils in the Flinz beds, Barthel (1978) concluded that these were deposited rapidly, with the bulk of sediments washed in during storms. Based on fish taphonomy, Viohl (1994) similarly inferred very rapid deposition of the Flinz, with several laminae representing less than a year. Flinz periods were followed by quiet, marly Fäule episodes. During such times, cyanobacteria, whose spherical remains occur in some of the Fäule beds, could have formed mats (Hess, 1999), which probably explains the exceptional preservation of several fossils in the Fäule beds and the low preservation of mayflies in the Flinz layers.

Similarly to the Flinz and Fäule cycles of the Solnhofen limestones, the Crato Formation also shows different types of limestones related to different sedimentation conditions. The limestones of the Crato Formation bear two laminated facies, the clay-carbonate rhythmite ('dark'), and the laminated limestone ('pale', dominated by calcite crystals), that corresponds to a shift in the depositional balance of authigenic carbonate precipitation and terrigenous input (Neumann et al., 2003; Heimhofer and Martill, 2007). However, within the microfacies the limestones also cyclically differ in mineralogy between yellowish and grayish limestones. Pyritized insects mainly occur in yellowish limestones, while kerogenized fossils are preserved in grayish limestones (Barling et al., 2020), and mayflies are extremely rare in grayish limestones (unpublished results, and Dias et al., 2023). So, contrary to Solnhofen, mayfly fossils are associated with the limestone type that holds the best-preserved insects at the Crato Formation.

Conclusion

The application of analytical techniques of spectroscopy was paramount to further unravel the preservation in the studied Lagerstätten. An intimate relationship is observed between insect soft tissue preservation and fine-grained laminated carbonates, such as those of the Crato Formation and Solnhofen limestones (Martínez-Delclòs et al. 2004).

The preservation of Crato mayflies and crickets is more diverse, with fossils displaying calcium phosphate and iron oxides/hydroxides preserving internal features, and the majority of specimens being, in general, substituted by iron oxide after pyritization, though in rare cases they can be preserved by carbonaceous content in grayish limestone (Dias and Carvalho, 2020). Additionally, we could differentiate the mineralogy between the cuticle and the well-preserved internal organs of some specimens. The high-fidelity preservation of internal organs is due to smaller, closely-packed framboids compared to the cuticle and other less-preserved parts. In our results, Crato specimens presented microscopic features that suggest the presence of microbial mats during the fossilization process, such as micro cracks, wrinkles, and EPS-like amorphous matter covering and infilling voids.

In comparison to the Crato insects, the mayfly fossils of the Solnhofen limestones show, at their highest fidelity, that they are complete, fully-articulated, but with no submicron-scale replication of neither external and internal morphology. The preservational fabric is largely calcitic imprints with metal influence, which are, for the first time, described as a preservation mode for Solnhofen fossil insects. We recovered no phosphatic influence, as common for their vertebrates, for instance. Based on their biostratinomy, we hypothesize that the mayflies were deposited in a partly or completely closed system with restricted water flow and that the specimens arrived dead on the water surface or were brought by winds. Also, their low preservation probably occurred during episodic humid conditions, and with restricted or no action of microbial mats.

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References

Arai, M. and Assine, M.L. (2020) 'Chronostratigraphic constraints and paleoenvironmental interpretation of the Romualdo Formation (Santana Group, Araripe Basin, Northeastern Brazil) based on palynology,' *Cretaceous Research*, 116, p. 104610. https://doi.org/10.1016/j.cretres.2020.104610.

Assine, M.L. (2007) Bacia do Araripe. *Boletim de Geociências Petrobras*, 15(2), 371-389.

Assine, M., Perinotto, A., Custódio, M.A., Neumann, V., Varejão, F.G. and Mescolotti, P.C. (2014) Sequências Deposicionais do Andar Alagoas (Aptiano superior) da Bacia do Araripe, Nordeste do Brasil. *Boletim de Geociências Petrobras*, 22(1), 3-28.

ASTM International. (2002) Standard Guide for Raman Shift Standards for Spectrometer Calibration. Annu. B. ASTM Stand. 96, 1-11.

Barling, N., Martill, D.M., Heads, S.W. and Gallien, F. (2015) 'High fidelity preservation of fossil insects from the Crato Formation (Lower Cretaceous) of Brazil,' *Cretaceous Research*, 52, pp. 605–622. https://doi.org/10.1016/j.cretres.2014.05.007.

Barling, N., Martill, D.M. and Heads, S.W. (2020) 'A geochemical model for the preservation of insects in the Crato Formation (Lower Cretaceous) of Brazil,' *Cretaceous Research*, 116, p. 104608. https://doi.org/10.1016/j.cretres.2020.104608.

Barling, N.T., Heads, S.W. and Martill, D.M. (2021) 'Behavioural impacts on the taphonomy of dragonflies and damselflies (Odonata) from the Lower Cretaceous Crato Formation, Brazil,' *Palaeoentomology*, 4(2), pp. 141–155. https://doi.org/10.11646/palaeoentomology.4.2.3.

Barlow, L.A. *et al.* (2021) 'Laser-stimulated fluorescence reveals unseen details in fossils from the Upper Jurassic Solnhofen Limestones,' *Royal Society Open Science*, 8(12). https://doi.org/10.1098/rsos.211601.

Barthel, K.W. (1978) Solnhofen – Ein Blick in die Erd-geschichte. Ott Verlag, Thun, 393 pp.

Barthel, K.W., Swinburne, N.H.M. and Conway-Morris, S. (1990) Solnhofen: a study in Mesozoic palaeontology. *Cambridge University Press*, Cambridge, 236 pp.

Bausch, W.M. (2004) Geochemie des Profils Maxberg/ Solnhofen. Erlanger Beitr Petr Min, 14, 35-51.

Bausch, W.M., Viohl, G., Bernier, P., Barale, G., Bourseau, J.P., Buffetaut, E., *et al.* (1994) Eichstätt and Cerin: geochemical comparison and definition of two different Plattenkalk types. *Geobios*, 16, 107-125.

Bechly, G. (2015) Insekten (Hexapoda). In: Arratia, G., Schultze, H.P., Tischlinger, H. and Viohl, G. eds. Solnhofen. Ein Fenster in die Jurazeit. Munich, Verlag Dr. Friedrich Pfeil, 239-270.

Bergmann, U., Morton, R.W., Manning, P.L., Sellers, W.I., Farrar, S., Huntley, K.G., *et al.* (2010) 'Archaeopteryx feathers and bone chemistry fully revealed via synchrotron imaging,' *Proceedings of the National Academy of Sciences of the United States of America*, 107(20), pp. 9060–9065. https://doi.org/10.1073/pnas.1001569107.

Beurlen, K.A. (1962) Geologia da Chapada do Araripe. *Anais da Academia Brasileira de Ciencias,* 34, pp. 365–70.

Bezerra, F.I., Da Silva, J.H., Agressot, E.V.H., Freire, P.T.C., Viana, B.C. and Mendes, M. (2023) 'Effects of chemical weathering on the exceptional preservation of mineralized insects from the Crato Formation, Cretaceous of Brazil: implications for late diagenesis of fine-grained Lagerstätten deposits,' *Geological Magazine*, 160(5), pp. 911–926. https://doi.org/10.1017/s0016756823000043.

Bezerra, F.I., Da Silva, J.H., De Paula, A.J., Oliveira, N.C., Paschoal, A.R., Freire, P.T.C., *et al.* (2018) 'Throwing light on an uncommon preservation of Blattodea from the Crato Formation (Araripe Basin, Cretaceous), Brazil,' *Revista Brasileira De Paleontologia*, 21(3), pp. 245–254. https://doi.org/10.4072/rbp.2018.3.05.

Bezerra, F.I., Da Silva, J.H., Miguel, E.C., Paschoal, A.R., Nascimento Jr, D.R., Freire, P.T.C., *et al.* (2020) 'Chemical and mineral comparison of fossil insect cuticles from Crato Konservat Lagerstätte, Lower Cretaceous of Brazil,' *Journal of Iberian Geology*, 46(1), pp. 61–76. https://doi.org/10.1007/s41513-020-00119-y.

Bezerra, F.I. and Mendes, M. (2024) 'A palaeoecological analysis of the Cretaceous (Aptian) insect fauna of the Crato Formation, Brazil,' *Palaeogeography, Palaeoclimatology, Palaeoecology*, 641, p. 112134. https://doi.org/10.1016/j.palaeo.2024.112134.

Bezerra, F.I., Solórzano-Kraemer, M.M. and Mendes, M. (2021) 'Distinct preservational pathways of insects from the Crato Formation, Lower Cretaceous of the Araripe Basin, Brazil,' *Cretaceous Research*, 118, p. 104631. https://doi.org/10.1016/j.cretres.2020.104631.

Bragg, W.L. (1924) 'The structure of aragonite,' *Proceedings of the Royal Society of London. Series a, Containing Papers of a Mathematical and Physical Character*, 105(729), pp. 16–39. https://doi.org/10.1098/rspa.1924.0002.

Brandão, N.C., Bittencourt, J.S., Calor, A.R., Mendes, M. and Langer, M.C. (2021) 'The Ephemeroptera (Hexapoda, Insecta) from the Lower Cretaceous Crato Formation (NE Brazil): a new genus and species, and reassessment of *Costalimella zucchii* Zamboni, 2001 *and Cratogenites corradiniae* Martins-Neto, 1996,' *Cretaceous Research*, 127, p. 104923. https://doi.org/10.1016/j.cretres.2021.104923.

Briggs, D.E.G. (2003) The role of biofilms in the fossilization of non-biomineralized tissues. In: Krumbein, W.E., Paterson, D.M., Zarvarzin, G.A. eds. Fossil and recent biofilms: a natural history of life on Earth. Dordrecht, *Kluwer Acad. Publ*, pp. 281-290.

Briggs, D.E.G., Kear, A.J., Martill, D.M. and Wilby, P. (1993) Phosphatization of softtissue in experiments and fossils. *Journal of the Geological Society*, 150:1035e1038.

Brito, I.M. (1987) Nota preliminar sobre uma nova Efemera do Cretaceo do Ceara (Insecta Ephemeroptera). Anais do X Congresso Brasileiro de Paleontologia, pp. 593-597.

Catto, B., Jahnert, R.J., Warren, L.V., Varejão, F.G. and Assine, M.L. (2016) 'The microbial nature of laminated limestones: Lessons from the Upper Aptian, Araripe Basin, Brazil,' *Sedimentary Geology*, 341, pp. 304–315. https://doi.org/10.1016/j.sedgeo.2016.05.007.

Chen, L., Xiao, S., Pang, K., Zhou, C. and Yuan, X. (2014) 'Cell differentiation and germ–soma separation in Ediacaran animal embryo-like fossils,' *Nature*, 516(7530), pp. 238–241. https://doi.org/10.1038/nature13766.

Coimbra, J.C. and Freire, T.M. (2021) 'Age of the Post-rift Sequence I from the Araripe Basin, Lower Cretaceous, NE Brazil: implications for spatio-temporal correlation,' *Revista Brasileira De Paleontologia*, 24(1), pp. 37–46. https://doi.org/10.4072/rbp.2021.1.03.

de Buisonjé, P.H. (1985) Climatological conditions during deposition of the Solnhofen limestones. 45-65. In Hecht, M.K., Ostrom, J.H., Viohl, G., Wellnhofer, P. eds. The beginnings of birds. Proceedings of the International *Archaeopteryx* Conference, Eichstätt, 1984. Freunde des Jura-Museums Eichstätt, Eichstätt, 382 pp.

Delgado, A.O., Buck, P.V., Osés, G,L., Ghilardi, R.P., Rangel, E.C. and Pacheco, M.L. (2014) 'Paleometry: A brand new area in Brazilian science,' *Materials Research*, 17(6), pp. 1434–1441. https://doi.org/10.1590/1516-1439.288514.

Delsett, L.L., Friis, H., Kölbl-Ebert, M. and Hurum, J.H. (2022) 'The soft tissue and skeletal anatomy of two Late Jurassic ichthyosaur specimens from the Solnhofen archipelago,' *PeerJ*, 10, p. e13173. https://doi.org/10.7717/peerj.13173.

Dias, J.J. and Carvalho, I.S. (2020) 'Remarkable fossil crickets preservation from Crato Formation (Aptian, Araripe Basin), a Lagerstätten from Brazil,' *Journal of South American Earth Sciences*, 98, p. 102443. https://doi.org/10.1016/j.jsames.2019.102443.

Dias, J.J. and Carvalho, I.S. (2022) 'The role of microbial mats in the exquisite preservation of Aptian insect fossils from the Crato Lagerstätte, Brazil,' *Cretaceous Research*, 130, p. 105068. https://doi.org/10.1016/j.cretres.2021.105068.

Dias, J.J., Carvalho, I.S., Buscalioni, A.D., Umamaheswaran, R., López-Archilla, A.N., Prado, G., *et al.* (2023) 'Mayfly larvae preservation from the Early Cretaceous of Brazilian Gondwana: Analogies with modern mats and other Lagerstätten,' *Gondwana Research*, 124, pp. 188–205. https://doi.org/10.1016/j.gr.2023.07.007.

Duncan, I.J. and Briggs, D.E.G. (1996) 'Three-dimensionally preserved insects,' *Nature*, 381(6577), pp. 30–31. https://doi.org/10.1038/381030b0.

Duncan, I.J., Briggs, D.E.G., Archer, M. (1998) Three-dimensionally mineralised insects and millipedes from the Tertiary of Riversleigh, Queensland, Australia. *Palaeontology*, 41(5), pp. 835-851.

Eriksson, M., Terfelt, F., Elofsson, R. and Marone, F. (2012) Internal Soft-Tissue Anatomy of Cambrian 'Orsten' Arthropods as revealed by Synchrotron X-Ray Tomographic Microscopy. *PLoS ONE*, 7(8), e42582.

Fielding, S., Martill, D.M. and Naish, D. (2005) Solnhofen-style soft-tissue preservation in a new species of turtle from the Crato Formation (Early Cretaceous, Aptian) of north-east Brazil. *Palaeontology*, 48(6), pp. 1301-1310.

Frey, D., Tishlinger, H., Buchy, M. and Martill, D.M. (2003) New specimens of Pterosauria (Reptilia) with soft parts with implications for pterosaurian anatomy and locomotion. 233-266. In Buffetaut, E. and Mazin, J.M. eds. Evolution and palaeobiology of pterosaurs. *Geological Society*, London, Special Publication, 217, 347 pp.

Frickhinger, K.A. (1994) Die Fossilien von Solnhofen: Dokumentation der aus den Plattenkalken bekannten Tiere und Pflanzen (The fossils of Solnhofen). Goldschneck: Korb., p. 336.

Frickhinger, K.A. (1999) Die Fossilien von Solnhofen, 2, Korb: Goldschneck, p. 190.

Gerdes, G. (2003) Biofilms and macroorganisms. In: Krumbein, W.E., Paterson, D.M. and Zavarzin, G.A. eds. Fossil and Recent Biofilms: A Natural History of Life on Earth. *Springer-Science & Business Media*, Dordrecht, pp. 197-216.

Gobbo, S.R. and Bertini, R.J. (2013) Tecidos moles (não resistentes): como se fossilizam? *Terrae didatica*, 10:2-13.

Grimaldi, D.A. (1990) Insects from the Santana Formation, Lower Cretaceous, of Brazil. *B Am Mus Nat Hist*, 195, pp. 1-191.

Grimaldi, D.A. and Engel, M.S. (2005) Evolution of the Insects. *Cambridge University Press*, New York, 755 p.

Grimes, S.T., Davies, K.L., Butler, I.B., Brock, F., Edwards, D., Rickard, D., *et al.* (2002) 'Fossil plants from the Eocene London Clay: the use of pyrite textures to determine the mechanism of pyritization,' *Journal of the Geological Society*, 159(5), pp. 493–501. https://doi.org/10.1144/0016-764901-176.

Heimhofer, U. and Martill, D.M. (2007) The sedimentology and depositional environment of the Crato Formation. In: Martill, D.M., Bechly, G. and Loveridge, R.F. eds. The Crato Fossil Beds of Brazil. *Cambridge University Press*, Cambridge, pp. 44-62.

Heimhofer, U., Ariztegui, D., Lenniger, M., Hesselbo, S.P., Martill, D.M., Rios-Netto, A.M. *et al.* (2010) 'Deciphering the depositional environment of the laminated Crato fossil beds (Early Cretaceous, Araripe Basin, North-eastern Brazil),' *Sedimentology*, 57(2), pp. 677–694. https://doi.org/10.1111/j.1365-3091.2009.01114.x.

Hess, H., Ausich, W.I., Brett, C.E. and Simms, M.J. eds. (1999) Fossil Crinoids. *Cambridge University Press.*

Heyng, A., Rothgaenger, M. and Röper, M. (2015) Die Grabung Brunn. In: Arratia, G., Schultze, H-P., Tischlinger, H. and Viohl, G. eds. Solnhofen. Ein Fenster in die Jurazeit. Munich, Verlag Dr. Friedrich Pfeil, pp. 114-118.

Iniesto, M., Lopez-Archilla, A.I., Fregenal-Martínez, M., Buscalioni, A.D., Carmen-Guerrero, M. *et al.* (2013) 'Involvement of microbial mats in delayed decay: An experimental essay on fish preservation,' *Palaios*, 28(1), pp. 56–66. https://doi.org/10.2110/palo.2011.p11-099r.

Iniesto, M., Laguna, C., Florín, M., Guerrero, M.C., Chicote, A., Buscalioni, A.D, *et al.* (2015) 'The impact of microbial mats and their microenvironmental conditions in early decay of fish,' *Palaios*, 30(11), pp. 792–801. https://doi.org/10.2110/palo.2014.086.

Iniesto, M., Gutierrez-Silva, P., Dias, J.J., Carvalho, I.S., Buscalioni, A.D. and López-Archilla, A.I. (2021) 'Soft tissue histology of insect larvae decayed in laboratory experiments using microbial mats: Taphonomic comparison with Cretaceous fossil insects from the exceptionally preserved biota of Araripe, Brazil,' *Palaeogeography, Palaeoclimatology, Palaeoecology*, 564, p. 110156. https://doi.org/10.1016/j.palaeo.2020.110156.

Keupp, H. (1977) Ultrafazies und Genese der Solnhofener Plattenkalke. Abh. Naturhist. Ges. Nürnberg e.V., 37, pp. 1-127.

Klug, C., Fuchs, D., Schweigert, G., Röper, M. and Tischlinger, H. (2015) 'New anatomical information on arms and fins from exceptionally preserved Plesioteuthis (Coleoidea) from the Late Jurassic of Germany,' *Swiss Journal of Palaeontology*, 134(2), pp. 245–255. https://doi.org/10.1007/s13358-015-0093-y.

Kundrát, M., Nudds, J., Kear, B.P., Lü, J. and Ahlberg, P. (2018) 'The first specimen ofArchaeopteryxfrom the Upper Jurassic Mörnsheim Formation of Germany,' *Historical Biology*, 31(1), pp. 3–63. https://doi.org/10.1080/08912963.2018.1518443.

Lafuente, B., Downs, R.T., Yang, H. and Stone, N. (2015) The power of databases: the RRUFF project. In: Armbruster, T. and Danisi, R.M. eds. Highlights in Mineralogical Crystallography, Berlin, Germany, W. De Gruyter, pp. 1-30.

Leakey, L.S.B. (1952) Lower Miocene invertebrates from Kenya. *Nature,* 169, pp. 624-625.

Li, J., Pei, R. and Xu, X. (2020) 'Micro-XRF study of the troodontid dinosaur Jianianhualong Tengi reveals new biological and taphonomical signals,' *Atomic Spectroscopy*, 41(6), pp. 1–11. https://doi.org/10.46770/as.2021.01.001.

Li, S. and Hihara, L.H. (2015) 'A Micro-Raman Spectroscopic study of marine atmospheric corrosion of Carbon Steel: The effect of Akaganeite,' *Journal of the Electrochemical Society*, 162(9), pp. C495–C502. https://doi.org/10.1149/2.0881509jes.

Lima, F.J., Pires, E.F., Saraiva, A.Á.F., Sayão, J.M., Jasper, A. and Uhl, D. (2021) 'Early Cretaceous (Aptian–Albian) wildfires in the Araripe basin, northeast Brazil: palaeoclimatic and palaeoenvironmental implications,' in *Springer eBooks*, pp. 1–12. https://doi.org/10.1007/978-3-319-90913-4_32-1.

Maas, A., Braun, A., Dong, X.-P., Donoghue, P. C. J., Müller, K. J., Olempska, E., *et al.* (2006) The 'Orsten'–More than a Cambrian Konservat-Lagerstätte yielding exceptional preservation. *Palaeoworld*, 15, pp. 266-282.

Martill, D.M and Wilby, P.R. (1993) Stratigraphy. In: Martill, D.M. ed. Fossils of the Santana and Crato Formations, Brazil. Field Guides to Fossils No. X. *Palaeontological Association*, London, p. 159.

Martill, D.M., Loveridge, R. and Heimhofer, U. (2007) 'Halite pseudomorphs in the Crato Formation (Early Cretaceous, Late Aptian–Early Albian), Araripe Basin, northeast Brazil: further evidence for hypersalinity,' *Cretaceous Research*, 28(4), pp. 613–620. https://doi.org/10.1016/j.cretres.2006.10.003.

Martill, D.M., Loveridge, R.F. and Heimhofer, U. (2008) 'Dolomite pipes in the Crato Formation fossil lagerstätte (Lower Cretaceous, Aptian), of northeastern Brazil,' *Cretaceous Research*, 29(1), pp. 78–86. https://doi.org/10.1016/j.cretres.2007.04.007.

Martins-Neto, R.G. (1996) New mayflies (Insecta, Ephemeroptera) from the Santana Formation (Lower Cretaceous), Araripe Basin, northeastern Brazil. *Revista Española de Paleontologia*, 11(2), pp. 177-192.

Martins-Neto, R.G. (2005) Estágio atual da paleoartropodologia Brasileira: hexápodes, miriápodes, crustáceos (Isopoda, Decapoda, Eucrustacea e Copepoda) e quelicerados. *Arquivos do Museu Nacional*, 63(3), pp. 471-494.

Martins-Neto, R.G. (2006) Insetos fósseis como bioindicadores em depósitos sedimentares: um estudo de caso para o Cretáceo da Baciado Araripe (Brasil). *Revista Brasileira de Zoociências*, 8(2), pp. 155-183.

Martínez-Delclòs, X., Briggs, D.E.G. and Peñalver, E. (2004) 'Taphonomy of insects in carbonates and amber,' *Palaeogeography, Palaeoclimatology, Palaeoecology*, 203(1–2), pp. 19–64. https://doi.org/10.1016/s0031-0182(03)00643-6.

Martínez-Delclòs, X. and Martinell, J. (1993) Insect taphonomy experiments: their application to the Cretaceous outcrops of lithographic limestones from Spain. *Kaupia*, 2, pp. 133-144.

Mccafferty, W.P. (1990) Chapter 2. Ephemeroptera. *Bulletin of the American Museum of Natural History*, 195, pp. 20-50.

McCobb, L.M.E., Duncan, I.J., Jarzembowski, E.A., Stankiewicz, B.A., Wills, M.A. and Briggs, D.E.G. (1998) 'Taphonomy of the insects from the Insect Bed (Bembridge Marls), late Eocene, Isle of Wight, England,' *Geological Magazine*, 135(4), pp. 553–563. https://doi.org/10.1017/s0016756898001204.

Melo, R.M., Guzman, J., Almeida-Lima, D., Piovesan, E.K., Neumann, V.H.M.L. and Sousa, A.J. (2020) 'New marine data and age accuracy of the Romualdo Formation, Araripe Basin, Brazil,' *Scientific Reports*, 10(1). https://doi.org/10.1038/s41598-020-72789-8.

Menges, F. (2022) 'Spectragryph - optical spectroscopy software', Version 1.2.16.1.

Menon, F. and Martill, D.M. (2007) Taphonomy and preservation of Crato Formation arthropods. In: Martill, D.M., Bechly, G., Loveridge, R.F. eds. The Crato Fossil Beds of Brazil: Window into an ancient world. *Cambridge University Press*, pp. 79-96.

Moura-Júnior, D.A., Scheffler, S.M., Moreira, F.F., Nel, A. and Mejdalani, G. (2020) 'First record of a shore bug (Insecta, Hemiptera, Saldidae) from Gondwana,' *Journal of Paleontology*, 95(1), pp. 133–140. https://doi.org/10.1017/jpa.2020.61.

Munnecke, A., Westphal, H. and Kölbl-ebert, M. (2008) 'Diagenesis of plattenkalk: examples from the Solnhofen area (Upper Jurassic, southern Germany),' *Sedimentology*, 55(6), pp. 1931–1946. https://doi.org/10.1111/j.1365-3091.2008.00975.x.

Nel, A. and Pouillon, J.-M. (2020) 'The third skimmer dragonfly species from the Lower Cretaceous (Aptian) Crato Formation in NE Brazil (Odonata, Cavilabiata, Araripelibellulidae),' *Cretaceous Research*, 115, p. 104565. https://doi.org/10.1016/j.cretres.2020.104565.

Neumann, V.H. and Assine, M.L. (2015) Stratigraphic proposal to Post-Rift I Tectonic-Sedimentary Sequence of Araripe Basin, Northeastern Brazil. *Proceedings of the 2nd International Congress on Stratigraphy*, Graz, Austria.

Neumann, V.H., Borrego, A.G., Cabrera, L. and Dino, R. (2003) Organic matter composition and distribution through the Aptian–Albian lacustrine sequences of the Araripe Basin, northeastern Brazil. Int J Coal Geol. 2003; 54:21-40.

Neumann, V.H. and Cabrera, L. (1999) Una nueva propuesta estratigráfica para la tectonosecuencia post-rifte de la cuenca de Araripe, nordeste de Brasil. *Boletim do 5° Simpósio sobre o Cretáceo do Brasil*, pp. 279-85.

Niebuhr, B. and Pürner, T. (2014) Lithostratigraphie der Weißjura-Gruppe der Frankenalb (außeralpiner Oberjura) und der mittel- bis oberjurassischen Reliktvorkommen zwischen Straubing und Passau (Bayern). *Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften*, 83, pp. 5-72.

Noffke, N. and Awramik, S.M. (2013) 'Stromatolites and MISS—Differences between relatives,' *GSA Today*, 23(9), pp. 4–9. https://doi.org/10.1130/gsatg187a.1.

Oliveira, G.R. and Kellner, A.W.A. (2017) 'Rare hatchling specimens of Araripemys Price, 1973 (Testudines, Pelomedusoides, Araripemydidae) from the Crato Formation, Araripe Basin,' *Journal of South American Earth Sciences*, 79, pp. 137–142. https://doi.org/10.1016/j.jsames.2017.07.014.

Osés, G.L., Petri, S., Becker-Kerber, B., Romero, G.R., Rizzutto, M.A., Rodrigues, F., *et al.* (2016) 'Deciphering the preservation of fossil insects: a case study from the Crato Member, Early Cretaceous of Brazil,' *PeerJ*, 4, p. e2756. https://doi.org/10.7717/peerj.2756.

Osés, G.L., Petri, S., Voltani, C.G., Prado, G.M.E.M., Galante, D., Rizzutto, M.A., *et al.* (2017) 'Deciphering pyritization-kerogenization gradient for fish soft-tissue preservation,' *Scientific Reports*, 7(1). https://doi.org/10.1038/s41598-017-01563-0.

Palmer, A.R. (1957) Miocene arthropods from the Mojave Desert California. In: Geological survey professional paper 294-G. Washington: *United States Government Printing Office.*

Pan, Y., Hu, L. and Zhao, T. (2018) 'Applications of chemical imaging techniques in paleontology,' *National Science Review/National Science Review*, 6(5), pp. 1040–1053. https://doi.org/10.1093/nsr/nwy107.

Pan, Y., Sha, J. and Fursich, F.T. (2014) 'A model for organic fossilization of the Early Cretaceous Jehol Lagerstatte based on the taphonomy of '*Ephemeropsis trisetalis*,'' *Palaios*, 29(7), pp. 363–377. https://doi.org/10.2110/palo.2013.119.

Park, M.H. and Fürsich, F.T. (2002) Stable isotopes and trace element compositions of the Upper Jurassic Solnhofen platy limestone. *Geoscience Journal*, 6, pp. 269-276.

Parker, A.R. and McKenzie, D.R. (2003) 'The cause of 50 million-year-old colour,' *Proceedings - Royal Society. Biological Sciences/Proceedings - Royal Society. Biological Sciences*, 270(suppl_2). https://doi.org/10.1098/rsbl.2003.0055.

Perardi, A., Zoppi, A. and Castellucci, E. (2000) Micro-Raman spectroscopy for standard and in situ characterisation of painting materials. *Journal of Cultural Heritage*, 1, pp. 269-272.

Ponomarenko, A.G. (1985) Fossil insects from the Tithonian „Solnhofener Plattenkalke" in the Museum of Natural History, Vienna. *Annalen Naturhistorische Museum Wien*, 1985; 87(A), pp. 135-144.

Pouillon, J.-M. and Nel, A. (2020) 'The oldest representative of the modern clade Aeshnodea from the Lower Cretaceous Crato Formation, Araripe Basin, NE Brazil (Odonata: Anisoptera),' *Cretaceous Research*, 116, p. 104580. https://doi.org/10.1016/j.cretres.2020.104580.

Prieto-Barajas, C.M., Valencia-Cantero, E. and Santoyo, G. (2018) 'Microbial mat ecosystems: Structure types, functional diversity, and biotechnological application,' *Electronic Journal of Biotechnology*, 31, pp. 48–56. https://doi.org/10.1016/j.ejbt.2017.11.001.

Prado, G., Arthuzzi, J.C.L., Osés, G.L., Callefo, F., Maldanis, L., Sucerquia, P., *et al.* (2021) 'Synchrotron radiation in palaeontological investigations: Examples from Brazilian

fossils and its potential to South American palaeontology,' *Journal of South American Earth Sciences*, 108, p. 102973. https://doi.org/10.1016/j.jsames.2020.102973.

Ribeiro, A.C., Ribeiro, G.C., Varejão, F.G., Battirola, L.D., Pessoa, E.M., Simões, M.G., *et al.* (2021) 'Towards an actualistic view of the Crato Konservat-Lagerstätte paleoenvironment: A new hypothesis as an Early Cretaceous (Aptian) equatorial and semi-arid wetland,' *Earth-science Reviews*, 216, p. 103573. https://doi.org/10.1016/j.earscirev.2021.103573.

Röper, M. (2005) Field Trip B: East Bavarian plattenkalk – different types of Upper Kimmeridgian to Lower Tithonian plattenkalk deposits and facies. *Zitteliana*, B, 26, pp. 57-70.

Röper, M. and Rothgaenger, M. (1995) Eine neue Fossillagerstätte in den ostbayerischen Oberjura-Plattenkalken bei Brunn/Oberpfalz. Erster Forschungsbericht. Freunde der Bayerischen Staatssammlung für Paläontologie und Historische Geologie eV, Jahresbericht und Mitteilungen, 24, pp. 32-46.

Santos, M.F.A., Mattos, I., Mermudes, J.R.M., Scheffler, S.M. and Reyes-Castillo, P. (2021) 'A new passalid fossil (Insecta: Coleoptera) from the Santana Formation (Crato member, Lower Cretaceous), Araripe Basin, NE Brazil: Paleoecological and paleobiogeographic implications,' *Cretaceous Research*, 118, p. 104664. https://doi.org/10.1016/j.cretres.2020.104664.

Saraiva, A.A.F., Lima, F.J., Barros, O.A. and Bantim, R.A.M. (2021) Guia de fósseis da Bacia do Araripe. 378 pp. Crato: Ceará.

Schlüter, T., Kohring, R. and Gregor, H-J. (2002) Dragonflies preserved in transparent gypsum crystals from the Messinian (Upper Miocene) of Alba, northern Italy. *Acta Zoologica Cracoviensia*, 46, pp. 373-379.

Schweigert, G. (2007) Ammonite biostratigraphy as a tool for dating Upper Jurassic lithographic limestones from South Germany – first results and open questions. *Neues Jahrbuch für Geologie und Paläontologie*, 245, pp. 117-125.

Schweigert, G. (2015) Biostratigraphie der Plattenkalke der südlichen Frankenalb. In: Arratia, G., Schultze, H.P., Tischlinger, H. and Viohl, G. eds. Solnhofen. Ein Fenster in die Jurazeit. Munich, Verlag Dr. Friedrich Pfeil. pp. 63-66.

Schwermann, A.H., Rolo, T.S., Caterino, M.S., Bechly, G., Schmied, H., Baumbach, T., *et al.* (2016) 'Preservation of three-dimensional anatomy in phosphatized fossil arthropods enriches evolutionary inference,' *eLife*, 5. https://doi.org/10.7554/elife.12129.

Seilacher, A., Reif, W.E. and Westphal, F. (1985) Sedimentological, ecological and temporal patterns of Fossil-Lagerstätten. *Philosophical Transactions of the Royal Society of London*, 311(B), pp. 5-23.

Staniczek, A.H. (2007) Ephemeroptera: mayflies. In: Martil, D.M., Bechly, G. and Loveridge, R.F. eds. The Crato Fossil Beds of Brazil. Cambridge: *Cambridge University Press*, pp. 163-184.

Staniczek, A.H., Storari, A.P. and Godunko, R.J. (2022) 'Revised systematics, phylogeny, and paleontology of the mayfly family Baetiscidae (Insecta: Ephemeroptera),' *Arthropod Systematics & Phylogeny*, 80, pp. 389–409. https://doi.org/10.3897/asp.80.e82845.

Storari AP, Godunko RJ, Salles FF, Saraiva AAF, Staniczek AH, Rodrigues T. (2021) 'An overview of the Hexagenitidae (Ephemeroptera) from the Crato Formation (Aptian, Lower Cretaceous) of Brazil, with the description of a new species,' *Historical Biology*, 34(5), pp. 875–884. https://doi.org/10.1080/08912963.2021.1952196.

Storari, A.P., Osés, G.L., Almeida-Lima, D.S.D., Rizzutto, M.A., Bantim, R.A.M., Lima, F.J., *et al.* (2024) 'Exceptionally well-preserved orthopteran proventriculi from the Cretaceous Crato Formation of Brazil,' *Journal of South American Earth Sciences*, 133, p. 104737. https://doi.org/10.1016/j.jsames.2023.104737.

Storari, A.P., Rodrigues, T., Bantim, R.A.M., Lima, F.J. and Saraiva, A.A.F. (2021) 'Mass mortality events of autochthonous faunas in a Lower Cretaceous Gondwanan Lagerstätte,' *Scientific Reports*, 11(1). https://doi.org/10.1038/s41598-021-85953-5.

Tałanda, M. (2018) 'An exceptionally preserved Jurassic skink suggests lizard diversification preceded fragmentation of Pangaea,' *Palaeontology*, 61(5), pp. 659–677. https://doi.org/10.1111/pala.12358.

Tischlinger, H. (2001) Bemerkungen zur Insekten-Taphonomie der Solnhofener Plattenkalke. *Archaeopteryx*, 19, pp. 29-44.

Tischlinger, H. and Unwin, D. (2004) UV-Untersuchungen des Berliner Exemplars von *Archaeopteryx lithographica* H.v.Meyer 1861 und der isolierten *Archaeopteryx*-Feder. *Archaeopteryx*, 22, pp. 17-50.

Varejão, F.G., Warren, L.V., Simões, M.G., Buatois, L.A., Angano, G.M. and Bahniuk-Rumbelsperger, A.N. (2021) 'Mixed siliciclastic–carbonate sedimentation in an evolving

epicontinental sea: Aptian record of marginal marine settings in the interior basins of north-eastern Brazil,' *Sedimentology*, 68(5), pp. 2125–2164. https://doi.org/10.1111/sed.12846.

Varejão, F.G., Warren, L.V., Simões, M.G., Fürsich, F.T., Matos, S.A. and Assine, M.L. (2019) 'Exceptional preservation of soft tissues by microbial entombment: Insights into the taphonomy of the Crato Konservat-Lagerstätte,' *Palaios*, 34(7), pp. 331–348. https://doi.org/10.2110/palo.2019.041.

Viana, M.S. and Neumann, V.H.L. (2002) Membro Crato da Formação Santana, Chapada do Araripe, CE - Riquíssimo registro de fauna e flora do Cretáceo. In: Schobbenhaus, C., Campos, D.A., Queiroz, E.T., Winge, M. and Berbert-Born, M.L.C. eds. Sítios Geológicos e Paleontológicos do Brasil. DNPM/CPRM - *Comissão Brasileira de Sítios Geológicos e Paleobiológicos*, pp. 113-120.

Viohl, G. (1990) Taphonomy of Fossil-Lagerstätten. Solnhofen Lithographic Limestones. In Briggs, D.E.G. and Crowther, P.R. eds. Palaeobiology. A synthesis. *Oxford: Blackwell*, pp. 285–289

Viohl, G. (1994) Fish taphonomy of the Solnhofen Plattenkalk. An approach to the reconstruction of paleoenvironment. *Géo Mém Spéc*. 16, pp. 81-90.

Viohl, G. (2015) Die Plattenkalk-Typen der südlichen Frankenalb. In: Arratia, G., Schultze, H.P., Tischlinger, H. and Viohl, G. eds. Solnhofen. Ein Fenster in die Jurazeit. Munich, Verlag Dr. Friedrich Pfeil, pp. 72-77.

Wang, B., Zhang, H.C., Wappler, T. and Rust, J. (2010) 'Palaeontinidae (Insecta: Hemiptera: Cicadomorpha) from the Upper Jurassic Solnhofen Limestone of Germany and their phylogenetic significance,' *Geological Magazine*, 147(4), pp. 570–580. https://doi.org/10.1017/s0016756809990896.

Wang, B., Zhao, F., Zhang, H., Fang, Y. and Zheng, D. (2012) 'Widespread pyritization of insects in the Early Cretaceous Jehol biota,' *Palaios*, 27(10), pp. 707–711. https://doi.org/10.2110/palo.2012.p12-029r.

Wappler, T. and Ben-Dov, Y. (2008) 'Preservation of Armoured Scale Insects on Angiosperm Leaves from the Eocene of Germany,' *Acta Palaeontologica Polonica*, 53(4), pp. 627–634. https://doi.org/10.4202/app.2008.0407.

Warren, L.V., Varejão, F.G., Quaglio. F., Simões. M.G., Fürsich, F.T., Poiré, D.G., *et al.* (2016) 'Stromatolites from the Aptian Crato Formation, a hypersaline lake system in the Araripe Basin, northeastern Brazil,' *Facies*, 63(1). https://doi.org/10.1007/s10347-016-0484-6.

Wilby, P.R. and Briggs, D.E.G. (1997) Taxonomic trends in the resolution of detail preserved in fossil phosphatized soft-tissues. *Geobios*, 20, 493e502.

Chapter 3 - Taphonomy of aquatic insects from the Crato Formation Lagerstätte (Aptian, Lower Cretaceous) under an actualistic look

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Abstract

The Crato Formation holds some of the most interesting fossiliferous deposits in the world, representing a lacustrine paleoenvironment, and therefore, important for the study of aquatic insects and their interaction with this past environment. However, only a few investigations have analyzed the general insect taphonomy of this deposit so far, yet none used actualistic data. Here, we present the results of experiments performed to determine the nature of the taphonomic processes and patterns responsible for the exceptional preservation seen in the larvae of mayflies and dragonflies and winged mayflies preserved in the Crato Formation. The experiments were performed with 253 extant Ephemeroptera, and 236 extant Odonata, including survival rates tests, tests on flotation under different salinity rates, and disarticulation tests. We then compared our results with a total of 306 fossil mayflies and dragonflies from the Crato Formation. During disarticulation tests, the thorax of the extant mayfly larval carcasses was the most likely element to disarticulate first, but the Hexagenitidae larvae from the Crato Formation rarely present a disarticulated thorax, indicating that their carcasses suffered little disturbance, typical of an autochthonous assemblage. We also noticed that alate fossil specimens lacking their wings'

margins and/or with their wings withered, which corresponded to longer decay time in our observations, were extremely rare. During our experiments, once dead or close to dying, the dragonflies assumed a characteristic position, with the hind legs distended laterally and the forelegs extended anteriorly, that changed as soon as any mechanical disturbance was applied. Several gomphid fossil larvae from the Crato Formation were also preserved in this characteristic position, indicating minimal transport after death. Moreover, since we rarely found the labial mask disarticulated in the fossil dragonflies (the most common structure to disarticulate first during the experiments), we assume these larvae were not transported for long distances, representing parautochthonous assemblages. In our experiments, the larvae of mayflies in lower salinity conditions were defecating in unusually large amounts long prior to death, probably to directly release salt in their feces. Although we have not seen anything similar in the Crato fossils, this does not exclude the possibility that the larvae were living in low-salinity conditions, since the digestive tract and feces are extremely delicate and difficult to preserve. We also noticed during experimentation that all carcasses immediately floated under hypersaline conditions and that carcasses immersed in saline conditions went through slower decomposition. Finally, our experiments have shown that when small insects, such as mayflies, die in sub-aerial conditions, there are few possibilities of overcoming the surface tension and sinking. Thus, we propose for the first time for the Crato Formation that microbial biofilms on the surface of the water were acting during carcass sinking.

Keywords: Biostratinomy; Odonata; Ephemeroptera; mayflies; dragonflies; Araripe Basin

Introduction

The Crato Formation holds some of the most interesting fossiliferous deposits in the world (Carvalho and Santos, 2005). It is especially important for palaeoentomology due to its Lower Cretaceous age, location, and the terrestrial origin of most of its fossils, since the majority of other important deposits for the study of insects are both of non-terrestrial origin and occur in the Northern Hemisphere (Grimaldi and Engel, 2005; Penney and Jepson, 2014). Nevertheless, since it mostly represents a lacustrine paleoenvironment (Ribeiro et al., 2021) it is particularly important for the study of its aquatic insects and their interaction with this past environment. The most prominent aquatic insects occurring in the Crato Formation are Ephemeroptera and Odonata,

which present outstanding abundance, diversity, and exceptionally preserved specimens (Menon and Martill, 2007).

However, most studies on the fossil Ephemeroptera and Odonata of the Crato Formation focused only on taxonomy (e.g., Brito, 1987; McCafferty, 1990; Martins-Neto and Caldas, 1990; Nel and Escuillié, 1994; Martins-Neto, 1996; Bechly, 1997, 1998; Jarzembowski et al., 1998; Polegatto and Zamboni, 2001; Bechly et al., 2001; Martins-Neto, 2005; Bechly, 2007, 2010; Pouillon and Nel, 2020; Storari et al., 2020; Storari et al., 2021a; Staniczek et al., 2022). Recently, several investigations that analyzed the general insect taphonomy of that unit been published (Menon & Martill, 2007; Barling et al., 2015; Osés et al., 2016; Varejão et al., 2019; Bezerra et al., 2020; Barling et al., 2021; Ribeiro et al., 2021; Storari et al., 2021b; Dias and Carvalho, 2022; Bezerra and Mendes, 2024), due to the fact that arthropod assemblages can provide important clues about the nature of the paleoenvironment. Freshwater taxa are especially informative, and fossil mayflies from the Crato Formation are ideal for taphonomical studies as their diversity and abundance could allow quantitative analyses of the paleolake (Storari et al., 2021b). Among them, the Hexagenitidae is the most common family of arthropods found in that deposit, with their larvae representing 85% of the total number of insect specimens in a controlled excavation (Storari et al., 2021b). The Odonata are also well-represented, comprising approximately 2% of this deposit's total number of insect fossil specimens (Bechly, 1998). Therefore, the study of the taphonomy of these groups can provide insights into the nature of the Crato paleoenvironment.

In this work, we focus on the taphono-biostratinomic study of the aforementioned aquatic groups, since the sedimentary history of organic remains includes processes that can be inferred after the biostratinomic analysis of fossils. The disarticulation and transport, for instance, tend to be two closely related parameters, as the first often occurs due to the latter, although disarticulation can occur naturally with no disturb (Martínez-Delclòs and Martinell, 1993; Briggs and McMahon, 2006). Disarticulation is also associated with a prolonged period between the death of an organism and its burial, so if burial occurs before complete necrolysis of the soft tissue, a carcass could be preserved practically intact and articulated (Schäfer, 1972).

A branch of taphonomy, traditionally underexplored for the Crato Formation fauna, is the actualistic taphonomy, also investigated in this work. It uses comparative studies between the fossil records and experiments on the preservation and decomposition of animal and plant carcasses and can provide important palaeoenvironmental data (Ritter et al., 2023). There are only a few studies

which explored experimental data on modern fauna as a proxy for studying the fossils from Crato. For arthropods, spider leg flexion was used by Downen et al. (2016) for inferring salinity in the paleolake; while Iniesto et al. (2021) studied the role of microbial mats in the decay of the larvae of an extant moth and mealworm species; recently, Dias et al. (2023) also compared microscopic signatures supposedly left by microbial mats in mayfly larvae fossils with signatures left by modern microbial mats in salt pans. Arthropods are good candidates for actualistic analyses because many experiments can be done under controlled laboratory environments, and most groups have modern equivalents. For instance, the overall body shape of larval stages of Hexagenitidae is similar to representatives of Tridentiseta (*sensu* Kluge, 2004) or Pisciforma (*sensu* McCafferty, 1991), which usually have streamlined bodies (Ogden et al., 2009). Among them, the extant family Baetidae is widely distributed on all continents, except Antarctica (Edmunds et al., 1976; Chinery, 1986) and, since it is the only Tridentiseta clade occurring in Brazil (Salles et al., 2018), it is the appropriate group for taphono-comparative actualistic studies in the country. Since Hexagenitidae is by far the most abundant arthropod group of the Crato Formation and the only well-represented one with available controlled excavation data, it was our focus during this study. However, to broaden our understanding of the aquatic biota and to compare the results among the most prominent aquatic insect groups occurring in that unit, we also assessed the taphonomical patterns of the Odonata and examined data from extant larval individuals. Thus, we performed experiments to determine the nature of the taphonomic processes responsible for the biostratinomic preservation of larval and winged mayflies and larval dragonflies of the Crato Formation.

Material and Methods

Extant specimen collection

To perform the experiments with extant specimens, a total of 253 *Callibaetis capixaba* Cruz, Salles & Hamada larvae (Ephemeroptera: Baetidae), and 236 Odonata larvae (vast majority of them Gomphidae - Anisoptera, but also the Anisoptera families Aeshnidae, and Libellulidae; and the Zygoptera families Calopterygidae, and Coenagrionidae) were collected in Santa Teresa municipality, Espírito Santo (Brazil), in the locations Nova Lombardia (S 19° 52' 31.6" and W 40° 31' 49.1") and Augusto Ruschi Biological Reserve (S 19° 53' 20.6" and W 40° 32' 41.5") under sampling authorization number SISBIO 81584-1 (three sampling trips in the years of 2021 and

2022). Besides its morphological similarities with Hexagenitidae, we chose *C. capixaba* among other baetids because it presents a reasonable size for macroscopic observations (ca. 10 mm), and can be easily collected in its preferred habitat, sandbanks (Figure 1). The odonatan groups collected were available while sampling in the preferred habitat of *C. capixaba* and share similarities in body plan with Odonata taxa occurring in the Crato Formation.

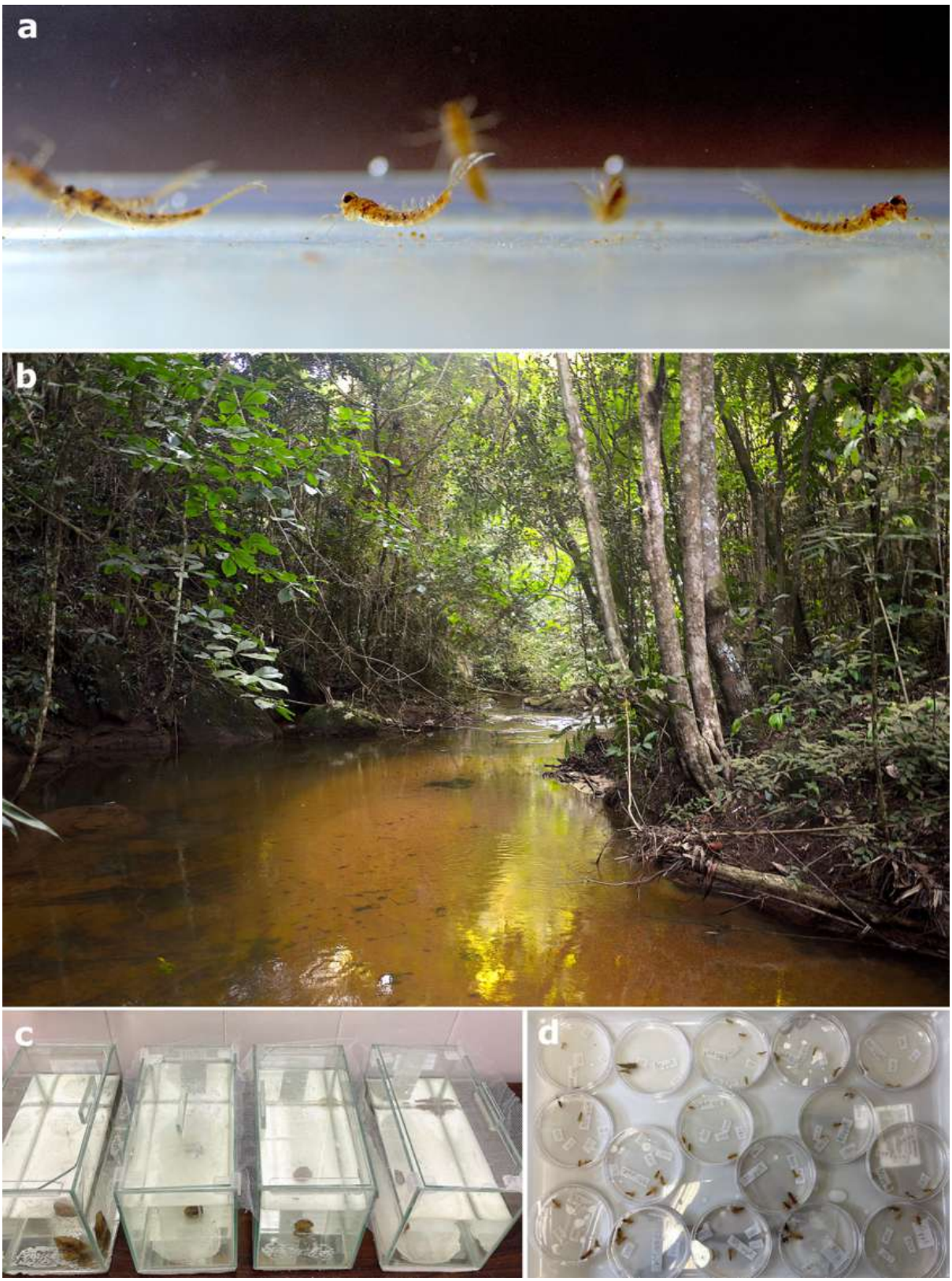
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Figure 1. a) Living *Callibaetis capixaba* larvae (Ephemeroptera: Baetidae) during the experiments. b) One of the sampling points in the Atlantic forest of Nova Lombardia, Santa Teresa municipality, Espírito Santo, Brazil. The preferred habitat of *C. capixaba* larvae were sand banks and overall sandy substrate of semi-lotic streams. c) Aquariums where experiments were conducted with the sampled specimens: two aquariums for salinity tests and two for temperature tests. d) Petri dishes with dragonfly carcasses during disarticulation tests.

Collections were made using sieves and nets with mesh openings of 0.5 and 1.0 mm. The taxonomic identifications were based on Cruz et al. (2017). Salinity, pH, and temperature conditions on the field were measured to establish values for the control groups using a Yieryi Portable refractometer, disposable PRODAC pH tests, and a Boyu glass thermometer. The specimens were placed in individual plastic containers for transport, following the protocol of Boldrini and Cruz (2013), and reared in glass aquariums with filtered water from the collection site (Figure 1). After the end of the experiments, the specimens were deposited at the entomological collection of the Federal University of Viçosa in a collective batch (Storari sampling, Santa Teresa, 2021).

Experiments

Experiments were done over the course of six months and included three replicates (R1, R2, and R3). We performed the main experiments with mayflies (including survival rates and disarticulation tests) and additional experiments with dragonflies (tests on disarticulation and flotation under different salinity rates) (see Figure 2 for simplified schematization of experiments; and Supplementary Tables S1 and S2 for results with extant Ephemeroptera and Odonata specimens, respectively).

During and after the experiments, the specimens were examined using a Leica binocular microscope, and photographs were taken with a Nikon D800 digital camera. When possible, serial photographs with different focal planes were taken, and the photo stacks were posteriorly processed with Helicon Focus Pro 6.4.1.

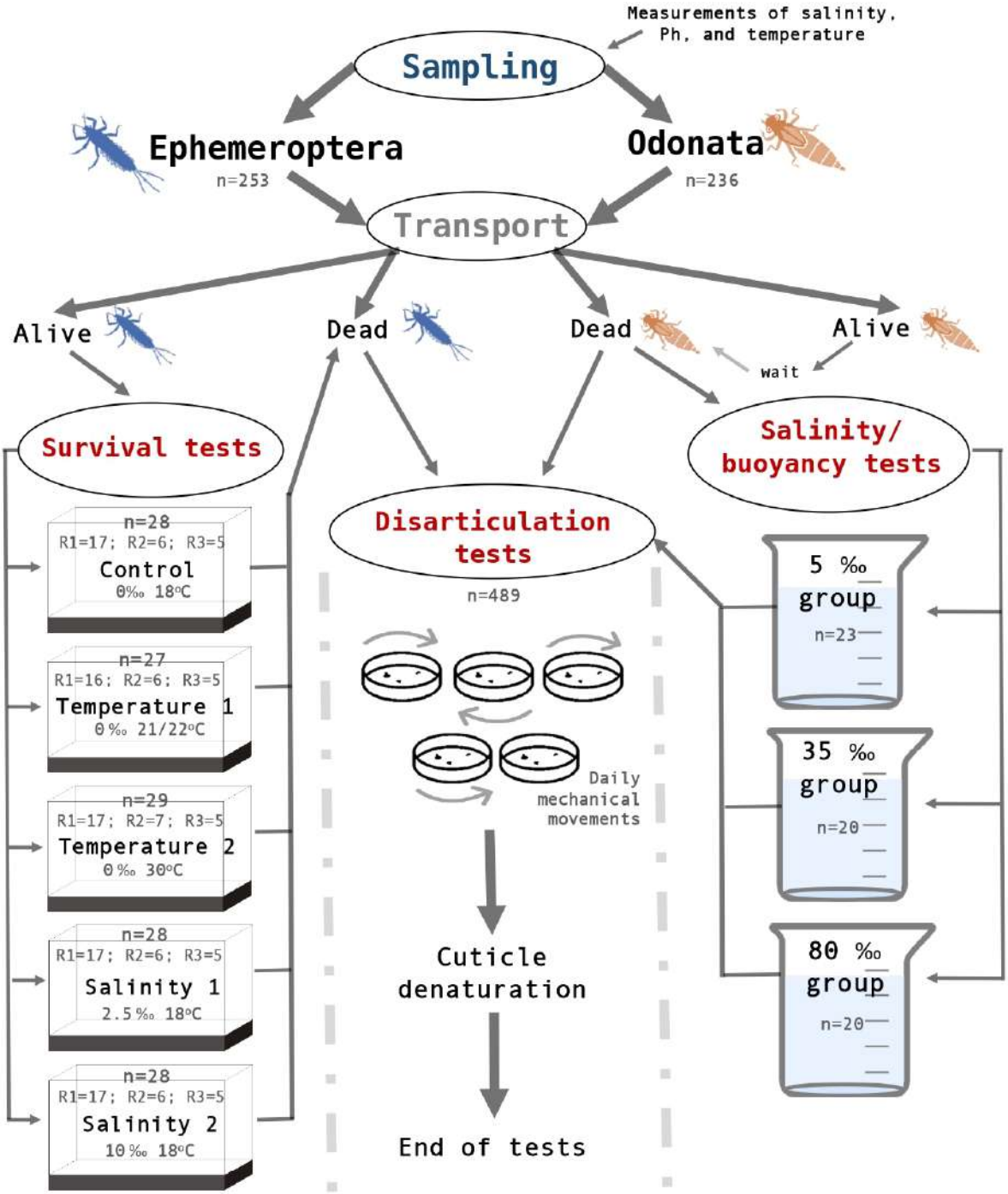

Figure 2. Schematic drawing of the experiments. Simplified infographic of our experiments' steps, from sampling to the end of tests. 'n' is the total sample number (from the three replicates together, which represents three sampling trips). R= replicate.

Survival rate tests: This test was performed to evaluate whether mayflies would present different trends of post-mortem attitudes under different abiotic conditions.

An aquarium was assigned to the control group (with 28 *C. capixaba* larvae in total for the three replicates - R1=17; R2=6; R3=5), in which controlled salinity (‰), pH, and temperature (°C) conditions were established based on those of the collection site (water conditions of 0‰; 5.5 pH; and 18°C) (Figure 2).

Four aquariums were destined for the test groups: two for salinity tests – one with 2.5‰, 5.5 pH, 18°C, with 28 *C. capixaba* larvae at total for the three replicates (R1=17, R2=6, R3=5); and the other with 10‰, 5.5 pH, 18°C, with 28 *C. capixaba* larvae at total for the three replicates (R1=17, R2=6, R3=5); and two for temperature tests – one with 21–22°C, 0‰, 5.5 pH, with 27 *C. capixaba* larvae at total for the three replicates (R1=16, R2=6, R3=5); and the other with 30°C, 0‰, 5.5 pH, with 29 *C. capixaba* larvae at total for the three replicates (R1=17, R2=7, R3=5) (Figure 2).

For allocation from the transport containers to the aquariums, a pipette with a thick tip was carefully used to handle specimens one by one (alive or carcasses). No substrate was placed in the aquariums as it would make observation of the small larvae difficult, and the aquariums were covered with a protective net adhered to their glass top (Figure 1). Dead leaves whose tissues had already been destroyed by bacteria were added to feed the larvae (Nikita Kluge, personal communication). The first batch of aquarium water was collected at the sampling site. The water was renewed with filtered water every 2–3 days to prevent bacteria and algae from proliferating (Simith and Diele, 2008).

In the salinity tests (two experimental groups), the larvae were subjected to an increase in the water's salinity, with dilution of different concentrations of saline solutions using Instant Ocean® Sea Salt, a mixture commonly used in saltwater aquariums, and measured using the refractometer. The laboratory's overall temperature was controlled daily with an air conditioner to keep the aquariums' water temperature at 18°C, while the two aquariums with increased temperature each had a Roxin aquarium heater (25W).

All larvae were reared until their death or until they reached the winged stage (i.e., subimagos). The aquariums were check daily at the same time for taking notes on death numbers and/or emerging specimens. In the latter case, we simply waited till the specimen died naturally attached to the aquarium net or drowned after falling into the water. After the death and/or molting

of the nymphs, we counted the number of individuals that reached their final winged stages (subimago or imago) in each experiment. We registered the post-mortem positions of all larvae and alate forms in the aquariums. Unfortunately, specimens escaped through the aquarium's upper net holes in many circumstances and could not be recovered for accounting. Each replicate ended once all the larvae died or emerged, and after all of them were accounted for in the disarticulation tests (see below), a new field collection was performed.

Salinity versus flotation tests: This test aimed to evaluate how dragonfly carcasses behaved with the increase of salinity in regards to flotation time and leg flexure - similarly to the test of Downen et al. (2016) with spiders.

Sixty-three odonatan larval specimens were selected immediately after their death for immersion in saline water with different concentrations (freshwater – 0.5‰, 5.5 pH, and 18°C, with 23 specimens; saltwater – 35‰, 5.5 pH, and 18°C, with 20 specimens; and hypersaline water – 80‰, 5.5 pH, and 18°C, with 20 specimens) to assess carcass flotation (Downen et al., 2016). The larvae from each group were placed in the same glass beaker, and the saline solution was poured over them to break the surface tension. Each vial was filled with the solution until full and then covered with plastic. Saline and hypersaline solutions were created using Instant Ocean® Sea Salt. The glasses were kept completely still and were photographed and observed every day to control if specimens started floating or changed the angle of their legs. After the end of tests, the carcasses were also evaluated under disarticulation tests (see below).

Disarticulation tests: This test was performed to evaluate disarticulation trends of different body parts of Ephemeroptera and Odonata.

All the collected specimens were quantified regarding their disarticulation rates. Besides the larvae, we also examined 15 winged Ephemeroptera, including ten *C. capixaba* and five representatives of Leptophlebiidae that were collected by accident. We chose to also analyze them since their adults possess the same overall morphotype as *C. capixaba*, though their larvae differ.

Odonatans were also allocated in aquariums with water conditions of 0‰, 5.5 pH, and 18°C. The specimens were kept until their natural death; after that, some of them were placed in the salinity/flotation test and the remaining in the disarticulation test (Figure 2).

After the death of the *C. capixaba* and of the odonatan larvae, we assessed disarticulation steps by carefully leaving the specimens to decompose in Petri dishes filled with water from the control group aquariums under different time slots. The specimens that died during transport and did not participate in the survival tests were also allocated to the petri dishes for the disarticulation observations. Specimens were subject to controlled, gentle movement on the water to evaluate the most sensitive parts to disarticulate under low mechanical movements. Every second day, we took notes on new elements disarticulating at hand scale (since specimens were only analyzed individually and in detail with a binocular microscope after the end of tests). Once all the analyzed specimens (or the majority of specimens in an Petri dish) started to show signs of cuticle denaturation (i.e., cuticle presented as faded and transparent), we took notes on the state of the carcass with a binocular microscope (i.e., which limbs were disarticulated at that moment), and preserved them in 70% alcohol as vouchers.

Fossil material

We analyzed a total of 306 fossil specimens: 230 Hexagenitidae larvae, 31 Hexagenitidae alates (subimagos and imagos), and 45 Odonata larvae (28 Proterogomphidae and 17 Nothomacromiidae). In order to prevent bias towards well-preserved individuals only, most of the Hexagenitidae larvae of the Crato Formation we analyzed were from the controlled excavations at the Mine Antônio Finelon (S 07^{o} 07' 22,5" e W 39^{o} 42' 01"), Nova Olinda, Ceará State (225 specimens). Since few alate Hexagenitidae specimens were recovered in that excavation, we also included literature data in our study (Brito, 1987; McCafferty, 1990; Martins-Neto, 1996; Bechly, 1998; Zamboni, 2001; Bechly, 2007; Staniczek, 2007; Brandão et al., 2021; Barling et al., 2021) and direct observation of specimens of the Paleontological collection of the Universidade Regional do Cariri – LPU URCA (Crato, Brazil), the Paleontological collection of the Museu de Paleontologia Plácido Cidade Nuvens – MPPCN (Santana do Cariri, Brazil), and the Scientific Palaeontological Collection of the Institute of Geosciences of the Universidade de São Paulo – IG USP (São Paulo, Brazil) (Supplementary Tables S3,S4).

To carry out biostratinomic analyses, the following taphonomic signatures were assessed and quantified: ontogeny (larva or adult and their body length), attitude (fossil preserved dorsally, ventrally, or laterally), and disarticulation. Disarticulation was measured qualitatively and

quantitatively, accounting for the total body elements missing from each of the specimens (e.g., specimen x is missing 1 head + 2 legs + 3 gills).

Results

Actualistic data: Mayflies

We observed that the usual *postmortem* position of the *C. capixaba* larvae in all survival rate test groups was either in dorsal view (i.e. ventral decubitus, 47% of specimens) or in ventral view (i.e. dorsal decubitus, 31% of specimens), and individuals in lateral view/decubitus after death (22% of specimens) were observed being disturbed by the activity of other larvae and/or minor currents in the aquarium (Figures 3a–d, Table 1). When the larvae died in dorsal decubitus (ventral view), they remained in a characteristic position with their legs not flexed but extended laterally near the body, with the fore legs extended forward (Figures 3a,d). The legs were flexed over the body when in dorsal view (Figure 3b).

Table 1. Postmortem position of larval mayflies. Number of specimens recovered in different postmortem attitudes (dorsal, lateral or ventral view), for each group of survival tests (control, 2.5‰ salinity, 10‰ salinity, 21–22°C temperature, and 30°C temperature).

Group	Post-mortem attitude	Number of specimens
Control	Dorsal	11
Control	Lateral	2
Control	Ventral	13
2.5‰ salinity	Dorsal	13
2.5‰ salinity	Lateral	6
2.5‰ salinity	Ventral	8
10‰ salinity	Dorsal	15
10‰ salinity	Lateral	5
10‰ salinity	Ventral	8
21–22°C temperature	Dorsal	16
21–22°C temperature	Lateral	4
21–22°C temperature	Ventral	7
30°C temperature	Dorsal	11
30°C temperature	Lateral	2
30°C temperature	Ventral	16

Figure 3. *Callibaetis capixaba* larvae during survival tests. a) Two *Callibaetis capixaba* carcasses, in dorsal view (right) and right lateral view (left). b) *C. capixaba* carcass in ventral view.

c) *C. capixaba* carcass in right lateral view. d) Five larval carcasses of *C. capixaba* that died under high temperature, showing a reddish coloration. e,f) *C. capixaba* larvae allocated at lower salinity levels and defecating in unusual amounts; 'f' already after death. Black arrows point to the excess of excrement eliminated by the larvae.

Fifteen specimens of the control group survived and reached the winged stage, combining the three replicates (R1=13, R2=1, R3=1). No specimens allocated in the experimental groups reached the winged stage, varying only on the time the larvae could stand alive before dying. All specimens allocated under a higher temperature (30°C) survived at most two days after allocation, and after death, their bodies displayed no natural curvature and had a reddish coloration (Figure 3d). The specimens allocated at 21–22°C survived the longer in comparison with the other experimental groups, with 6 specimens surviving 3 days, 1 specimen surviving 4 days, 11 specimens surviving 6 days, 3 specimens surviving 7 days, 3 specimens surviving 8 days, 2 specimens surviving 9 days, and 1 specimen surviving 10 days.

Specimens allocated in more brackish water (10‰) also died quickly, with most of them dying around one day after allocation in the aquarium (n=25), while 3 specimens from the R1 survived for two days. Only 3 out of 28 specimens allocated at lower salinity levels (2.5‰) were alive for one week (all from R1), but the remaining 25 died within four days (5 specimens died after 2 days, 6 after 3 days, and 11 after 4 days). In the latter group, the larvae were defecating in unusual amounts long before death, always one/two days after allocation to the aquarium (Figures 3e,f).

Due to difficulties in handling and keeping emerging alate forms in a controlled environment, we only observed the natural death of seven winged individuals; the remaining eight alate specimens that emerged had to be euthanized in alcohol when about to escape. After that, the specimens were submerged in water for cleaning and then placed in the Petri dishes for subsequent disarticulation tests. From those seven that died naturally, five specimens emerged and then drowned, presenting their wings open and spread out, and two subimagos died while attached to the aquarium protective net without falling into the water, and presented their wings adducted (Figures 4a,b). Without any water disturbance, the winged carcasses did not sink to the bottom of the aquarium and went through decomposition without ever sinking. The soft-tissued abdomen began to decay immediately after death and bloating, likely due to fermentation gases in the

abdomen, facilitated buoyancy. On average, 15 days after allocation in the disarticulation tests, the carcasses had a shrunk abdomen and completely withered wings, then we ceased the disarticulation observations - one specimen had this condition after 4 days, one after 5 days, two after 7 days, two after 8 days, one after 11 days, one after 14 days, one after 21 days, five after 23 days, and one after 25 days (Figure 4c,d; S1 Table).

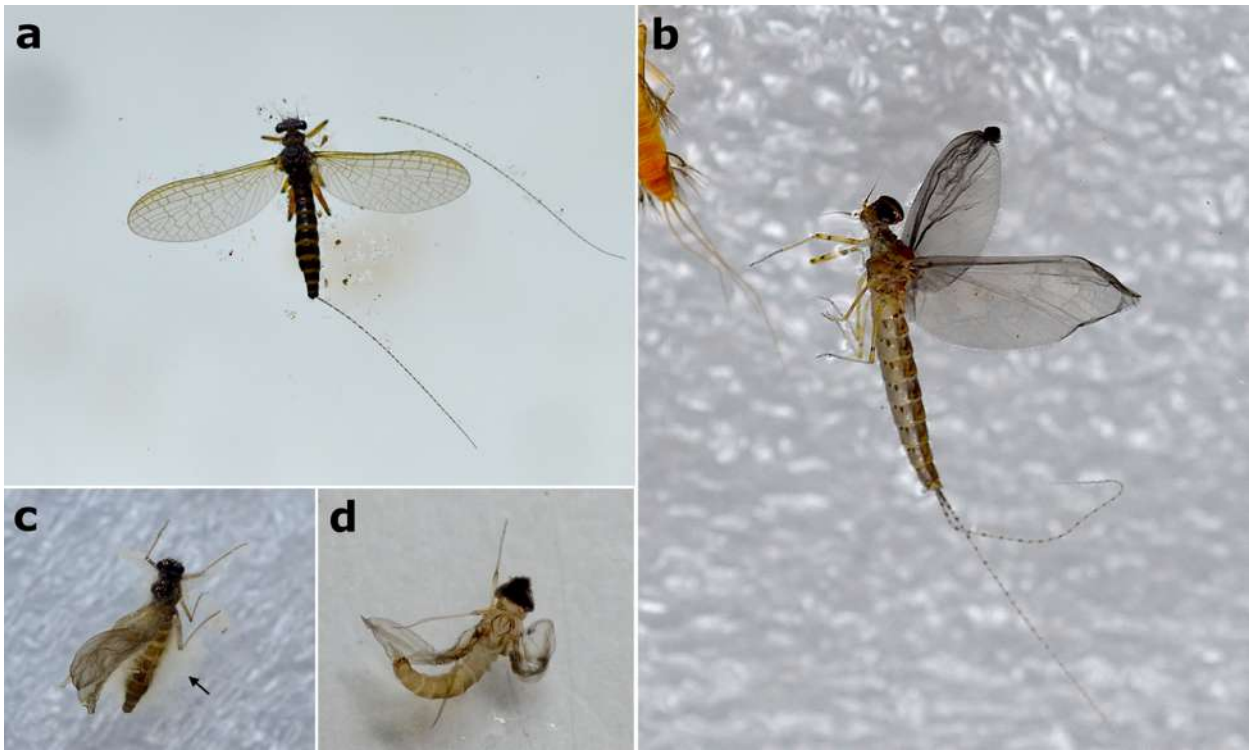

Figure 4. Winged mayflies during disarticulation tests. a) The most frequent postmortem position for winged mayflies; *Askola* female imago floating on the water surface with wings open and spread out, in dorsal view. b) Winged male *Callibaetis capixaba* subimago that died while still attached to the protective net trying to molt to imago stage, floating in the water surface with wings adducted. c,d) Female and male *Askola* subimagos, respectively, floating after days of decomposition, with their wings withered and abdomen shrunk, as well as whitish microbial proliferation on the abdomen in 'c' (black arrow).

Regarding the disarticulation observations, the darkening and detachment of the digestive tract were one of the first processes that occurred during larval decomposition (usually less than a day after death) (Figures 2d and 5a–e) and possibly influenced the disarticulation of other corporal

elements, such as the thorax. The latter was the most likely element to disarticulate first naturally; 26% of specimens had their thorax disarticulated, on average 3.5 days after their death (Figures 5a,b, Table 2). The disarticulation of the thorax occurred faster in situations of denaturation acceleration, such as in warmer waters in which, in most cases, it disarticulated just one day after the death of the individual (even before allocation to Petri dishes, when the specimen was noticed dead in the daily check). Many specimens started to show early signs of cuticle denaturation when decomposing in water (when disarticulation tests ceased), with 108 specimens within 2 to 4 days only, with the average number of days for all the larval mayflies analyzed being 4 (see S1 Table). The redder and more transparent the cuticle was, the more decomposed the specimens were.

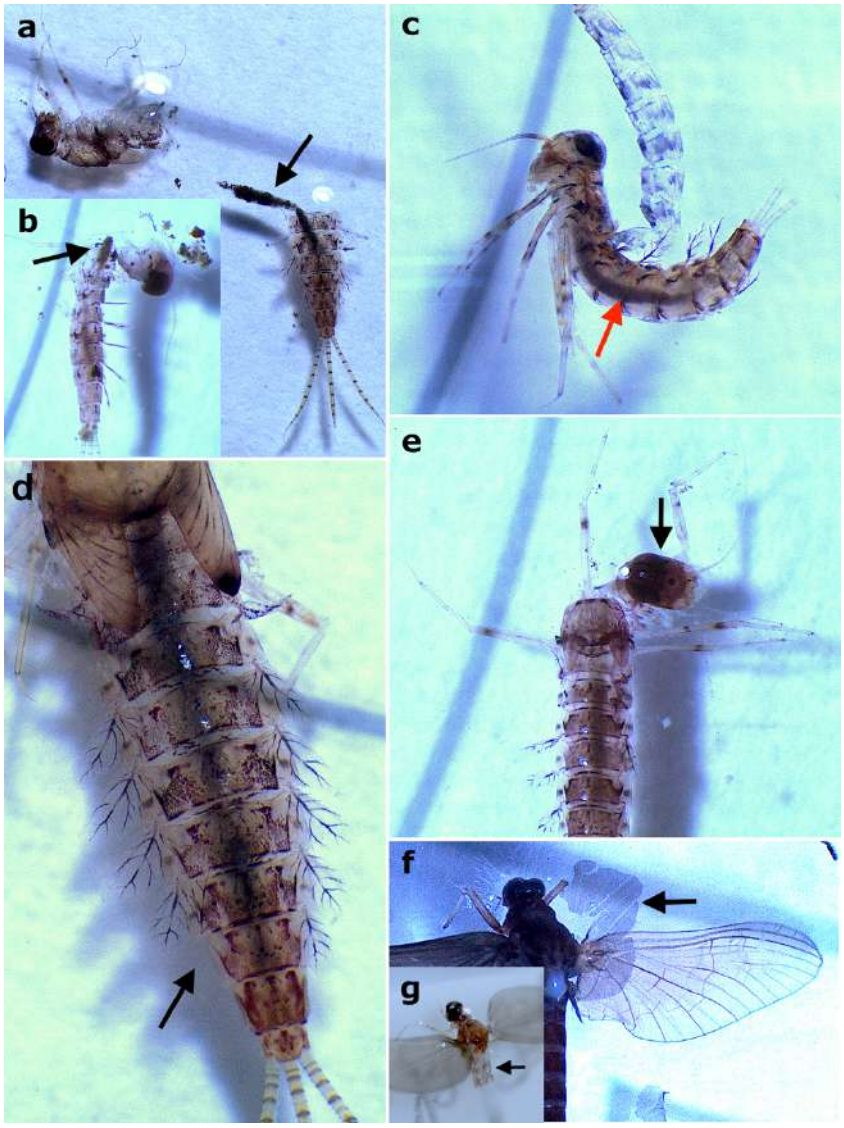

Figure 5. Larval and winged mayflies during disarticulation tests. a,b) *Callibaetis capixaba* carcasses with thorax disarticulated; darkening and detachment of the digestive tract are also evident; digestive tracts indicated by black arrows. c) *C. capixaba* carcass with darkened digestive tract and part of caudal filaments disarticulated; digestive tract indicated by red arrow. d) *C. capixaba* carcass with one final gill missing, indicated by black arrow. e) *C. capixaba* carcass with head disarticulated; head indicated by black arrow. f) Leptophlebiidae carcass with subimaginal membrane of forewing detaching; piece of membrane indicated by black arrow. g) *C. capixaba* male subimago with missing abdomen (except by first segments indicated by arrow).

Table 2. Disarticulation rates of larval mayflies. List of body parts disarticulated and the total number of specimens with this part disarticulated when observations ceased (see text), and what percentage this number represents. Percentages are rounded up.

Body part(s) disarticulated	Total number of specimens	Percentage of specimens
Head	11	4%
Antennae	40	20%
One antenna	16	7%
Thorax	66	26%
All legs	10	4%
Five legs	5	2%
Four legs	9	3%
Three legs	18	7%
Two legs	29	10%
One leg	30	11%
Abdominal segments	18	8%
All the gills	66	26%
All three caudal filaments	33	13%
One or two caudal filaments	17	7%
Parts of caudal filaments	36	15%

We observed that carcasses that went through natural disarticulation at the Petri dishes more frequently had the thorax as the first body part to disarticulate or at least weaken, while in the larvae that died in the aquariums while there were still several alive and active larvae (disturbing the carcasses before the specimen was noticed dead in the daily check) the legs, gills, caudal filaments, and antennae (most delicate body parts) were the first body parts to disarticulate. In the specimens that lost only some of their gills, the last ones were more prone to disarticulate

first (Figures 5c,d). For complete percentages of disarticulated body parts, see Table 2.

In alate forms, the first elements to disarticulate were the thorax and wings. The membrane of the subimago's wings also detached easily after the carcass decomposed (Figures 5f,g). The abandoned larval exuvia tended to disarticulate in the weak spots between thorax and head, and thorax and abdomen (Table S1).

Actualistic data: Dragonflies

When dragonfly larvae were stressed and/or close to dying, they usually stayed in ventral view and without any movement. After dying either in dorsal or ventral view (more frequently in ventral; 64% of specimens, n=151), they stayed in a characteristic position with the hind legs extended laterally and the forelegs extended close to the body (S2 Table). After a disturbance took place, the extended hind legs completely disarticulated, either at the base or between femur and tibia likely because they were more exposed (Figures 6a–d). All the larvae tended to float after dying, probably due to the gases released inside the abdomen by the decomposition; dense white microbial mycelia quickly appeared in 3 to 4 days (similarly to the mayflies but in smaller amounts in the latter - which were not quantitatively analyzed as for dragonflies), which seemly helped to keep the labium and legs articulated; followed by the darkening and denaturation of the head. After the microbial chitinofag mycelia attacked, the denaturation accelerated, and after only one week the animal was very disintegrated (signs of disintegration are visible when the cuticle starts to become transparent by the decomposition of chitin) (Figures 6e–h), upon which tests were ended; the average time for disarticulation tests to end were 14 days (see S2 Table). We also recorded one case of cannibalism, possibly caused by stress, 15 days after the allocation of specimens in the aquariums.

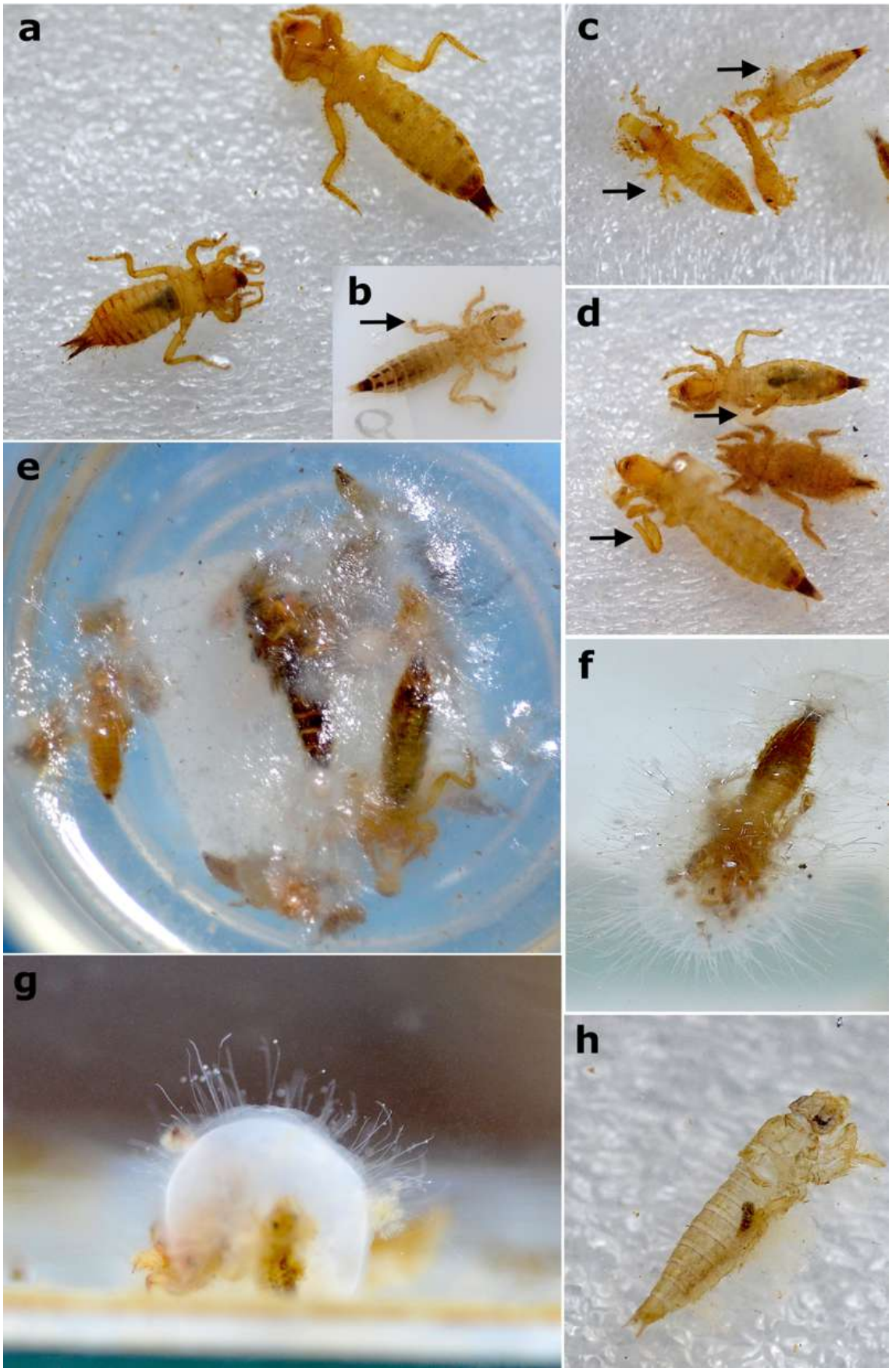
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Figure 6. Dragonfly larvae during experiments. a) Gomphidae larvae stressed in ventral view and close to die, exhibiting the characteristic position with the hind legs distended laterally and the forelegs extended anteriorly. b,c,d) Gomphidae carcasses after minor disturbance; black arrows point to the disarticulated hind legs (in different points: in 'b' tibia, 'c' tibia and femur, and 'd' coxa and femur. e,f) White microbial mycelia covering floating carcasses. g) Carcasses at the bottom after descending from flotation due to the weight of microbial mycelia. h) Carcass showing signs of chitin denaturation in which the cuticle starts to become transparent.

During the salinity versus flotation tests, we found that the angle of the legs of the carcasses was not affected by the increase in salinity, but floatation did. Under hypersaline conditions (80‰), all Odonata carcasses immediately floated, and at lower salinities (0.5‰ and 35‰) they all sank to the bottom of the glass after the saline solution was poured over them. The 20 carcasses allocated at 35‰ salinity only started to float in the vials two days after immersion, and the 23 carcasses allocated at 0.5‰ salinity, after three days (Figure 7).

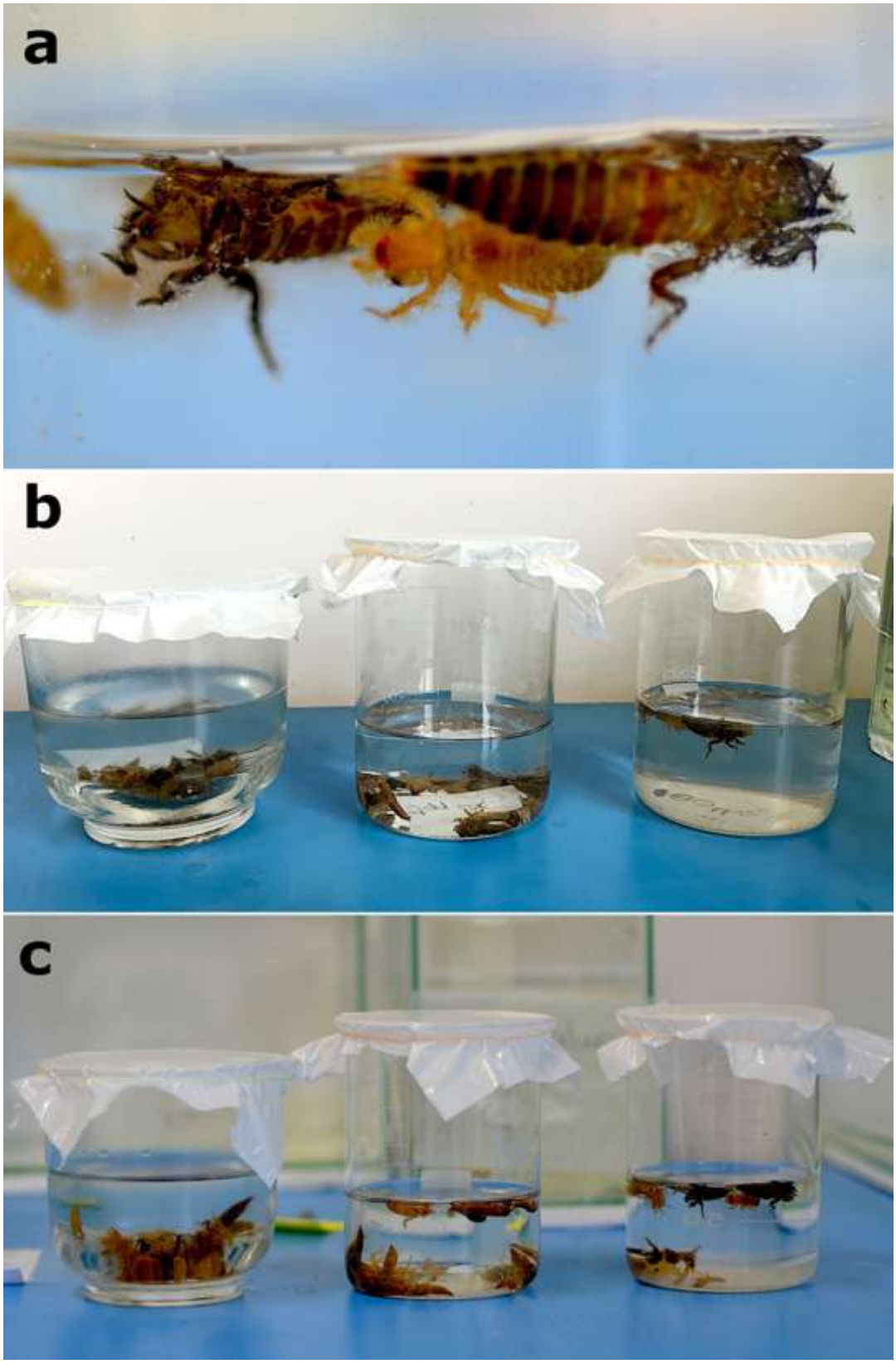

Figure 7. Dragonfly larvae during salinity tests. a) Close-up of floating carcasses during

immersion tests in saline water. b) First day of the immersion tests with saline water; from left to right groups 0.5‰, 35‰, and 80‰ (in which the carcasses immediately floated). c) Second day of experimentation, in which carcasses of the 35‰ group came to float, while carcasses from the 80‰ group started to descend back to the bottom.

Regarding the disarticulation observations, the first element to disarticulate in the larvae was the labium; almost half of all the specimens analyzed (n=93) had their labium disarticulated at the end of the experiments (Tables 3, S2), and it tended to disarticulate within one week after death in the majority of the specimens in which the labium disarticulated (n=62; Figures 7a–b). We considered the labium disarticulated when it was distended enough to observe its tip (i.e. prementum) on dorsal view (Figures 8c–e). Regarding only the specimens from the flotation tests, 46% of the carcasses of the freshwater group (0.5‰) showed labium disarticulation (n=11), 26% of the saltwater group (35‰, n=5), and only 10% of the carcasses in hypersaline water (80‰, n=2).

Table 3. Disarticulation rates of larval odonatans. List of main body parts disarticulated with total number of specimens with this part disarticulated, and what percentage this number represents. Percentages are rounded up.

Body part(s) disarticulated	Total number of specimens	Percentage of specimens
Head	2	1%
Labium	93	39%
All legs	1	0.5%
Five legs	1	0.5%
Four legs	4	2%
Three legs	6	2%
Two legs	20	8%
One leg	27	11%
Abdomen	3	1%
All the gills	3	1%

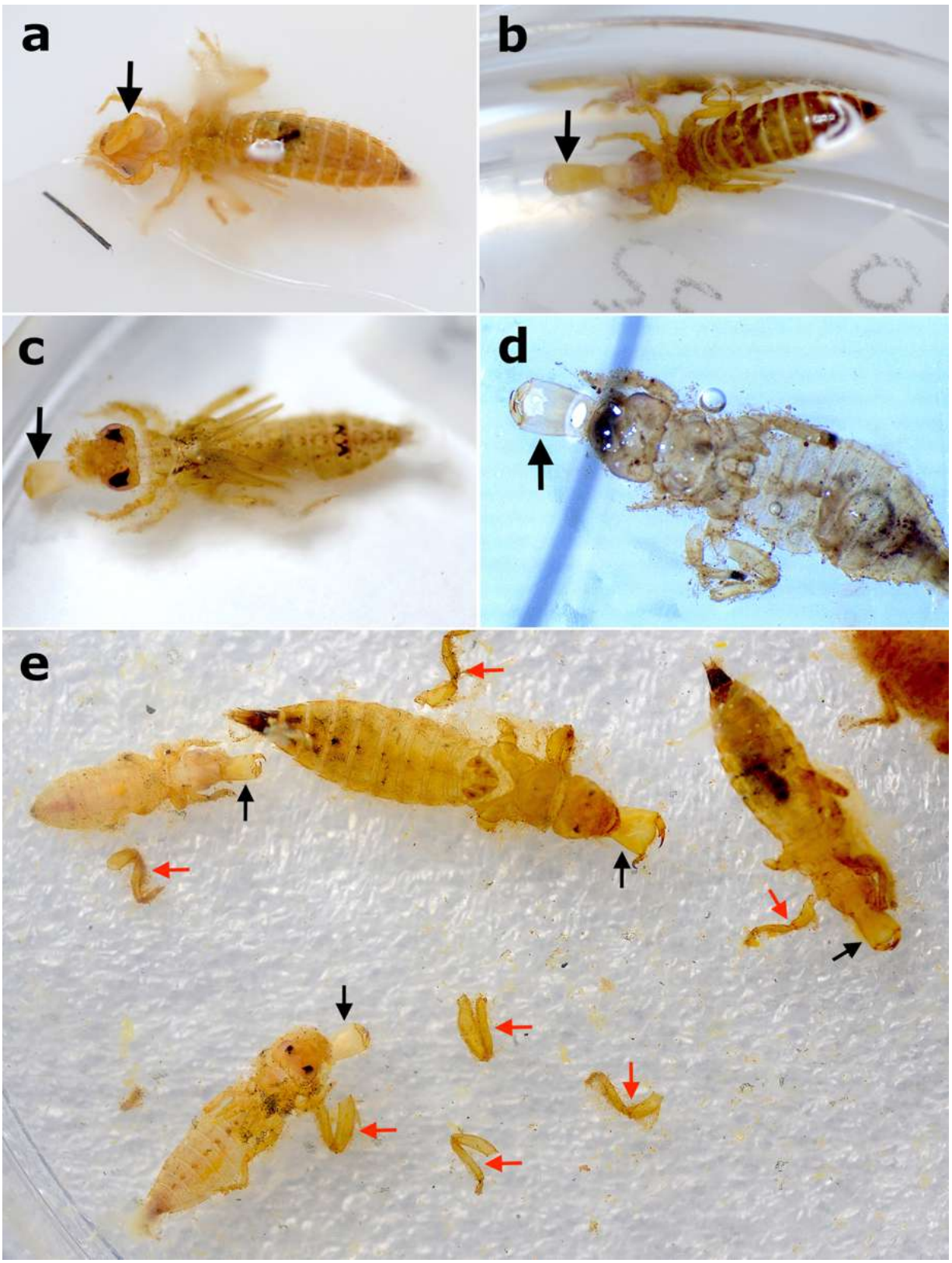

Figure 8. Gomphidae larvae during disarticulation tests. a) Larva in ventral attitude with

labium in early process of disarticulation; labium indicated by black arrow. b) Larva in ventral attitude with labial mask completely disarticulated; labium indicated by black arrow. c,d) Larva in dorsal attitude with labium completely disarticulated; in image 'd' specimen also in late state of decomposition evidenced by the transparent cuticle; labium indicated by black arrows. e) Four larval carcasses with legs and labial masks disarticulated; labium indicated by black arrows, and fragments of legs (mainly hind) indicated by red arrows.

Among anisopteran groups (n=233), besides the labium, the legs were also the first to disarticulate, especially under mechanical disturbance (25% of specimens had at least one leg missing at the end of disarticulation observations, n=59) (Figure 8e), and the disarticulation of the abdomen from the thorax was rare, occurring in only three specimens. Regarding the three analyzed damselflies larvae (Zygoptera), the caudal gills were the most easily disarticulated elements, with all specimens losing them. Raw data on the experiments with Odonata are available in Table S2.

Biostratinomic data: Mayflies

Most winged Hexagenitidae preserved in the Crato Formation limestones are preserved either in dorsal or ventral view and have their wings open and spread out (70% of specimens, n=22), 25% of specimens (n=8) are in lateral position and have their wings adducted, and only one specimen was preserved as mere isolated wings (Table S3). Among larval Hexagenitidae from the same unit, most are preserved in dorsal view (61% of the specimens, n=140), followed by ventral view (22% of specimens, n=50), and lateral view (10% of specimens, n=24) (Figure 9). A total of 23% of the Hexagenitidae larval specimens of the Crato Formation (n=53) preserved in dorsal or ventral view are similar to the death position we observed in the larvae of *C. capixaba* (in dorsal view, legs not flexed but extended laterally near the body, with the anterior legs extended forward; and in ventral view, legs flexed over the body) (Figure 9; Table S3).

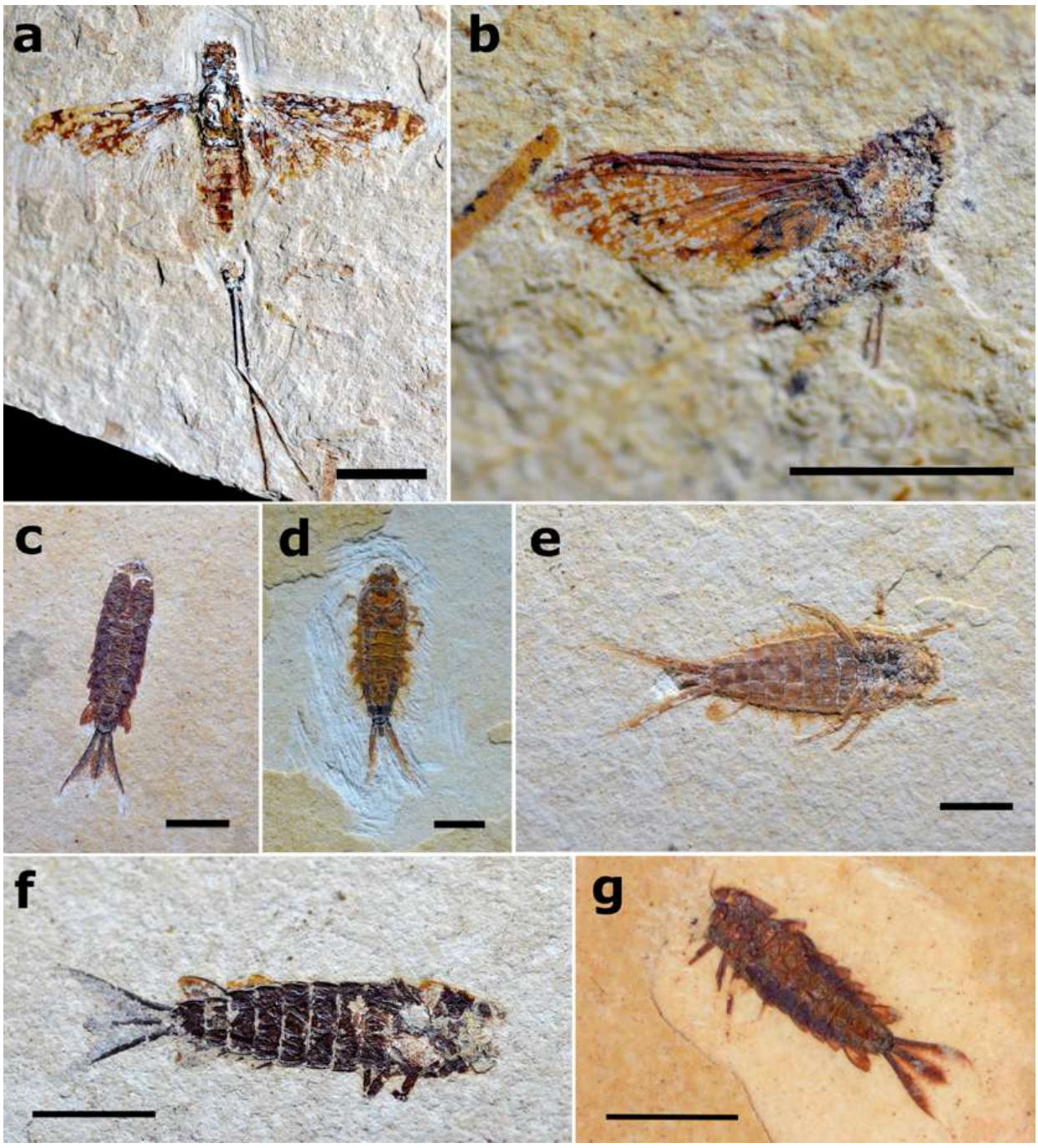

Figure 9. Fossils of Hexagenitidae from the Crato Formation. a) Winged specimen, MPPCN I 4287, preserved in dorsal attitude with wings open and spread out. Scale 5 mm; b) Winged specimen, MPPCN I 409, preserved in lateral attitude with wings closed. Scale 5 mm; c) Larval specimen, MPPCN I 5229, preserved in dorsal attitude. Scale 3 mm; d) Larval specimen, LPU 1698, preserved in dorsal attitude. Scale 3 mm; e) Larval specimen, MPPCN I 5230, preserved in ventral attitude and displaying the typical death position recovered in extant larvae. Scale 3 mm;

f) Larval specimen, LPU 1697, preserved in ventral attitude. Scale 4 mm; g) Larval specimen, MPPCN I 5227, preserved dorsolateral attitude. Scale 5 mm.

Hexagenitidae are often completely articulated (81% of all specimens, n=214, including larvae and wingeds), and less than 5% of larval specimens (n=13) have a disarticulated thorax (Figures 10a,b); several specimens have fragile structures preserved, like setae on caudal filaments (31% of specimens, n=71) (Figures 10c–f). Also, a small percentage of Hexagenitidae larvae (14%, n=34) are recovered without the cuticle preserved and showing their inner digestive tube (Figures 10a,g,h; Table S3).

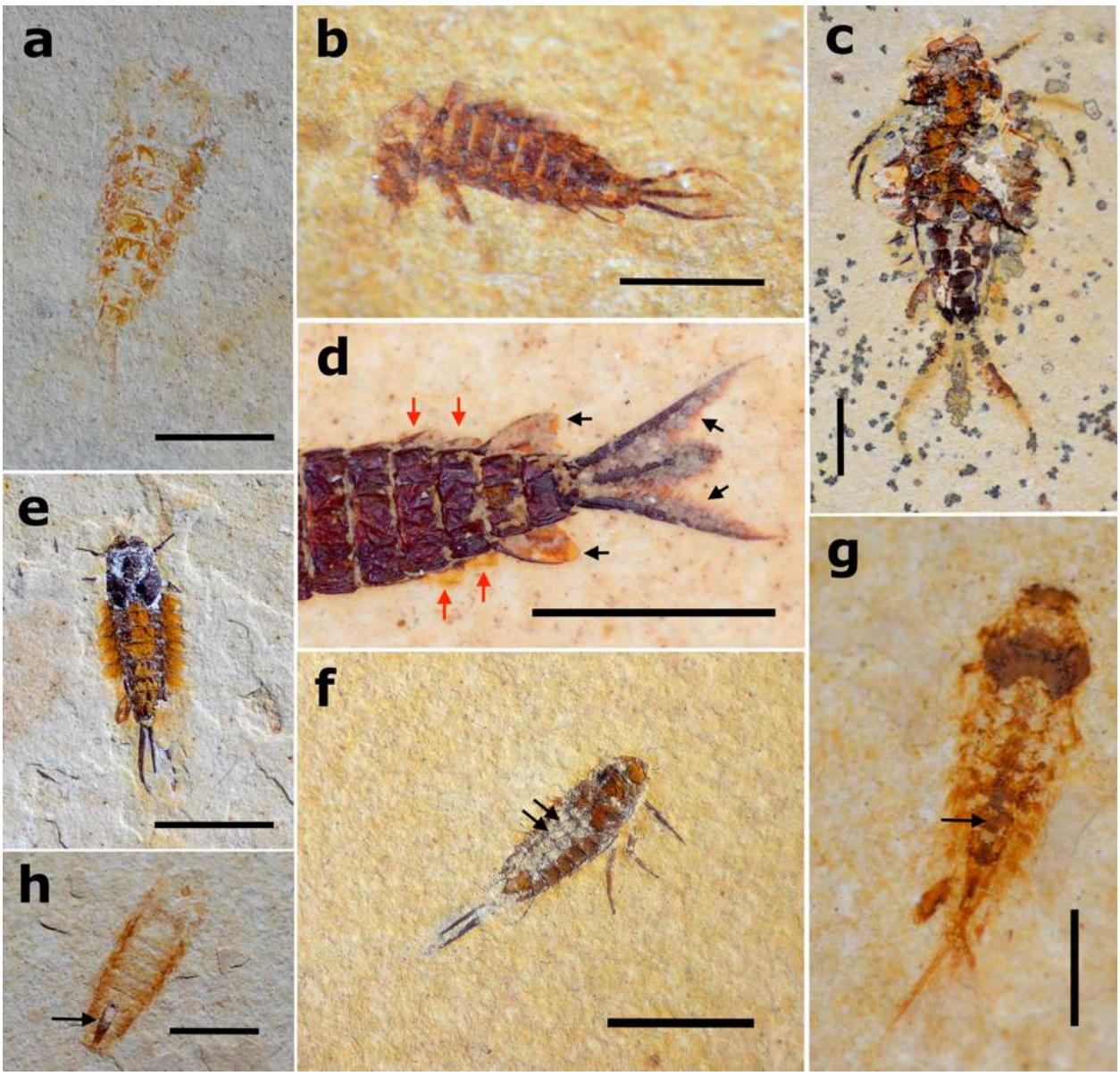

Figure 10. Hexagenitidae larval specimens in different disarticulation degrees. a) Unnumbered LPU specimen with disarticulated thorax. Scale bar 3 mm; b) Specimen MPPCN I 293 with thorax about to disarticulate. Scale bar 5 mm; c) Specimen GP/1T 2583, an example of a specimen considered completely articulated in our study - even if the preservation is hampered by weathering, for instance. Scale bar 3 mm; d) Part of specimen LPU 1697 evidencing its well-preserved details such as the abdominal cuticle texture, gills V–VI closed (indicated by red arrows) and VII (indicated by black arrow), and setae on caudal filaments (indicated by black arrows). Scale bar 5 mm; e) Another example of an unnumbered LPU specimen considered completely articulated in this study. Scale bar 5 mm; f) Specimen MPPCN I 5224, considered completely

articulated in this study and preserving micro textures on its dorsal cuticle (indicated by black arrows). Scale bar 5 mm; g) Unnumbered LPU specimen showing signs of cuticle denaturation but still articulated; digestive tube well visible in its all length (indicated by black arrow). Scale bar 5 mm; h) Unnumbered LPU specimen showing signs of cuticle denaturation, remaining only its lower digestive tube preserved (indicated by black arrow); caudal filaments, gills, and thorax disarticulated. Scale bar 3 mm.

In alate forms, the legs are the body part most likely to be absent (i.e., more easily subject to disarticulation), with at least four legs absent in 90% of specimens (n=28) with body parts absent (four legs absent in 4 specimens; five legs absent in 7 specimens; and all legs absent in 17 specimens), followed by the caudal filaments, with at least one of them being absent in 42% of the analyzed specimens (n=13). In 25% of the specimens (n=8), there is at least one disarticulated segment in the abdomen. The less frequently absent body elements are the hind wings (absent in one specimen), the whole body, remaining only isolated wings (absent in one specimen), and one of the forewings (absent in one specimen) (Table S3). Also, 18% of winged fossil (n=7) present their wings withered in different degrees, similar to the dehydrated and decomposed state observed during our actualistic experiments (see above) (Figure 11).

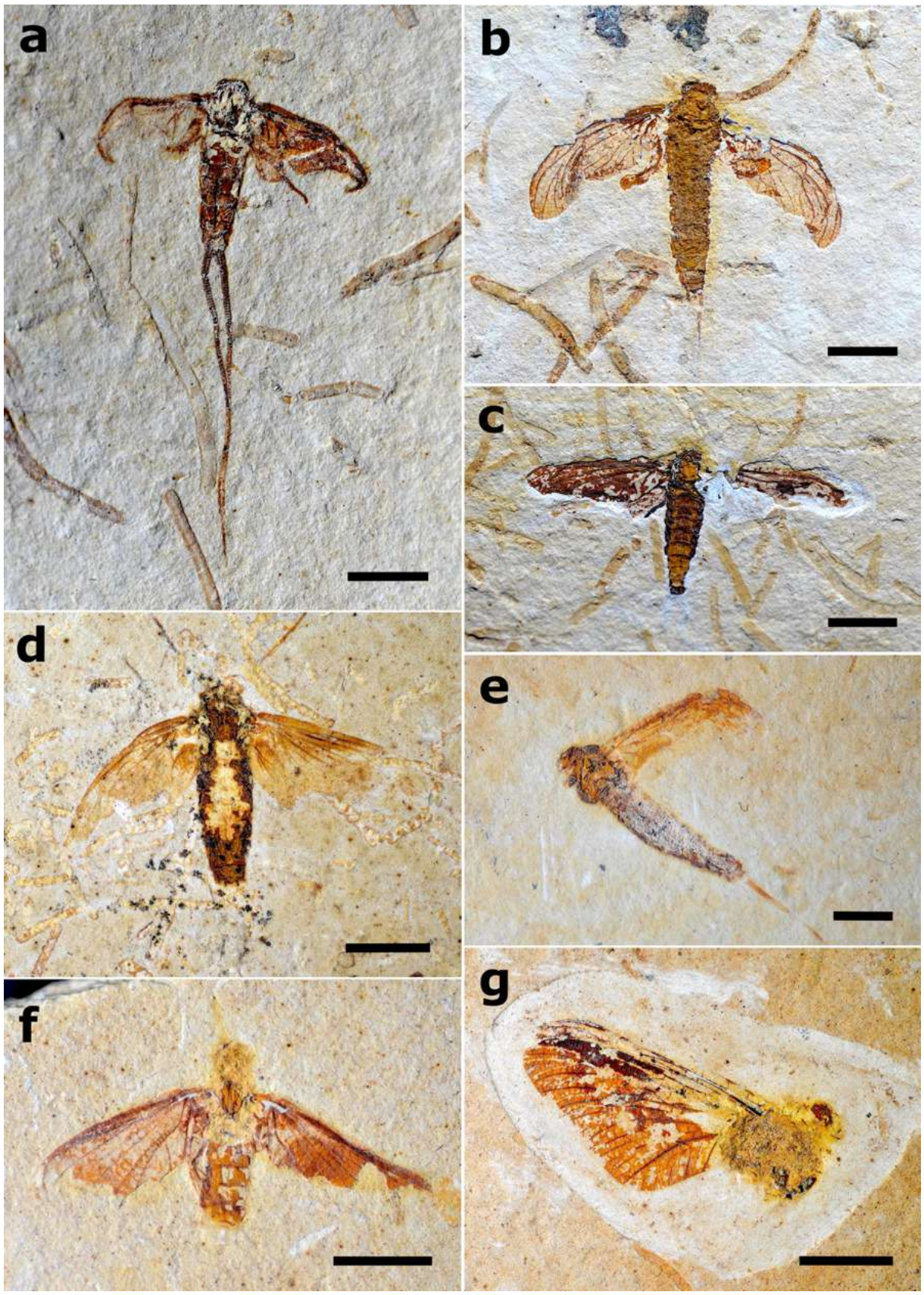
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Figure 11. Hexagenitidae winged specimens in different disarticulation degrees. a) Specimen MPPCN I 4286 with four legs missing and wings withered. Scale bar 4 mm; b) Unnumbered LPU specimen with all legs and part of caudal filaments missing; wings are also partially withered. Scale bar 4 mm; c) Specimen MPPCN I 4422 with five legs, caudal filaments, and right hind wing absent, as well as border of forewings. Scale bar 4 mm; d) Specimen GP/1E 9562 with four legs, caudal filaments, and border of forewings absent. Scale bar 4 mm; e) Specimen GP/1E 7221 with all legs (except one pair of coxae) absent and with wings withered. Scale bar 3 mm; f) Specimen GP/1E 6763 with all legs and end of abdominal segments missing; the borders of all wings are also absent. Scale bar 5 mm; g) Specimen GP/1E 9034 with all legs, hind wings, and abdomen missing. Scale bar 5 mm.

In the larvae, the legs are the body part more frequently absent, with at least one leg missing in 88% (n=202) of those specimens with body parts absent (one leg absent in 7% of all specimens, n=16; two legs absent in 5% of all specimens, n=11; three legs absent in 12% of all specimens, n=28; four legs absent in 9% of all specimens, n=22; five legs absent in 6% of all specimens, n=13; and all legs absent in 50% of all specimens, n=115). They are followed by the antennae (absent in 64% of the specimens, n=147), at least two gills which are absent in 37% of the specimens (n=85), and at least parts of the caudal filaments are absent in 34% (n=78) of the incomplete specimens (parts of caudal filaments missing in 5% of all specimens, n=11; paracercus absent in 6% of all specimens, n=13; one cercus absents in 2% of all specimens, n=5; cerci absent in 3% of all specimens, n=6; and all the caudal filaments absent in 19% of all specimens, n=43). The less frequently absent body elements among the larvae are abdominal segments (absent in only one specimen), cuticle (absent in two specimens), the head (absent in 4% of specimens, n=12), and the head and thorax (absent in 5% of the specimens, n=13) (Figure 12). We also found that 15% of the specimens (n=34) are preserved with signs of cuticle denaturation in different degrees, made evident by the apparent digestive tube due to cuticle tissue loss (Figures 10g–h, 12; Table S3).

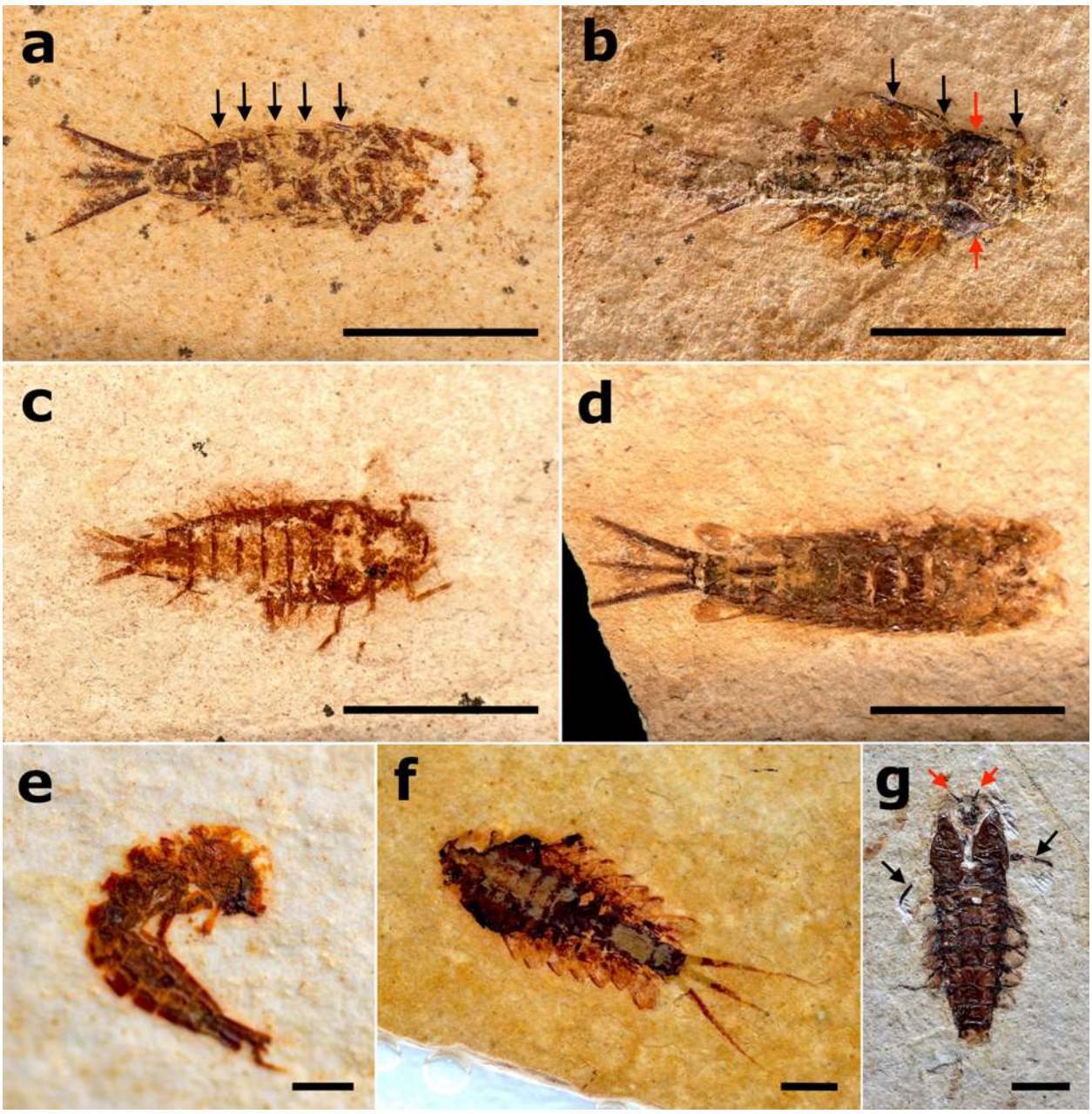

Figure 12. Hexagenitidae larval specimens in different disarticulation degrees. a) Specimen MPPCN I 2507 in ventral attitude with antennae missing; gills II-VI preserved close to the body and left ones (indicated by black arrows). Scale bar 5 mm; b) Specimen MPPCN I 2533 in dorsal attitude; three legs are partially preserved (indicated by black arrows), as well as all their gills in yellowish coloration (except gill VII), wing pads are also preserved and indicated by red arrows. Scale bar 5 mm; c) Specimen MPPCN I 2509 preserved in ventral attitude; five legs are almost completely preserved in a natural postmortem position. Scale bar 5 mm; d) Specimen MPPCN I

2516 preserved in ventral attitude with all legs missing. Scale bar 5 mm; e) Specimen MPPCN I 1336 was preserved with the abdomen about to disarticulate in curved lateral attitude; part of caudal filaments is also missing. Scale bar 2 mm; f) Unnumbered LPU specimen preserved in dorsal attitude, evidencing all gills preserved and all legs missing. Scale bar 2 mm; g) Unnumbered LPU specimen preserved in dorsal attitude; two legs are partially preserved (indicated by black arrows) as well as antennae (indicated by red arrows) and wing pads, while part of its gills and caudal filaments were disarticulated. Scale bar 3 mm.

Biostratinomic data: Dragonflies

Fossil dragonfly larvae from the Crato Formation are often completely articulated (64% of specimens, n=29). Similarly to the actualistic experiments with anisopterans, the analyzed fossils never displayed thorax, head, or abdomen disarticulated since those are extremely robustly attached structures in dragonflies, differing from the damselflies (Figures 13,14; Table S4).

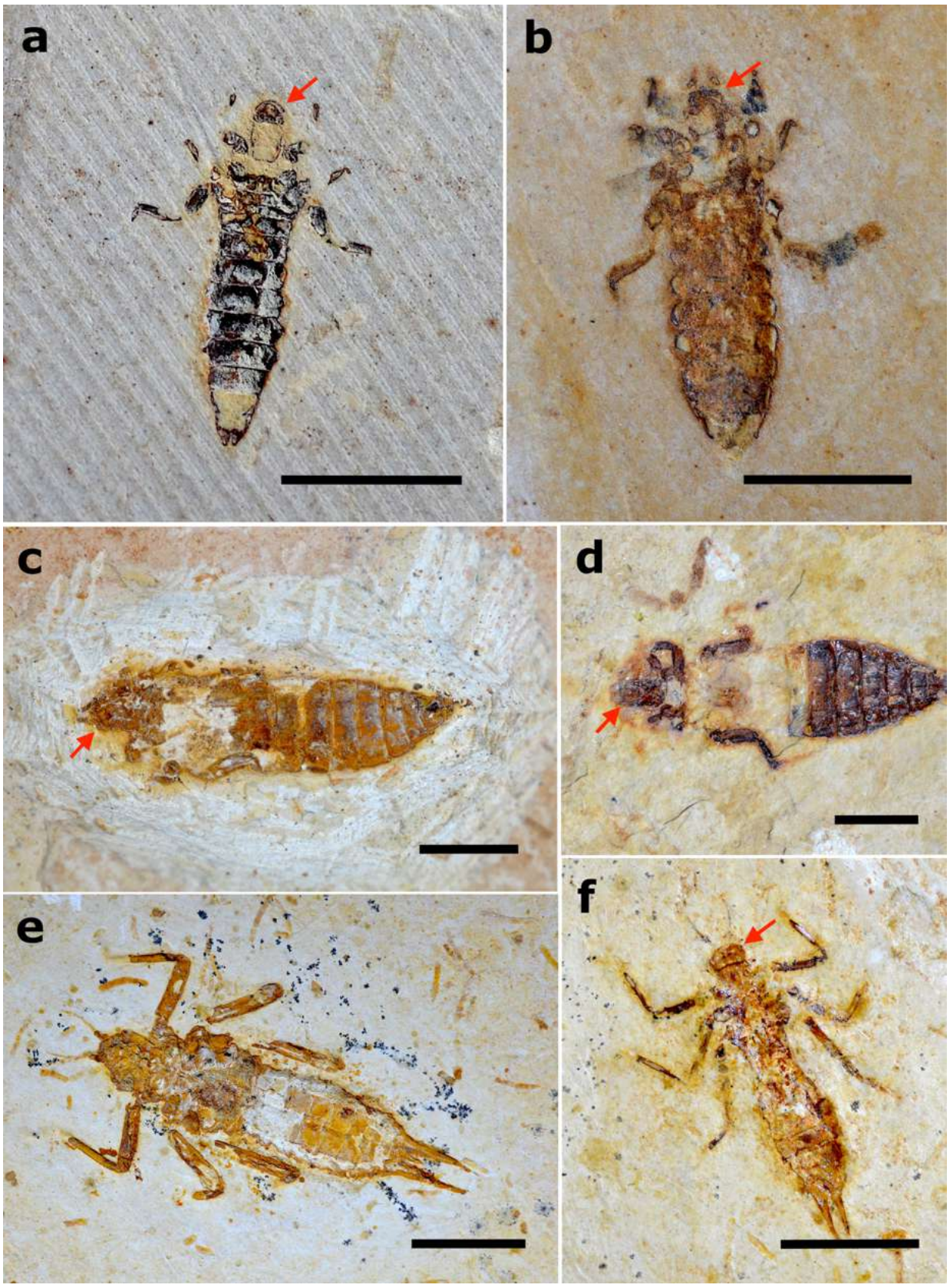
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Figure 13. Dragonfly larval specimens with labial masks articulated in different degrees. a) Proterogomphidae specimen LPU 1102 in ventral attitude evidencing labial mask totally disarticulated/distended (indicated by red arrow); specimen was also preserved in a natural death position. Scale bar 5 mm; b,c) Proterogomphidae specimens MPPCN I 388 and GP/1E 6891 in ventral attitude showing labial mask still articulated or only partially disarticulated, evidenced by the visualization of antennae in both specimens. Scale bar 5 mm; d) Proterogomphidae specimen MPPCN I 384 in ventral attitude evidencing disarticulated labial mask (indicated by red arrow). Scale bar 5 mm; e) Nothomacromiidae specimen MPPCN I 709 in dorsal attitude. Scale bar 10 mm; f) Nothomacromiidae specimen in ventral attitude with labial mask still articulated (indicated by red arrow). Scale bar 10 mm.

In contrast to the observed actualistic results with extant specimens, we rarely found disarticulated labial masks in the fossils from the Crato Formation, being disarticulated in only 2 specimens (Figure 13). In relation to the other corporal elements, 31% of the fossils (n=14) have at least the final segments of the legs missing (3 specimens missing only the tarsi of some legs, 4 specimens missing one leg, 2 missing two legs, 3 missing three legs, 1 missing four legs, and 1 missing five legs), and 17% of specimens (n=4) have their antennae absent. 47% of the analyzed dragonfly larvae (n=21) from the Crato Formation display the same natural postmortem position that we observed in the extant larvae, with the delicate hind legs still preserved, although in five cases at least one segment from the hind leg disarticulated (Figures 13a,b;14; Table S4).

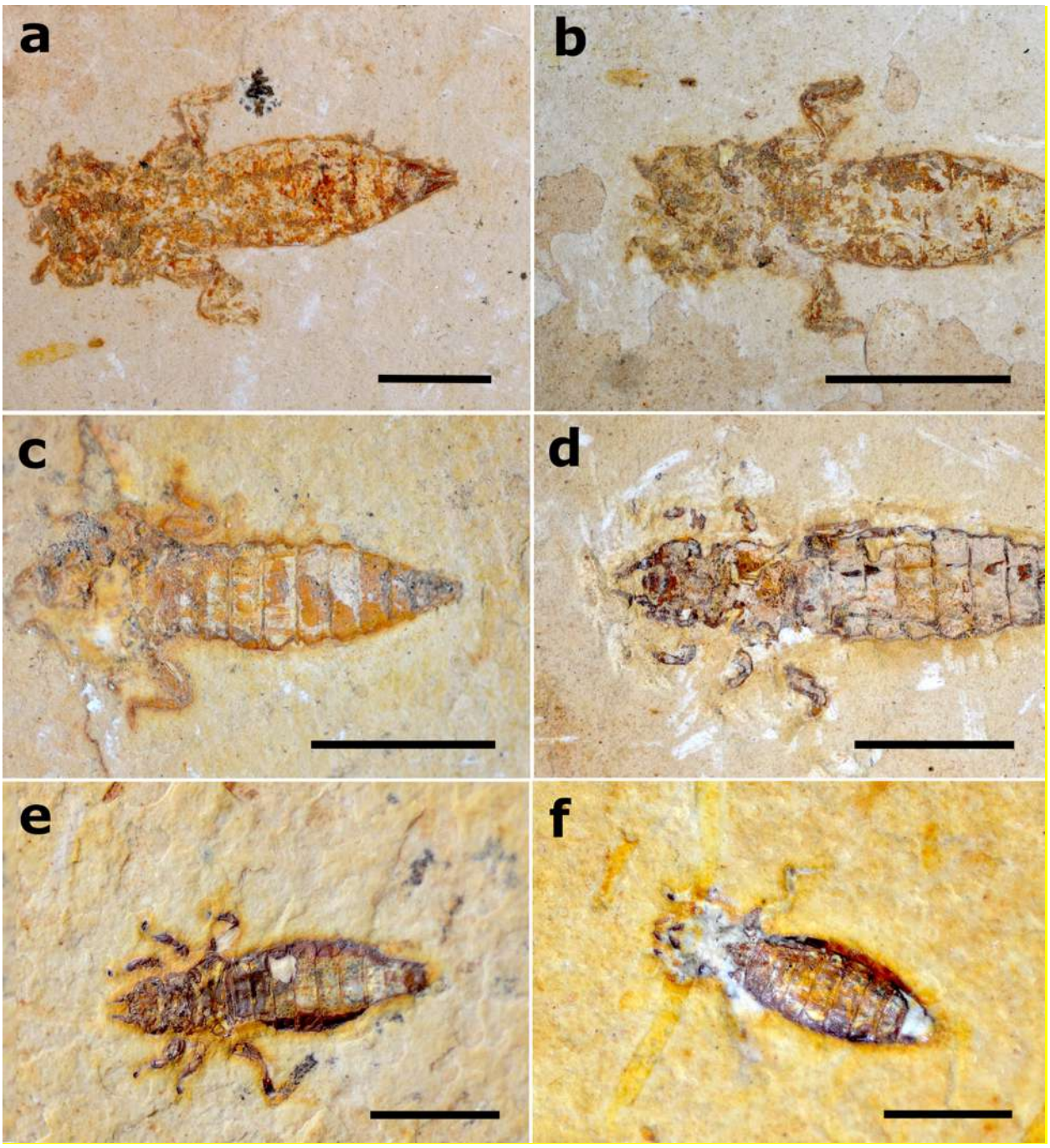

Figure 14. Proterogomphidae fossils displaying natural postmortem position. a) Specimen GP/1E 9220; b) Specimen GP/1E 6807; c) Specimen GP/1E 6806; d) Specimen GP/1E 8699. The natural death position was slightly modified in this specimen since the right hind leg is close to the body and not spread open as the left leg; e) Specimen MPPCN I 389. Hind legs showing signs of minor transport since the right one is disarticulated at posterior segments, and the left is about to disarticulate; f) Specimen MPPCN I 1355. Scale bars 5 mm.

Discussion

Proxies used for this differentiation of assemblages that suffer transport to the depositional site usually involve the study of abundance, the degree of breakage, disarticulation, and fragmentation of individuals, together with the attitude of fossils in sediments, although disarticulation can occur naturally over time (Martínez-Delclòs and Martinell, 1993; Martínez-Delclòs et al., 2004). During our experiments, the thorax was the most likely element to disarticulate first in *C. capixaba* larvae (even more easily in their exuvia), especially in situations of denaturation acceleration, such as in warmer water. As the Hexagenitidae larvae from the Crato Formation rarely present a disarticulated thorax, this indicates that their carcasses suffered little or no disturbance and that the decomposition process must also have been slow (Martínez-Delclòs et al., 2004), but also could suggest that the carcasses suffered minimal transport, corroborating that the group was living in the depositional site (i.e. autochthonous), as pointed out before by Storari et al. (2021b).

Another set of biostratinomic data additionally supports that Hexagenitidae carcasses suffered little or no disturbance. All extant mayflies that died in the experiments under higher temperatures presented straighter bodies, which could be related to the acceleration of cuticle denaturation at high temperatures (Gibbs and Rajpurohit, 2010). But in the fossil record, the few larvae that are preserved laterally display a natural curvature in their body. Only a few fossil Hexagenitidae larvae were recovered without cuticle preserved and showing their inner digestive tube; here, we interpret them as larvae that went through enough decomposition to lose their cuticle before going through diagenesis, similar to the experiments in which the specimens went through cuticle denaturation after many days (when disarticulation tests ceased). Finally, fossil specimens lacking their wings' margins and/or with their wings withered, which could indicate longer decay time, are rare in the Crato fossil record; although these latter could also simply represent subimagos that did not complete the full opening of wings after emergence, since some degree of wings' withering is common in this winged stage (Kluge, 2004).

All the Odonata groups recovered in the Crato Formation have been presumed in the past as allochthonous to the depositional site (Bechly, 1998; Bechly, 2007; Menon and Martill, 2007). However, we assume otherwise for Proterogomphidae and Nothomacromiidae, which are the groups of dragonflies with the marjority of larval representatives preserved in the Crato Formation

(Bechly, 2007). During our experiments, we observed that, once dead, the dragonflies stayed in a characteristic position with the hind legs distended laterally and the forelegs extended anteriorly. However, as soon as any mechanical disturbance occurs, the segments of the hind legs easily disarticulate. Seventeen of the 27 gomphid larvae from the Crato Formation analyzed also presented this pre-disturbance pose, and 14 of them still had all segments of the delicate hind legs attached, indicating minimal disturbance of the carcasses after death (e.g. transport). Moreover, since we rarely found the labial mask preserved as visibly disarticulated, we could at first hypothesize that this structure disarticulates so early that it is the first part of the body to completely detach, thus being absent from the fossil record. However, it is possible to see the structure still attached, but not distended nor disarticulated, in at least 14 of the analyzed fossils. Also, in none of our experiments did the strongly adhered labium detach from the body as in the case of legs. Proterogomphidae and Nothomacromiidae larvae are not so commonly found in the Crato Formation limestones like Hexagenitidae larvae (Storari et al., 2021a,b). For instance, only two specimens were recovered in the controled excavation. However, they could be more abundant in different strata than those excavated at the Antonio Minelon Mine, and pertaining to smaller assemblages. In any case, not considering controlled data, their overall abundancy is still inferior to Hexagenitidae, which should be a factor when considering how far from the depositional site a assemblage was occurring in life, especially the aquatic forms.

On the other hand, in our experiments, we noticed that all dragonfly carcasses immediately floated under hypersaline conditions and that carcasses immersed in hypersaline conditions had slower decomposition and disarticulation times, while the carcasses immersed in freshwater or lower salinity levels took days to float. This late buoyancy, which started in the abdomen, was probably due to the time taken to accumulate decomposition gases. Salinity levels, therefore, must play a key role in increasing the buoyancy of the carcasses and thus their transport, but mostly improving the preservation of carcasses. Finally, we also observed that the angle of the legs of dragonflies' carcasses was not affected by hypersaline conditions, which probably would only act shrinking the specimens' legs if the individuals were still alive (similarly with results of Downen et al., 2016 with spiders), and therefore could only be a useful proxy for individuals that reached the depositional site alive and not transported carcasses. Thus, our results suggest that, although some authors have interpreted the Odonata larvae as allochthonous to the Crato Formation depositional sites (Bechly, 1998; Menon and Martill, 2007), at least the Proterogomphidae and

Nothomacromiidae individuals, probably lived close and were not transported for long distances. Up to now, no larvae of the suborder Zygoptera have been discovered among the hundreds of fossil odonatan larvae from the Crato Formation (Bechly, 2007).

There is a good deal of hypotheses in favor of a brackish or saline paleolake environment for the Crato Formation, at least in certain temporal phases of and/or certain strata of the water body (Martill and Wilby, 1993; Neumann et al., 2003; Martill et al., 2007; Martill et al., 2008; Heimhofer et al., 2010; Oliveira and Kellner, 2017; Warren et al., 2017; Varejão et al., 2019; Storari et al., 2021b), and some that support an exclusive lacustrine water setting (Grimaldi, 1990; Barling et al., 2020; Ribeiro et al., 2021). In our experiments, specimens allocated at lower salinity levels defecated in unusually large amounts long before death. These nymphs were probably releasing a lot of salt directly in their feces since, in insects, the excretory system is linked to the digestive system by the Malpighian tubules (Bradley, 2009). Unfortunately, we have not seen anything that could be related to this process in the Crato fossils, since the feces are extremely delicate and difficult to preserve.

Our experiments have shown that when small insects such as mayflies die in sub-aerial conditions and are transported to the water, there are few possibilities of overcoming the surface tension and sinking. In mayflies, the subimagos present wings covered with microtrichia that could keep them afloat, and males potentially could float more easily since their abdomen is filled with air, instead of eggs (Kluge, 2004). Our results agree with actualistic data from Martínez-Delclòs and Martinell (1993) who studied different terrestrial insects and concluded that it is difficult for small insects to sink. Their sinking could be generated by some disturbance (e.g., strong water currents), but the Crato Formation's limestones with fine-scale parallel lamination instead indicate a calm depositional site (Heimhofer and Martill, 2007). Thus, another phenomenon probably occurred during carcass sinking, such as the formation of floating microbial mats or biofilms which, when lump around the carcass, make it denser, breaking the water tension and making the carcass descend (see Figure 6). This adds to the hypothesis that the formation of benthonic microbial mats, directly on the bottom of the depositional site (after sinking), improves the preservation of carcasses (Heimhofer et al., 2010; Iniesto et al., 2016; Varejão et al., 2019; Barling et al., 2020; Dias and Carvalho, 2022). We hypothesize that, additionally, entombment by microbial mats while floating was also important to improve the conservation of articulated insect fossils in the Crato Formation, as already acknowledged as important for the preservation of insects

of the Florissant Fossil Beds, United States (Harding and Chant, 2000; Briggs, 2003).

The degradation of chitin is catalyzed by chitinases, which are found in organisms such as fungi and bacteria (Veliz et al., 2017). Insect cuticles can be decomposed by bacteria from the genus *Chitinophaga* (or chitinolytic bacteria), which are almost exclusively aerobic, found in a wide range of habitats, and can form superficial mats (Martínez-Delclòs and Martinell, 1993). *Streptomyces* species, which have been thoroughly studied, decompose solid chitin pieces rapidly, in large part because of their ability to penetrate substrates (or water) in association with fungal hyphae, such as those of *Saprolegnia* sp. (Boer et al., 1999; Bai et al., 2016). The microbial growth we saw taking over the studied carcasses had definetely fungi in their composition (if not solely), giving their hyphae-like morphology. Similar bacteria/fungi could potentially decompose carcasses in the Crato paleolake while, at the same time, acting as an anchor, because they increase density and thus help the insect to overcome the surface tension and sink to the bottom. They also act as a type of cohesion mechanism for the remains, hindering fragmentation and disarticulation (Briggs, 2003). However, cuticular decomposition by chitinophag bacteria is greatly hindered by anoxia and low temperatures (Bunch, 2009), so the decomposition could stop or be delayed as soon as the carcass reached the anoxic bottom of the Crato Formation's depositional site. Additionaly, in regard of exceptional preservation, new studies suggest that not only bacteria could be acting in their formation, but also fungi (Luo et al., 2023).

Martínez-Delclòs and Martinell (1993) observed in laboratory conditions that, without biological or physical disturbing agents, when reaching the bottom, insects could remain there for one year without becoming disarticulated, but with the passing of time disarticulation occurs around the joint zones due to the natural decomposition of carcasses (Martínez-Delclòs and Martinell, 1993). This differed from our experiments, in which this process lasted roughly only a month. We also observed that the more delicate the body, the faster this occurred; for instance, it was faster in small-sized mayfly larvae than in the more robust dragonfly larvae.

Conclusion

Our biostratinomic data and actualistic approach help to explain the fossilization process and the past environmental conditions the aquatic insects from the Crato Formation went through. Our results corroborate the group Hexagenitidae (specifically *Protoligoneuria limai* assemblages) as autochthonous to the depositional site of the Crato Formation, a condition that was probably

only shared with small populations of gomphids, and seasonally with *Dastilbe* juvenile fish (Storari et al., 2021b). *Protoligoneuria limai* is the most abundant invertebrate group of the Crato Formation, presenting complete ontogenetic series and has already been reported in mass mortality events (Storari et al., 2021b). Only 4% of hexagenitid larvae we analyzed had a disarticulated thorax, which also agrees with autochthony. Based on our experimental evidence, we conclude that, at least for the studied mayfly larvae, the high completeness rate would not have been possible if the carcasses had been transported from outside the depositional site.

Our observations are also consistent with Proterogomphidae and Nothomacromiidae living close to the depositional site and not being transported for long distances, as evidenced by the completeness of their fossils. We assume this due to the absence of disarticulated labial masks in the fossils, which could indicate longer decomposition time. Also, several fossils we analyzed had their body preserved in a natural death pose, in some cases including all segments of the leg preserved (extremely delicate elements that are disarticulated by minimal currents or mechanical friction). However, a thorough review of dragonflies from the Crato Formation is crucial for further taphonomic elucidations, and future interpretations should be based, preferably, on specimens collected under controlled excavations to remove collection bias that privileges well-preserved specimens, a common problem when studying Crato fossils. The taphonomy of insects from the Crato Formation will be better understood only when we also study the least preserved fossils

One also may hypothesize some details on the paleoenvironment of the Crato Formation based on these comparisons between its aquatic insects' fossils and experimental actualistic data. The paleolake complex of the Crato Formation (Ribeiro et al., 2021), in which these autochthonous populations were living, had most probably floating microbial mats in its surface waters, instead of only in the bottom (as suggested by a variety of works - Heimhofer et al., 2010; Iniesto et al., 2016; Varejão et al., 2019; Barling et al., 2020; Dias and Carvalho, 2022), similarly to the ones that grew in extant carcasses during our experiments of disarticulation, helping the carcasses to sink and keeping their body parts cohesive.

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References

Bai Y, Eijsink VGH, Kielak AM, Veen JA, Boer W. 2016. Genomic comparison of chitinolytic enzyme systems from terrestrial and aquatic bacteria. Environmental Microbiology, 18:38–49. https://doi.org/10.1111/1462-2920.12545

Barling N, Heads SW, Martill DM. 2021. Behavioural impacts on the taphonomy of dragonflies and damselflies (Odonata) from the Lower Cretaceous Crato Formation, Brazil. Palaeoentomology, 004(2):141–155. https://doi.org/10.11646/palaeoentomology.4.2.3

Barling N, Martill DM, Heads SW. 2020. A geochemical model for the preservation of insects in the Crato Formation (Lower Cretaceous) of Brazil. Cretaceous Research, 116:104608. https://doi.org/10.1016/j.cretres.2020.104608

Barling N, Martill DM, Heads SW, Gallien F. 2015. High fidelity preservation of fossil insects from the Crato Formation (Lower Cretaceous) of Brazil. Cretaceous Research, 52:605e622. https://doi.org/10.1016/j.cretres.2014.05.007

Bechly G. 1997. Dragonflies from the Lower Cretaceous of Brazil. Meganeura, 1:27–28.

Bechly G. 1998. New fossil dragonflies from the Lower Cretaceous Crato Formation of north-east Brazil (Insecta: Odonata). Stuttgarter Beiträge zur Naturkunde, Serie B, 264, 166.

Bechly G. 2007. Odonata: damselflies and dragonflies. 184–222. In: Martill DM, Bechly G, Loveridge RF (eds). The Crato Fossil Beds of Brazil: Window into an ancient world. University of Cambridge Press.

Bechly G. 2010. Additions to the fossil dragonfly fauna from the Lower Cretaceous Crato Formation of Brazil (Insecta: Odonata). Palaeodiversity, 3:11–77.

Bechly G, Nel A, Martínez-Delclòs X, Jarzembowski EA, Coram R, Martill DM, Fleck G, Escuillié F, Wisshak MM, Maisch M. 2001. A revision and phylogenetic study of Mesozoic Aeshnoptera, with description of several new families, genera and species (Insecta: Odonata: Anisoptera). Neue Paläontologische, Abhandlungen, 4:1–219.

Behrensmeyer AK, Boaz DD. 1980. The Recent bones of Amboseli National Park, Kenya, in relation to East African paleoecology. 72–92. In: Behrensmeyer AK, Hill AP (eds). Fossils in the making; vertebrate taphonomy and paleoecology. University of Chicago Press. Chicago, Ill., United States.

Bezerra FI, Mendes M. 2024. A Palaeoecological Analysis of the Cretaceous (Aptian) Insect Fauna of the Crato Formation, Brazil. Palaeo Palaeo Palaeo, 641:112134. https://doi.org/10.1016/j.palaeo.2024.112134

Bezerra FI, Silva JH, Miguel EC, Paschoal AR, Nascimento Jr DR, Freire PTC, Viana B, Mendes M. 2020. Chemical and mineral comparison of fossil insect cuticles from Crato Konservat Lagerstätte, Lower Cretaceous of Brazil. Journal of Iberian Geology, 46. https://doi.org/10.1007/s41513-020-00119-y

Boer WD, Gerards S, Gunnewiek PJAK, Modderman R. 1999. Response of the chitinolytic microbial community to chitin amendments of dune soils. Biology and Fertility of Soils, 29:170–177.

Boldrini R, Cruz PV. 2013. Criação e Transporte de Ninfas de Ephemeroptera (Insecta) em Campo. EntomoBrasilis, 6(2):168–170. https://doi.org/10.12741/ebrasilis.v6i2.281

Bradley TJ. 2009. Excretion. 334–339. In: Resh VH, Cardé RT (eds). Encyclopedia of Insects. Academic Press, Oxford UK.

Brandão NCA, Bittencourt JS, Calor AR, Mendes M, Langer MC. 2021. The Ephemeroptera (Hexapoda, Insecta) from the Lower Cretaceous Crato Formation (NE Brazil): a new genus and species, and reassessment of *Costalimella zucchii* Zamboni, 2001 and *Cratogenites*

corradiniae Martins-Neto, 1996. Cretaceous Research, 127:104923. https://doi.org/10.1016/j.cretres.2021.104923

Briggs DEG. 2003. The role of biofilms in the fossilization of non-biomineralized tissues. 281–290. In: Krumbein WE, Paterson DM, Zavarzin GA (eds). Fossil and Recent Biofilms: A Natural History of Life on Earth. Springer-Science & Business Media, Dordrecht.

Briggs DEG, McMahon S. 2006. The role of experiments in investigating the taphonomy of exceptional preservation. Paleontology, 59:1–11.

Brito IM. 1987. Nota preliminar sobre uma nova Efêmera do Cretáceo do Ceará (Insecta Ephemeroptera). Anais do X Congresso Brasileiro de Paleontologia, 593–597.

Carvalho MSS, Santos MECM. 2005. Histórico das Pesquisas Paleontológicas na Bacia do Araripe, Nordeste do Brasil. Anuário do Instituto de Geociências – UFRJ, 28(1):15–34.

Chinery M. 1986. Insects of Britain and Western Europe. Collins Guide.

Cruz PV, Salles FF, Hamada N. 2017. Four new species of *Callibaetis* Eaton (Ephemeroptera: Baetidae), nymphal description of *Callibaetis* (*Abaetetuba*) *fasciatus* (Pictet) and keys for South American species of *Callibaetis*. Zootaxa, 4250(3):229–261. https://doi.org/10.11646/zootaxa.4250.3.3

Downen MR, Selden PA, Hasiotis ST. 2016. Spider leg flexure as an indicator for estimating salinity in lacustrine paleoenvironments. Palaeogeography, Palaeoclimatology, Palaeoecology, 445:115–123. https://doi.org/10.1016/j.palaeo.2015.11.028

Edmunds GF, Jensen SL, Berner L. 1976. The Mayflies of North and Central America. University of Minnesota Press, 330 pp.

Gibbs AG, Rajpurohit S. 2010. Cuticular lipids and water balance. 100–120. In: Bagnères AG, Blomquist GJ (eds). Insect hydrocarbons: Biology, biochemistry, and chemical ecology. Cambridge University Press.

Grimaldi DA. 1990. Insects from the Santana Formation, Lower Cretaceous, of Brazil. Bulletin of the American Museum of Natural History, 195:1–191.

Grimaldi DA, Engel MS. 2005. Evolution of the insects. Cambridge, Cambridge University Press, 755 pp.

Harding IC, Chant IC. 2000. Self-sedimented diatom mats as agents of exceptional fossil preservation in the Oligocene Florissant lake beds, Colorado, United States. Geology, 28:195–198.

Heimhofer U, Martill DM. 2007. The sedimentology and depositional environment of the Crato Formation. 44–62. In: Martill DM, Bechly G, Loveridge RF (eds). The Crato Fossil Beds of Brazil. Cambridge University Press, Cambridge.

Heimhofer U, Ariztegui D, Lenniger M, Hesselbo SP, Martill DM, Rios-Netto AM. 2010. Deciphering the depositional environment of the laminated Crato fossil beds (Early Cretaceous, Araripe Basin, North-eastern Brazil). Sedimentology, 57:677–694. https://doi.org/10.1111/j.1365-3091.2009.01114.x

Iniesto M, Buscalioni Á, Carmen Guerrero M, Benzerara K, Moreira D, López-Archilla AI. 2016. Involvement of microbial mats in early fossilization by decay delay and formation of impressions and replicas of vertebrates and invertebrates. Scientific Reports, 6:25716. https://doi.org/10.1038/srep25716

Jarzembowski EA, Martínez-Delclòs X, Bechly G, Nel A, Coram R, Escuillié F. 1998. The Mesozoic noncalopterygoid Zygoptera: description of new genera and species from the Lower Cretaceous of England and Brazil and their phylogenetic significance (Odonata, Zygoptera, Coenagrionoidea, Hemiphlebioidea, Lestoidea). Cretaceous Research, 19:403–444. https://doi.org/10.1006/cres.1997.0113

Kluge NJ. 2004. The Phylogenetic System of Ephemeroptera. Dordrecht: Kluwer Academic.

Martill DM, Loveridge R, Heimhofer U. 2007. Halite pseudomorphs in the Crato Formation (Early Cretaceous, Late Aptian-Early Albian), Araripe Basin, northeast Brazil: further evidence for hypersalinity. Cretaceous Research, 28:613–620. https://doi.org/10.1016/j.cretres.2006.10.003

Martill DM, Loveridge RF, Heimhofer U. 2008. Dolomite pipes in the Crato Formation fossil Lagerstätte (Lower Cretaceous, Aptian), of northeastern Brazil. Cretaceous Research, 29:78–86. https://doi.org/10.1016/j.cretres.2007.04.007

Martill DM, Wilby PJ. 1993. Stratigraphy. 20–50. In: Martill DM (ed). Fossils of Santana and Crato Formations, Brazil, Field Guide to Fossils 5. Cambridge University Press, Cambridge.

Martínez-Delclòs X, Martinell J. 1993. Insect taphonomy experiments. Their application to the Cretaceous outcrops of lithographic limestones from Spain. Darmstädter Beiträge zur Naturgeschichte, 2:133e144.

Martínez-Delclòs X, Briggs DEG, Peñalver E. 2004. Taphonomy of insects in carbonates and amber. Palaeogeography, Palaeoclimatology, Palaeoecology, 203(1–2):19–64. https://doi.org/10.1016/S0031-0182(03)00643-6.

Martins-Neto RG. 1996. New mayflies (Insecta, Ephemeroptera) from the Santana Formation (Lower Cretaceous), Araripe Basin, northeastern Brazil. Revista Española de Paleontologia, 11(2):177–192.

Martins-Neto RG. 2005. Estágio atual da paleoartropologia brasileira: Hexápodes, miriápodes, crustáceos (Isopoda, Decapoda, Eucrustacea e Copepoda) e quelicerados. Arquivos do Museu Nacional, 63:471–494.

Martins-Neto RG, Caldas EB. 1990. Efêmeras escavadoras (Insecta, Ephemeroptera, Ephemeroidea) na Formação Santana (Cretáceo Inferior), Bacia do Araripe Nordeste do Brasil: Descrição de três novos gêneros e três novas espécies (ninfas). Atas do I Simpósio sobre a Bacia do Araripe e Bacias Interiores do Nordeste Crato, 1990:265–275.

McCafferty WP. 1990. Chapter 2. Ephemeroptera. Bulletin of the American Museum of Natural History, 195:20–50.

McCafferty WP. 1991. Toward a phylogenetic classification of the Ephemeroptera (Insecta): A commentary on systematics. Annals of the Entomological Society of America, 84(4): 343–360.

Menon F, Martill DM. 2007. Taphonomy and preservation of Crato Formation arthropods. 79–96. In: Martill DM, Bechly G, Loveridge RF (eds). The Crato Fossil Beds of Brazil: Window into an Ancient World. Cambridge University Press.

Nel A, Escuillé F. 1994. A new dragonfly from the Lower Cretaceous of Brazil. Palaeontology, 37:923–930.

Neumann VH, Borrego AG, Cabrera L, Dino R. 2003. Organic matter composition and distribution through the Aptian–Albian lacustrine sequences of the Araripe Basin, northeastern Brazil. International Journal of Coal Geology, 54:21–40. https://doi.org/10.1016/S0166-5162(03)00018-1

Ogden TH, Gattolliat JL, Sartori M, Staniczek AH, Soldán T, Whiting MF. 2009. Towards a new paradigm in mayfly phylogeny (Ephemeroptera): combined analysis of morphological and molecular data. Systematic Entomology, 34(4):616–634. https://doi.org/10.1111/j.1365-3113.2009.00488.x

Oliveira GR, Kellner AWA. 2017. Rare hatchling specimens of *Araripemys* Price, 1973 (Testudines, Pelomedusoides, Araripemydidae) from the Crato Formation, Araripe Basin. Journal of South American Earth Sciences, 69:137–142. https://doi.org/10.1016/j.jsames.2017.07.014

Osés GL, Petri S, Becker-Kerber B, Romero GR, Rizzutto MA, Rodrigues F, Galante D, Silva TF, Curado JF, Rangel EC, Ribeiro RP, Pacheco MLAF. 2016. Deciphering the preservation of fossil insects: a case study from the Crato Member, Early Cretaceous of Brazil. PeerJ, 4:e2756. https://doi.org/10.7717/peerj.2756

Penney D, Jepson JE. 2014. Fossil insects: An introduction to palaeoentomology. Siri Scientific Press, Manchester, 222 pp.

Polegatto CM, Zamboni JC. 2001. Inferences regarding the feeding behavior and morphoecological patterns of fossil mayfly nymphs (Insecta Ephemeroptera) from the Lower Cretaceous Santana Formation of northeastern Brazil. Acta Geologica Leopoldinense*,* 24:145–160.

Pouillon JM, Nel A. 2020. The oldest representative of the modern clade Aeshnodea from the Lower Cretaceous Crato Formation, Araripe Basin, NE Brazil (Odonata, Anisoptera). Cretaceous Research, 116:104580. https://doi.org/10.1016/j.cretres.2020.104580

Ribeiro AC, Ribeiro GC, Varejão FG, Battirola LD, Pessoa EM, Simões MG, Warren LV, Riccomini C, Poyato-Ariza FJ. 2021. Towards an actualistic view of the Crato Konservat-Lagerstätte paleoenvironment: A new hypothesis as an Early Cretaceous (Aptian) equatorial and semi-arid wetland. Earth-Science Reviews, 216: 103573. https://doi.org/10.1016/j.earscirev.2021.103573

Salles FF, Domíngues E, Molineri C, Boldrini R, Nieto C, Dias LG. 2018. Order Ephemeroptera. In: Hamada N, Thorp, Covich (eds). Thorp and Covich's Freshwater Invertebrates, Fourth Edition: Keys to Neotropical Hexapoda. Academic Press. https://doi.org/10.1016/C2015-0-01626-4

Schäfer W. 1972. Ecology and palaeoecology of marine environments. The University of Chicago Press, 568 pp.

Simith DJB, Diele K. 2008. O efeito da salinidade no desenvolvimento larval do caranguejo-uçá, *Ucides cordatus* (Linnaeus, 1763) (Decapoda: Ocypodidae) no Norte do Brasil. Acta Amazônica, 38(2):345–350. https://doi.org/10.1590/S0044-59672008000200019

Staniczek AH. 2007. Ephemeroptera: mayflies. 163–184. In: Martill DM, Bechly G, Loveridge RF (eds). The Crato Fossil Beds of Brazil: Window into an Ancient World. Cambridge University Press, Cambridge.

Staniczek AH, Storari AP, Godunko RJ. 2022. Revised systematics, phylogeny, and paleontology of the mayfly family Baetiscidae (Insecta: Ephemeroptera). Arthropod Systematics & Phylogeny, 80:389–409. https://doi.org/10.3897/asp.80.e82845

Storari AP, Godunko RJ, Salles FF, Saraiva AAF, Staniczek AH, Rodrigues T. 2021a. An overview of the Hexagenitidae (Ephemeroptera) from the Crato Formation (Aptian, Lower Cretaceous) of Brazil, with the description of a new species. Historical Biology, 34(5):875-884. https://doi.org/10.1080/08912963.2021.1952196

Storari AP, Rodrigues T, Bantim RAM, Lima FJ & Saraiva AAF. 2021b. Mass mortality events of autochthonous faunas in a Lower Cretaceous Gondwanan Lagerstätte. *Scientific Reports*, 11:6976. https://doi.org/10.1038/s41598-021-85953-5

Storari AP, Rodrigues T, Saraiva AAF, Salles FF. 2020. Unmasking a gap: a new oligoneuriid fossil (Ephemeroptera: Insecta) from the Crato Formation (upper Aptian), Araripe Basin, NE Brazil, with comments on *Colocrus* McCafferty. PLoS One, 15(10):e0240365. https://doi.org/10.1038/s41598-021-85953-5

Varejão FG, Warren LV, Simões MG, Fürsich FT, Matos SA, Assine ML. 2019. Exceptional preservation of soft tissues by microbial entombment: insights into the taphonomy of the Crato Konservat-Lagerstätte. Palaios, 34:331–348. https://doi.org/10.2110/palo.2019.041

Veliz EA, Matínez-Hidalgo P, Hirsch AM. 2017. Chitinase-producing bacteria and their role in biocontrol. AIMS MIcrobiology, 3(3):689–705. https://doi.org/10.3934/microbiol.2017.3.689

Warren LV, Varejão FG, Quaglio F, Simões MG, Fürsich FT, Poire DG, Catto B, Assine ML. 2017. Stromatolites from the Aptian Crato Formation, a hypersaline lake system in the Araripe Basin, northeastern Brazil. Facies, 63. https://doi.org/10.1007/s10347-016-0484-6

Zamboni JC. 2001. Contribution to the knowledge of the aquatic paleoentomofauna from Santana Formation (Araripe Basin, Lower Cretaceous, Northeast Brazil) with description of new taxa. Acta Geologica Leopoldinense, 24:129–135.

General conclusion

To study the preservational modes of the Crato Formation it is important to emphasize the exceptional preservation, like the well-preserved foreguts presented in Chapter 1, however, it is also important to highlight the modal preservational fidelity. In the Crato Formation, insect fossils are most commonly preserved with moderate-to-high relief, but can also vary greatly in preservational fidelity within a single fossil, like observed in the crickets from Chapter 1 that, while bearing preserved delicate inner organs like the three-dimensionally proventriculi, also present overall bad preservation of their outer morphological traits (such as wings and head structures, useful for taxonomic studies). Interestingly, it is common that insects with good traits preserved for taxonomic identifications (general body parts like head, thorax, wings, legs, and abdomen) to have bad preservation at micron-scale and *vice versa*.

In any case, remarkably well-preserved fossils like the crickets portrayed in Chapter 1 are not rare in the Crato Formation, which enabled a detailed characterization of internal organs and soft tissues preserved for this specific taxonomic group. Although it is not common to recover soft tissue preserved as these proventriculi, features from the insect cuticle (e.g. cuticular scales, setae, and spiracles), as shown in Chapter 2, are not so rare and demonstrate how singular the Crato Formation is in preserving exceptional fossils. The preservation of Crato insects is not only exceptional but also diverse, as shown in Chapter 2, with fossils displaying [1]calcium phosphate and iron oxides/hydroxides preserving internal features, which are, in general, substituted by [2]iron oxide after pyritization, though in rare cases they can be preserved by [3]carbonaceous content in grayish limestones. In our results, we also observed features that once again suggest the presence of microbial mats in the Lagerstätte strata of the Crato Formation, strengthening the hypothesis that they are a crucial proxy for soft tissue preservation.

For both chapters 1 and 2, analytical techniques were crucial to unravel the fine preservation of specimens, but also, in Chapter 2, to distinguish the insect preservation of two fossiliferous units, regarded as similar in the past, namely the Crato Formation and the Solnhofen limestones. But apart from diagenetic data provided from those analytic analyses, biostratinomic data and actualistic approaches also help to explain the fossilization process of insects and even past environmental conditions, which we show in Chapter 3 for aquatic insects of the Crato Formation. Our results demonstrate that *Protoligoneuria limai* assemblages were autochthonous

to the depositional site, a condition that was probably only shared with small populations of gomphids, and seasonally with *Dastilbe* juvenile fish. It is, however, important to replicate such experiments with additional taxonomic groups and additional abiotic conditions/tests.

The Crato Formation fossils are commonly described as exceptionally preserved, however, when specimens from controlled excavation are analyzed, actually, the fossil record present a wider range of preservation. This is due to collector's bias, as well as biased collections which favor well-preserved specimen and/or taxonomic groups related to the expertise of curators. However, it should be noted that for taphonomic studies (specially biostratinomy) it is pointless to study only the well preserved fossils. Considering this, for our results, we recognize some data might be affected by bias, and only the preservational data on Hexagenitidae larvae can be fully trusted since it comes entirely from controlled excavations.

The goals of this project were achieved, but there are still many questions regarding the taphonomy of the Crato Formation fossil insects that require further investigation. Next steps would be to expand the type of study performed in Chapter 3 to other taxonomic groups of the Crato Formation, performing similar experiments with different modern taxonomic groups. Most importantly, to focus from now on in the biostratinomic study of specimens from controlled excavations, since observations on the disarticulation rates can be completely mismatched if not including also the "badly preserved" specimens. Finally, apart from the taphonomy, many insect groups from the Crato Formation need significant review to start with (e.g. microanatomy). As noted above, the exceptional preservation of internal soft tissues may be more common than we first assumed. This means that many features could be uncovered with the reanalysis of described specimens using technologic analytical tools (e.g. SEM; MicroCT).

The novel results of this thesis can be summarized as follows:

1. First detailed description of fossilized proventriculi from Orthoptera;

2. Fossil crickets present proventriculus more robust than modern crickets, i.e. with more rows of sclerotised parallel divisions;

3. In Crato fossils, areas with the highest fidelity of preservation present smaller, and more closely-packed crystals when compared to the less-preserved parts;

4. New evidence based on preserved microstructures suggesting the presence of microbial mats during the fossilization process of insects from the Crato Formation, reinforcing previous hypotheses;

5. Reinforcement of the two main modes of preservation of Crato fossil insects: Pyritization and phosphatisation;

6. First detailed comparison between insect fauna of Crato and Solnhofen;

7. First geochemical and micromorphological characterization performed at any insect group from the Solnhofen;

8. First taphonomic study on fossil insects based on experimental data on modern groups present in the Crato Formation;

9. The thorax of the extant mayfly larvae carcasses is the most likely element to disarticulate first during decomposition;

10. Mayfly larvae from the Crato Formation rarely present a disarticulated thorax;

11. Reinforcement of hypothesis that *Protoligoneuria limai* assemblages were autochthonous to the Crato paleolake;

12. Identification of natural postmortem position of both mayfly and dragonfly larvae;

13. The labium of the extant dragonfly larvae carcasses is the most likely element to disarticulate first during decomposition;

14. Dragonfly larvae from the Crato Formation rarely present a disarticulated labium;

15. Opposition to hypothesis stating that Proterogomphidae and Nothomacromiidae dragonfly groups from Crato represent allochtonous assemblages;

16. Carcasses immersed in saline conditions go through slower decomposition;

17. Reinforcement of hypothesis that when small insects die in sub-aerial conditions, there are few possibilities of sinking;

18. For the first time, the hypothesis is proposed that floating microbial biofilms also acted during sinking and subsequent preservation of insect carcasses from the Crato Formation.