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Distinct preservational pathways of insects from the Crato Formation, Lower Cretaceous of the Araripe Basin, Brazil

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ABSTRACT

This study compares two different preservation pathways of fossil insects in the lacustrine deposits of the Crato Formation, Lower Cretaceous (Aptian) of the Araripe Basin, northeastern Brazil. Three hundred seventy-seven specimens were examined and separated into ten taxonomic groups. Of this total, one hundred twenty-three are kerogenized insects, and two hundred fifty-four are pyritized insects. We carried out quantitative analyses of their taphonomic characters, such as body articulation, and morphological preservational quality (e.g. discernible eyes). Of all morphological categories, the thorax presented the highest degree of preservation quality, while the antennae had the lowest. Our statistical results show significant differences in the preservation quality of individual morphological categories among the insect taxa. We expected that mineralized insects would have lower preservational quality than the kerogenized ones, but instead found the opposite pattern to be true. Counter to the findings of other studies, we found that pyritized insects had higher preservation quality than kerogenized insects. The expected lower preservation fidelity of pyritized fossils occurs due to longer time exposed to microbial decay before final burial. Few studies have presented a quantitative comparison of preservational/biostratinomic patterns in different insect taxa, especially within the same geological setting. In this context, the Crato Formation presents an intriguing and unique opportunity to understand the taphonomic bias that results from two different preservation pathways of insects.

1. Introduction

Since the Carboniferous insects have been a group that has experienced a nearly continuous increase in diversity. Today, they represent the most diverse and widespread animal clade in the history of life on Earth (Labandeira and Sepkoski, 1993; Grimaldi and Engel, 2005). The large insect fossil record extends from different terrestrial to marine environments (Smith et al., 2006; Smith and Moe-Hoffman, 2007; Smith, 2012). Among different geological settings in which fossil insects can occur, those preserved in amber and in lacustrine deposits have been highlighted for their quality of preservation (Henwood, 1992, 1993; Martínez-Declòs and Martinell, 1993; Smith, 2000, 2012; Martínez-Declòs et al., 2004; Smith et al., 2006; Edwards et al., 2007; Thoene Henning et al., 2012; Wang et al., 2013; Barling et al., 2015; McCoy et al., 2018; Bezerra et al., 2020). Insects preserved in amber display higher morphological fidelity, though the sticky resin only captures insects living in and around the resiniferous trees (Solórzano-Kraemer et al., 2015, 2018). Amber inclusions occur rarely in the Triassic (Schmidt et al., 2012). However, amber accumulations increase in importance only after the Early Cretaceous (Smith, 2012). Lacustrine deposits can be found since the Carboniferous, preserving taxa from distinct ontogenetic stages, distinct habits and variable sizes. The insect accumulation in lake sediments is a significant complement to the insect fossil record in terms of diversity. Hence, increasing our understanding about the taphonomic processes involved in these insect assemblages is essential to interpreting both the palaeoecology and palaeoenvironment (Martínez-Delclòs et al., 2004).

In this context, the Crato Formation (Aptian of the Araripe Basin, northeastern Brazil) is one of the world's premiere Fossil-lagerstätten (Seilacher, 1970), yielding an exceptionally well-preserved entomofauna in a series of lake deposits. The Crato Fm. consists of laminated limestones interbedded with a series of claystones, siltstones and sandstones, deposited in at lacustrine system (Heimhofer et al., 2010). Typically, Crato carbonate facies can be divdided into two different sub-facies: clay–carbonate rhythmites

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(CCR) and laminated limestones (LL) (Neumann et al., 2003). Normally, CCR facies yield dark-grey-colored layers while LL occurs in yellow-colored layers (Fig. 1). The Crato insects commonly appear as an orange to brown friable material or as a seemingly amorphous dark matter. Previous studies have hypothesized this difference in preservation as a result of rock weathering (Delgado et al., 2014; Osés et al., 2016). But Osés et al. (2017) reported two distinct taphonomic modes: carbonaceous compressions in dark limestones and iron oxy-hydroxide after pyrite in yellow-colored limestone. In addition, Bezerra et al. (2018) identified a cockroach preserved into carbonaceous compressions in dark limestones. Thus, we consider that the insects are preserved in a kerogenization zone (dark limestone) and a pyritization zone (yellow limestone).

Preservation by keroginization represents cyclic hydrocarbons and aliphatic components which have undergone chemical transformation from original organic material into the fossil record (Stankiewicz et al., 1998; Briggs, 1999; McNamara et al., 2013; Schiffbauer et al., 2014). According to Schiffbauer et al. (2014), this preservation style occurs when the carcass is placed most of time into a metanogenesis zone of the sediment column. In the bacterial sulfate reduction zone (BSR), an organism becomes pyritized when ions generated by bacterial activity produce minerals that replaces the body soft-tissue (Raiswell et al., 1993; Schiffbauer et al., 2014). Both keroginization and pyritization are able to preserve recalcitrant components of soft-bodied material. However, pyritized fossils tend to undergo further degradation than kerogenized ones, given the greater amount of time in the BSR which is more metabolically efficient than the Methanogenesis Zone (Anderson and Smith, 2017). Surprisingly, pyritized insects from the Crato Formation are preserved with ultrastructural cuticular details (Barling et al., 2015; Osés et al., 2016). Therefore, the Crato Fm. offers a rare opportunity to compare how two very different preservational pathways differ within the same geological setting. Thus we compared the preservational fidelity between keroginized and pyritized insects from the Crato Formation of the Araripe Basin in northeastern Brazil to understand which preservational style is superior for preserving soft-bodied fossils.

2. Geological setting

The Araripe Basin is one of the largest basins in the interior of the Brazilian northeast (Fig. 2). The Crato Fm. comprises a 70-m-thick succession and consists of carbonate layers interbedded with siliciclastic sediments (shales, claystones and sandstones), whose origin is attributed to transgressive-regressive events associated with the expansion and contraction of a lacustrine system

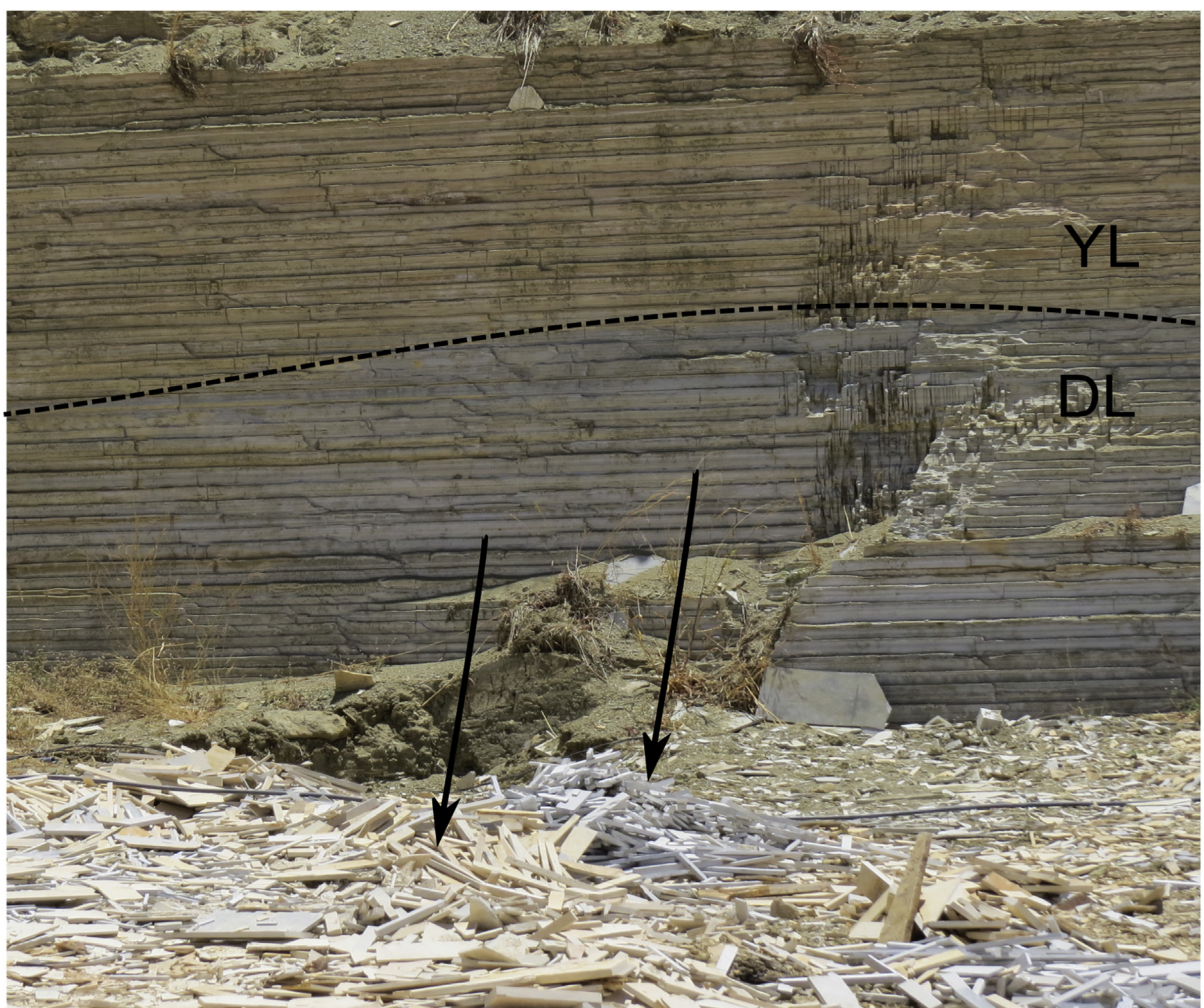

Fig. 1. View of the Crato limestone exposure in one of the quarries located between Nova Olinda and Santana do Cariri, Ceará. Here, the contact between the pyritization zone and kerogenized zone is shown. The arrows showing the two types that are left over after extraction of *Pedra Cariri*.

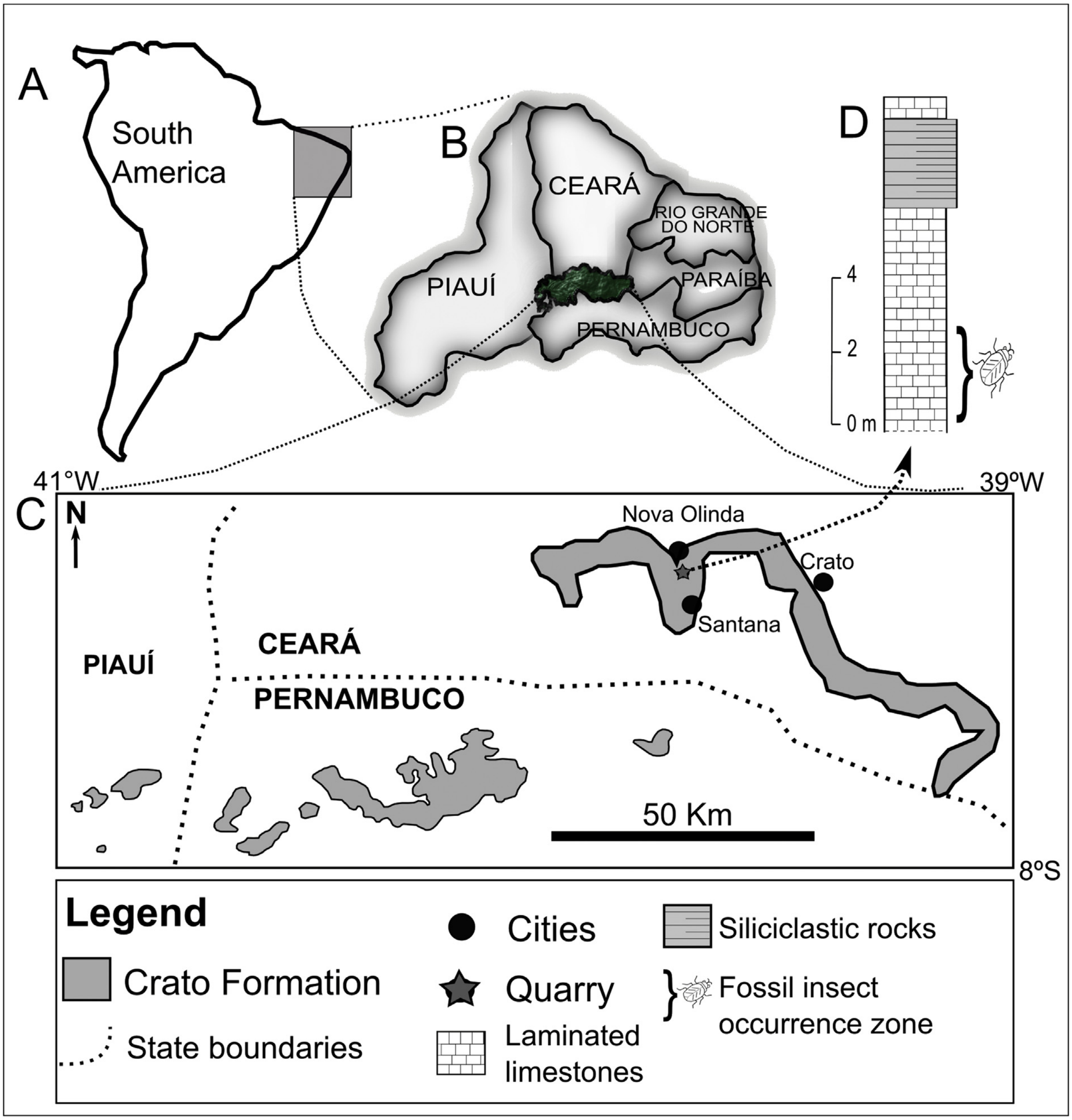

Fig. 2. Simplified map showing the location of the Crato Formation. A. The map of the South America continent shows the position of some states in northeastern Brazil. B. Black square showing the position of the Araripe Basin. C. Local map showing the main outcrops of the Crato Formation. D. Generalized stratigraphy of the Crato Formation in the Nova Olinda, Ceará.

(Neumann, 1999). Crato strata (Aptian) represent the second lacustrine phase in the development of the Araripe Basin (Assine et al., 2014).

The Crato carbonate facies have a micritic calcite composition with low magnesium content (Neumann, 1999; Catto et al., 2016). This unit is part of the Aptian post-rift I sequence (Assine, 2007). The origin of the Crato laminated carbonates has been attributed to chemical precipitation associated with fine clastic sediments (Heimhofer et al., 2010). Catto et al. (2016) investigated the evidence of microbial influence on the formation of the Crato limestones. The presence of halite pseudomorphs in some levels suggests deposition under fluctuating salinity conditions (Martill et al., 2007). The excellent preservation of Crato fossils have made it known worldwide as a Cretaceous Fossil Lagerstätte. Most of the outcrops of laminated limestones are exposed due to the commercial extraction of *pedra cariri* (*Pedra* = stone; *Cariri* = Southern region of Ceará) in small quarries.

3. Material and methods

All insects analyzed in this study are deposited in the Laboratório de Paleontologia from Universidade Federal do Ceará (UFC). The fossils were collected over several years during different fieldwork campaigns. All fieldwork activities involved geology and biological sciences undergraduate students. The collections focused on the materials left over after the process of separating the lucrative *Pedra Cariri*. Taxonomic classification was made to order level. The suborders Heteroptera and Auchenorrhyncha

(Hemiptera) and the suborders Caelifera and Ensifera (Orthoptera) were separated from their broad orders because we consider that the morphological differences between these suborders are sufficiently considerable for the purpose of this study. In order to avoid possible nomenclature problems we preferred to gather the specimens using a general term, taxon. All insects were analyzed using a standard quantitative method of scoring preservational quality.

The preservation-quality indicators were quantitatively assigned scores for different categories of insect morphology: head; antennae; eyes; legs; thorax; abdomen and wings. The maximum score for a category depended on the morphological variability within each category. The antennae, legs, thorax, abdomen have four potential scores, where 0: not preserved; 1: present without details; 2: present showing major segments; 3: present showing all segments. The wings have four potential scores, 0: absence; 1: present as an outline; 2: at least one wing with partially erased veins or folded; 3: at least one extended wings with all veins. The head and eyes have three potential scores, 0: not preserved; 1: present; 2: present with details. For comparisons of "presence" or "absence" of each morphological category between the pyritized and kerogenized insects, we assigned the score 0, when the trait is absent, and the score 1, when the trait is present even without anatomical details. For the "articulation" state, we considered articulated insects those which display the head, thorax, abdomen, and at least one articulated wing (if preserved in dorsal view), and head, thorax, abdomen, at least one leg, and at least one wing articulated (if preserved in both lateral and ventral views). Almost complete bodies such as specimens with head, thorax and wings; thorax and abdomen; head and abdomen; or winged specimens missing wings or specimens with no legs were considered "partially articulated". Isolated wings or legs represent the "disarticulated" specimens. The orientation of each fully/partially articulated specimen in the host slab, dorsoventral or lateral position, was also documented. The proportions of the quality scores of the articulation states were determined by comparing the completeness of entire insect bodies between the two preservation types, where 0: disarticulated (Fig. 5H), 1: partially articulated (Fig. 5D); 2: fully articulated (Fig. 5E).

To compare pyritized and kerogenized insects we used the chi square tests (Franke et al., 2012). A chi-square contingency test was used to compare the frequency distributions among all taxa and the number of samples within each insect taxon. The chi-square goodness of fit test was used to compare the observed distribution to the expected distribution of the maximum values (score of 3), zero values (score of 0) and the articulation state. The differences in the frequency distributions were tested with an α of 0.05. A list of these specimens and their associated data can be found in the online supplementary material. All statistical analyses were performed using EXCEL and PAST version 3.09.

The percolation of late diagenetic meteoric fluids leading to loss of fine details is a taphonomical factor that should be considered. Thus, intensely weathered specimens were not included in this study.

4. Results

4.1. Taxonomic and taphonomic distributions

In this study we analyzed 377 insects from the Crato Formation: 123 insects were described from the kerogenized zone and 254 insects were described from the pyritized zone. Only insects identifiable to orders were considered as a result, ten insect taxa were included in this study: Blattodea (Without Isoptera), Ensifera (Orthoptera), Caelifera (Orthoptera), Odonata, Ephemeroptera, Neuroptera, Coleoptera, Diptera, Auchenorrhyncha (Hemiptera) and Heteroptera (Hemiptera) (Table 1).

Table 1
Number of insects for each taxonomic group from the Crato Formation. Statistically significant values are given in bold.

Taxon	Kerogenized insects	Pyritized insects	Chi-squared tests	
			$\chi 2$	*P-value*
Blattodea	11	42	3.397	0.065
Neuroptera	36	17	**12.53**	**$<10^{-4}$**
Auchenorrhyncha	18	27	1.113	0.291
Caelifera	11	33	1.164	0.280
Ensifera	16	33	$<10^{-4}$	0.996
Odonata	12	27	0.061	0.804
Heteroptera	7	24	1.426	0.232
Ephemeroptera	0	27	–	–
Coleoptera	4	14	0.886	0.346
Diptera	8	10	1.143	0.284
Total	123	254		

Of the 123 kerogenized insects, the most abundant taxa are Neuroptera (28.8%), Auchenorrhyncha (14.4%), Ensifera (12.8%) and Blattodea (10.4%). There were no kerogenized Ephemeroptera found. Of 254 pyritized insects, the most abundant are Blattodea (17.2%), Caelifera (13.5%), Odonata (11%) and Ephemeroptera (11%) (Fig. 3).

Of the total of specimens, 59.2% were whole bodies with wings, 37.9% of bodies were not complete; they had lost delicate structures, such as their antennae, head or discernible legs. Only 2.1% of specimens were isolated wings, and only 0.8% were isolated legs. 55.4% of all specimens were preserved in a dorsal position, 14.6% and 27% were preserved in lateral and ventral positions, respectively. The antennae were the most affected feature, only 3.2% of insects exhibited complete antennae with all segments. Only 9.3% of all insects showed discernible eyes. 26% of insects displayed at least one articulated leg. One-third of the winged insects preserved in a lateral position have wings folded, while 83% of the winged insects preserved in a dorsal position were with forewings partially spread in flight position.

4.2. Comparison of preservational fidelity for individual morphological categories

We compared all kerogenized and all pyritized insects and the distribution of quality scores is significantly different between both types of preservation. The quantitative analyses revealed significant differences in the frequency distributions of scores for the head ($x^2 = 4.134$; $p = 0.042$; df = 1), the legs ($x^2 = 8.446$; $p = 0.014$; df = 2), the abdomen ($x^2 = 34.321$; $p < 10^{-4}$; df = 2) and the eyes ($x^2 = 9.596$; $p = 0.0019$; df = 1). There was no significant difference in the frequency of preservational fidelity scores of the antennae ($x^2 = 1.270$; $p = 0.529$; df = 2) and thoraces ($x^2 = 3.913$; $p = 0.141$; df = 2) between the two preservation types. The wings also showed no significant difference ($x^2 = 2.755$; $p = 0.252$; df = 2). For wings, the preservational process was not selective; both membranous and heavily sclerotized forewings (tegmina) are often present. Even membranous wings beneath the elytra of Coleoptera and hemelytra of Heteroptera are usually preserved for both the pyritized and kerogenized insects.

When we compared only individual insect taxa, the distributions of the individual morphological categories were often significantly different between the mineralized and kerogenized insects. In addition to the abdomen, the other morphological categories that had significant differences were wings, head, antennae and legs among the included taxa. For example, the comparison

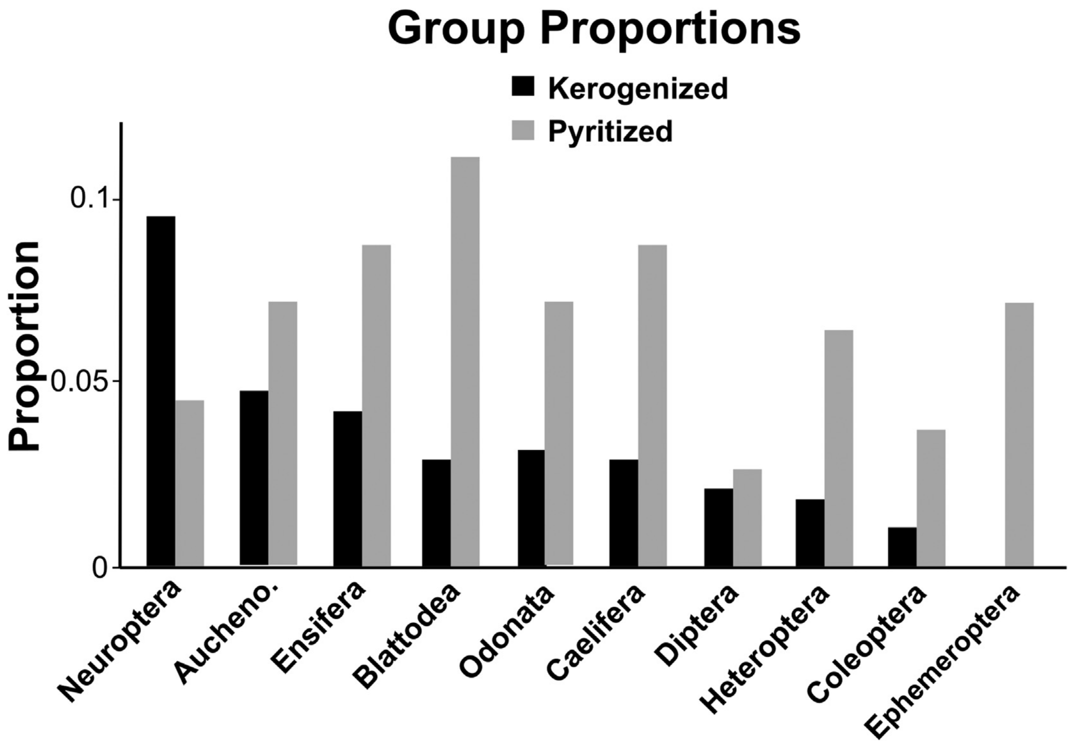

Fig. 3. The proportional distribution of identified kerogenized and pyritized insects from the Crato Formation.

between the kerogenized Ensifera and pyritized Ensifera revealed differences in the abdomen ($x^2 = 6.975$; $p = 0.030$; df = 2); head ($x^2 = 5.216$; $p = 0.022$; df = 1) and legs ($x^2 = 6.543$; $p = 0.037$; df = 2) categories. Besides Ensifera, Neuroptera, Odonata, Caelifera, Blattodea, Diptera and Auchenorrhyncha also displayed differences in the individual morphological categories. Neuroptera showed significant differences in the antennae ($x^2 = 6.371$; $p = 0.041$; df = 2) and wings ($x^2 = 9.990$; $p = 0.006$; df = 2). Auchenorrhyncha also presented significant differences in the head ($x^2 = 4.008$; $p = 0.045$; df = 1) and abdomen ($x^2 = 11.954$; $p = 0.002$; df = 2) categories. Diptera showed relevant differences in the thorax ($x^2 = 7.348$; $p = 0.025$; df = 2) and wings ($x^2 = 8.66$; $p = 0.013$; df = 2). Some individual insect taxa showed relevant differences in only one individual morphological category. For Caelifera, quantitative analysis revealed significant differences in the frequency distributions of scores for the wing ($x^2 = 8.249$; $p = 0.016$; df = 2) category. For Heteroptera, the distribution of scores for the abdomen preservation fidelity was statistically significant ($x^2 = 21.13$; $p < 10^{-4}$; df = 2). Lastly, the quantitative analysis also revealed significant differences in the frequency distributions of the abdomen preservation for pyritized Blattodea versus kerogenized Blattodea ($x^2 = 13.85$; $p < 10^{-4}$; df = 2).

When we compared only individual insect taxa, Ensifera presented the highest number of differences among the individual morphological categories between the two preservation types. When analyzing only the individual insect features, the thorax preservation-fidelity score stood out as the best-preserved morphological component, when all insects were considered (Fig. 4). In contrast, the antennae were the worst and the least frequently preserved for both pyritized and kerogenized insects.

4.2.1. *The distribution of top scores for the individual morphological categories*

The quality of preservation of insect exoskeletal materials in the Crato Formation can be classified as excellent (Fig. 5). This exceptional preservation makes it possible to easily identify specimens to the order level. Most insects have received at least one maximum-score-value (in this case 3) for at least one morphological category for both preservation types. For example, 18.8% of the specimens received a score of 3 for the head morphological category, considering all insects from the two preservation types (Fig. 6). Among all the insect taxa, 28.9% received a score of 3 for the abdomen. For legs, 25.9% of all specimens scored 3. Auchenorrhyncha have the highest proportion for the maximum score of the thorax and leg morphological categories, 75.5% and 64.4%, respectively. Considering all insects, 25.4% of the specimens received score 3 for wings. 46.1% of all Odonata specimens had this score for wings. Only 9.2% of all insects have discernible eyes, but 33.3% of the Odonata scored 3. Interestingly, considering the Odonata that received score 3 for eyes, 84.6% are pyritized specimens.

The thorax was the individual morphological category that received the highest number of maximum scores, 31% for insects preserved by both pathways. In contrast, the individual morphological category with the lowest number of maximum scores was the antenna. The antennae were lost more than any other feature. Only 3.1% of all insects scored a 3.

Comparing the mineralized and kerogenized insects separately, the proportions of the maximum-score value for the individual morphological categories show that the preservational quality of the mineralized insects was often higher than that of the kerogenized ones. Counting only the pyritized insects 35.4% of the specimens received a score of 3 for the abdomen, 33.5% for the thorax, 27.1% for the legs, 23.6% for the wings, 21.2% for the head, 12.6% for the eyes and 3.1% for the antennae. When we observe only the kerogenized insects, 15.4% of the specimens received a score of 3 for the abdomen, 26% for the thorax, 23.6% for the legs, 29.2% of specimens scored 3 for the wings, 13.8% for the head, 13.8% for the eyes and 3.2% for the antennae. The distribution of the maximum-score-value was significantly different

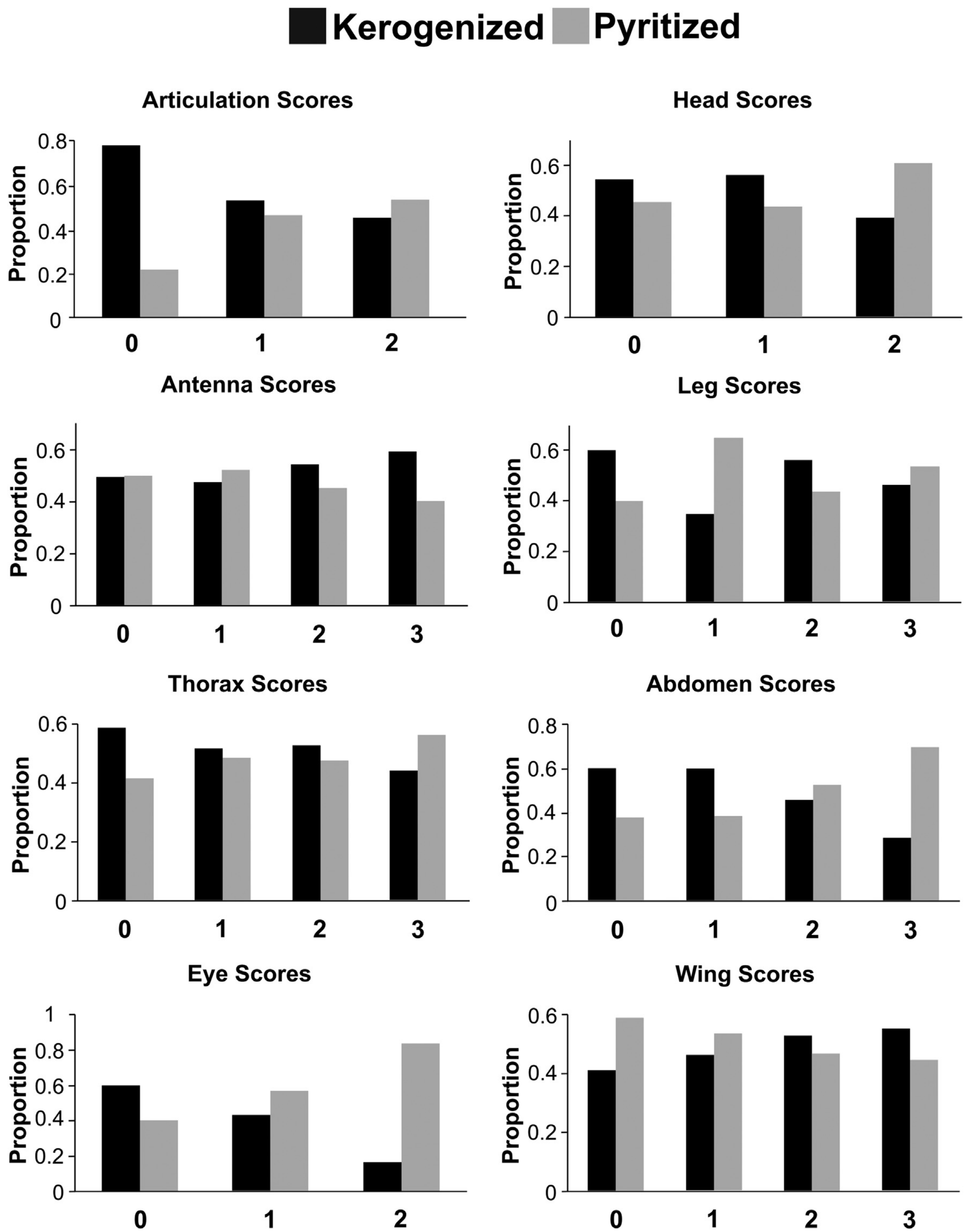

Fig. 4. The distribution of all quality scores for the individual morphological categories of all kerogenized and pyritized insects from the Crato Formation.

between the mineralized and kerogenized insects for eyes and abdomen (Table 2).

Considering the head morphological category, we noticed that 18.2% of the pyritized Caelifera scored 3, while no kerogenized Caelifera reached that mark. For thoraces, the highest difference among all insect taxa was attributed to Odonata and Diptera. 20.5% and 16.6% of the pyritized Odonata and Diptera scored a 3, respectively, while none of the kerogenized Odonata and Diptera scored a 3. Quantitative analysis revealed that the abdomen and eyes are the mostly completely preserved in the pyritized specimens in comparison to the kerogenized ones. For eyes, the highest difference among all insects was attributed to Odonata. Heteroptera, Blattodea, Caelifera, Ensifera, Diptera, Coleoptera and Neuroptera not reach the maximum value, when we considered the kerogenized insects (Fig. 7).

4.3. Articulation states

The majority of kerogenized and pyritized insects from the Crato Formation have complete articulated bodies and partially

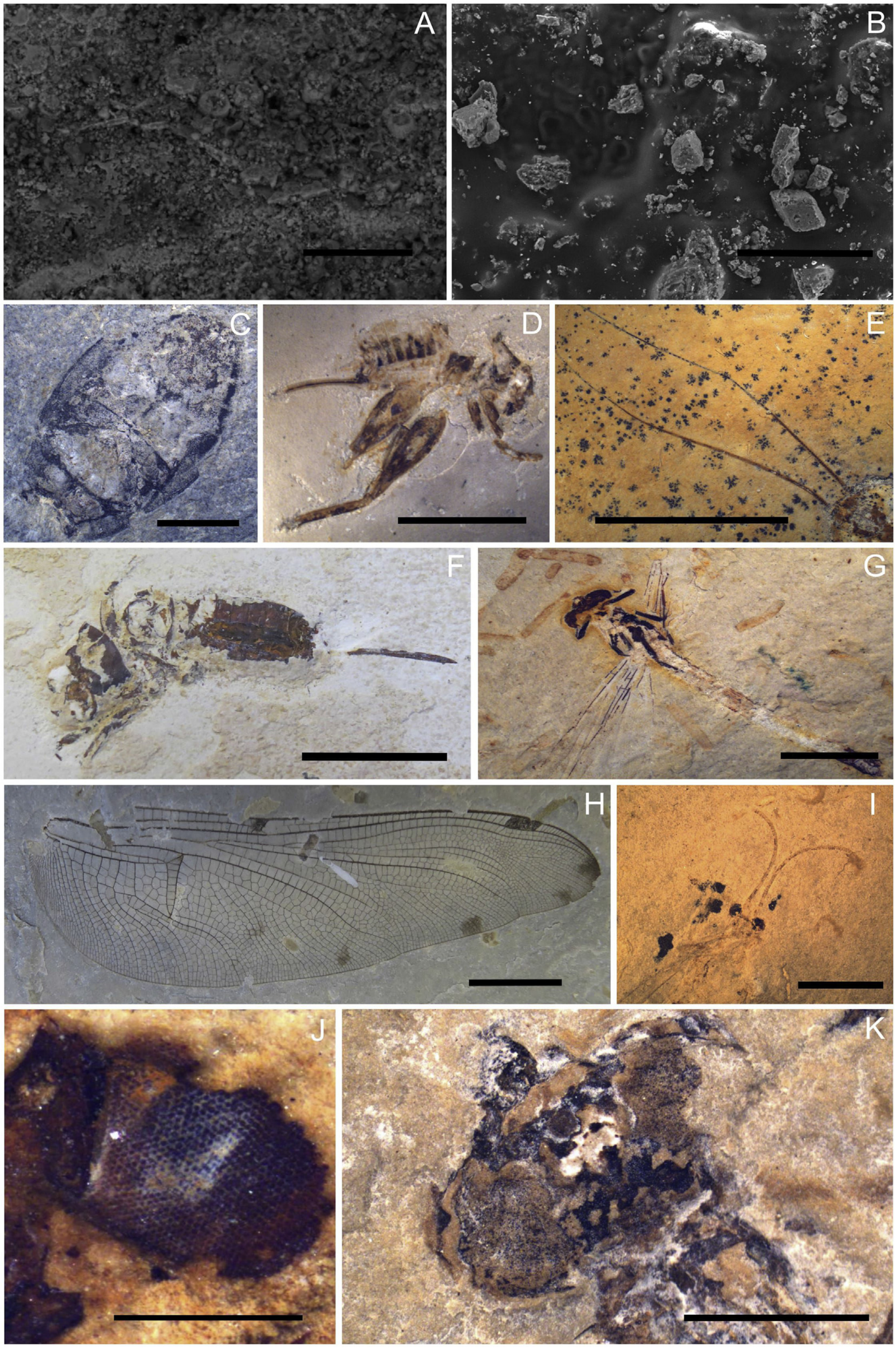

Fig. 5. Examples of Crato insects in varying degrees of preservation and completeness. A. Scanning electron micrograph of the pyritized cuticle of Ensifera (CRT/UFC 2659). B. Secondary electron micrograph of the kerogenized Ensifera showing no discernible microfabrics (CRT/UFC 2400). C. Kerogenized Heteroptera, the head missing (CRT/UFC 703). D. Kerogenized Ensifera, partially articulated (CRT/UFC 2303). E. Pyritized Blattodea showing antennae preserved spectacularly (CRT/UFC 2060). F. Pyritized Ensifera, abdomen and ovipositor preserved (CRT/UFC 2388). G. Pyritized Odonata, almost whole abdomen left as impression (CRT/UFC 1923). H. Kerogenized Odonata, isolated wing (CRT/UFC 1154). I. Kerogenized Neuroptera preserved as a faint impression, the eyes in particular (CRT/UFC 100). J. Pyritized Odonata displaying individual ommatidia (CRT/UFC 95). K. Kerogenized Odonata with preserved eyes and ommatidia (CRT/UFC 1213). Scales bars: A = 30 μm; B = 50 μm; C = 5 mm; D = 5 mm; E = 10 mm; F = 5 mm; G = 5 mm; H = 10 mm; I = 4 mm; J = 2 mm; K = 3 mm.

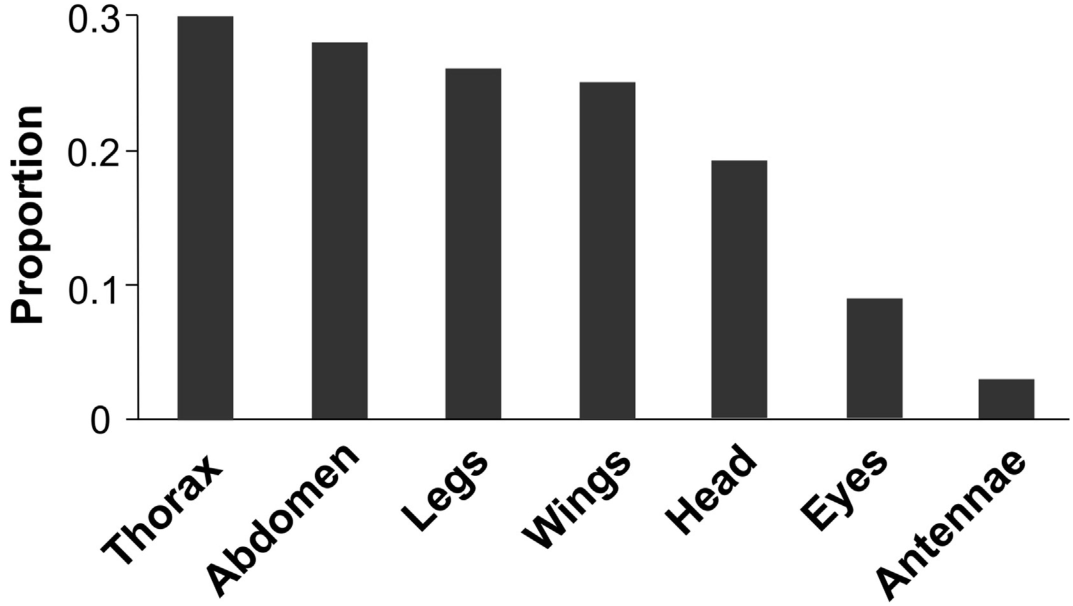

Fig. 6. Histogram showing the maximum-score value proportions for the individual morphological categories of all pyritized and kerogenized insects of the Crato Formation.

articulated bodies. The comparison of the distribution of scores for articulation state of the specimens was not significantly different between kerogenized and pyritized insects from the Crato Formation ($x^2 = 4455$; $p = 0.107$; df $= 2$). When we compared only individual insect taxon, the distribution of articulation state was significantly different between the mineralized and kerogenized Ensifera ($x^2 = 10.26$; $p = 0.0059$; df $= 2$) and Neuroptera ($x^2 = 28.66$; $p < 10^{-4}$; df $= 2$).

For all insects, fully articulated specimens are the most common (223) (Table 3). Considering all fully articulated insects, 158 are pyritized. The differences between pyritized and kerogenized insects were not significant ($x^2 = 1.227$; $p = 0.267$; df $= 1$) when only the fully articulated specimens were considered. 143 of all specimens are partially articulated, when we observe only the partially articulated insects, 92 are mineralized. For partially articulated specimens, the difference between pyritized and kerogenized insects was not significant ($x^2 = 0.6$; $p = 0.438$; df $= 1$). However, the comparison ($x^2 = 4.812$; $p = 0.028$; df $= 1$) of the proportion of disarticulated specimens (11) was significantly different between iron-oxide insects and kerogenous insects (Table 3).

When considering all specimens, 20.9% of insects were missing the head. When considering only pyritized insects, 19.6% were missing the head. On the other hand, 23.6% of specimens were assigned to this state when considering only kerogenized insects. 76.1% of all specimens received a score 0 for antennae and 49% for eyes. Counting only the pyritized insects, 76.4% of the specimens score 0 for antennae, and 42.1% of the specimens score 0 for eyes. Counting only the kerogenized insects, 75.6% and 63.4% score 0 for antennae and eyes, respectively. 10.6% of kerogenized insects are missing the thorax against 7.5% of pyritized insects. When we considered all specimens, 17.7% of the insects lost their abdomen. The abdomen is absent in 14.9% of the specimens, when considering only pyritized insects, and in 23.5% of specimens, when considering only kerogenized insects. For wings, the results were similar for both types of preservation, 10.6% of the pyritized insects and 7.3% of the kerogenized insects lost their wings. Only 9.5% of all insects score 0 for wings. The distribution of the zero-values was significantly different between the mineralized and kerogenized insects for eyes and legs (Table 4).

When comparing the proportion of the presence or absence of individual morphological categories, regardless of anatomical details, for all mineralized and kerogenized insects from the Crato Formation, the majority are fully articulated, while less than 3% are disarticulated. Of all the partially articulated specimens, only the pyritized insects have representatives missing the head and thorax, the head and abdomen or the head and wings: 3.9%, 2.9% and 2.9%, respectively. This suggests that pyritized insects have a greater number of disarticulation styles. However, when we investigated the percentages of individual morphological categories missing, the kerogenized insects show higher values. The presence/absence proportions were statistically significant for eyes, legs and abdomen morphological categories (Table 5).

Table 2
The distribution of the maximum-score values for the individual morphological categories, for pyritized and kerogenized insects of the Crato Formation.

Morphological Categories	All insects	Pyritized insects	Kerogenized insects	chi-squared tests	
				χ2	*P-value*
Head	71	54	17	2.434	0.118
Eye	35	32	3	**9.213**	**0.002**
Antenna	12	8	4	0.002	0.958
Thorax	117	85	32	1.481	0.223
Leg	98	69	29	0.410	0.521
Wing	96	60	36	1.037	0.308
Abdomen	109	90	19	**11.448**	**$<10^{-4}$**

Statistically significant values are given in bold.

5. Discussion

Our results show that the two modes of preservation have a significantly different quality of preservation. Surprisingly, the preservation quality of pyritized insects is consistently higher than that of the kerogenized insects of the Crato Formation. The kerogenized insects only showed higher preservation quality for the wing and antenna morphological categories. Contrary to expectations, keroginized insects do not show preservation quality that is as high as that of the specimens preserved by pyritization.

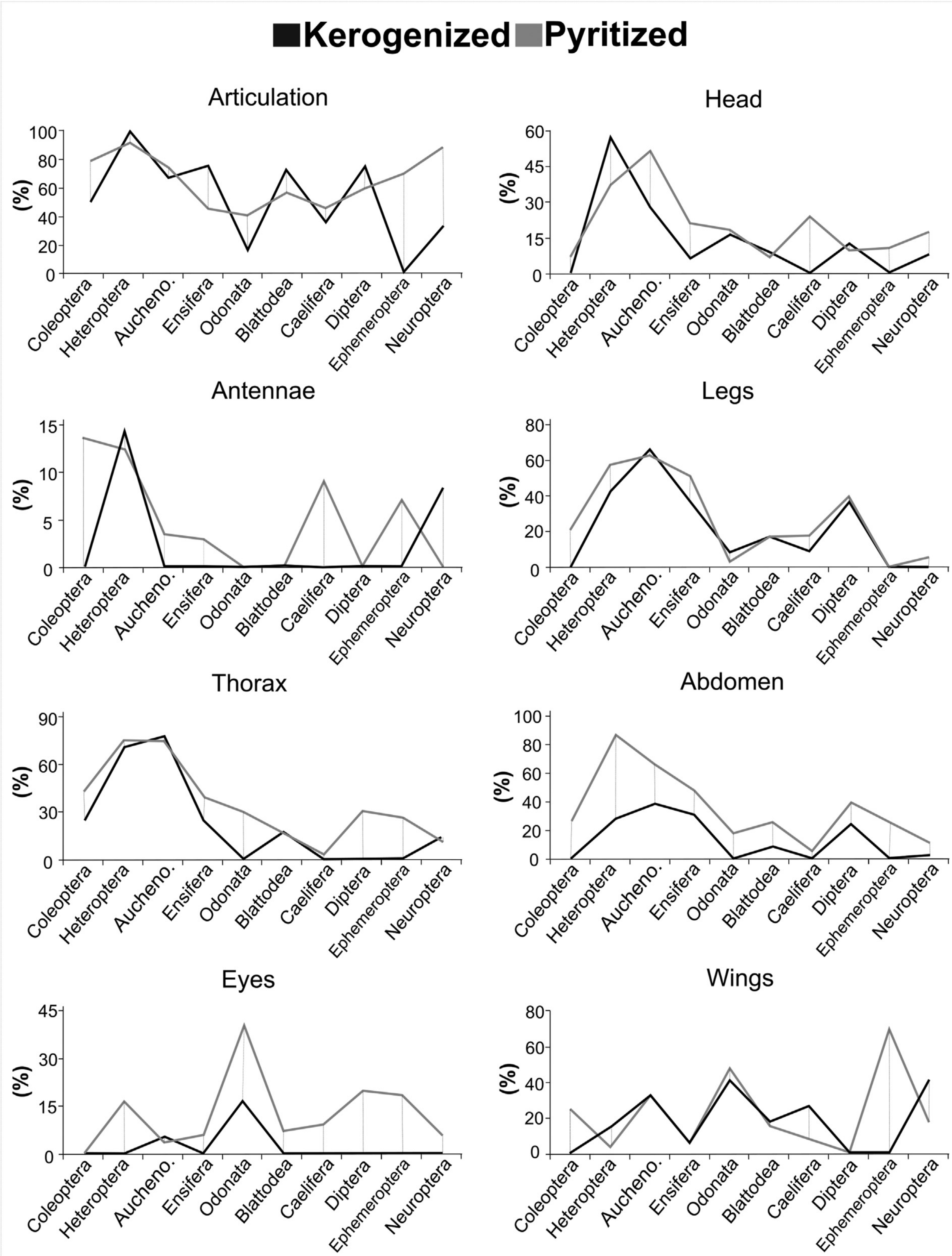

Fig. 7. The comparison of the proportion of the maximum quality-scores for the individual morphological categories between all the mineralized and kerogenized specimens for all the considered taxa.

Table 3
Percentages and results of chi-squared tests for articulation state differences between the kerogenized and pyritized insects from the Crato Formation. Statistically significant values are given in bold.

Articulation state	All insects	Pyritized insects	Kerogenized insects	chi-squared tests	
				χ2	*P-value*
Fully articulated	59.2%	62.2%	52.8%	1.227	0.267
Partially articulated	37.9%	36.2%	41.5%	0.600	0.438
Disarticulated	2.9%	1.6%	5.7%	**4.812**	**0.028**

Statistically significant values are given in bold.

Table 5
The results of the statistical analyses comparing the proportions of presence or absence for each morphological category of the insects from the two modes of preservation in the Crato Formation.

Morphological Categories	χ2	*P-value*
Head	0.853	0.355
Eye	**21.037**	**$<10^{-4}$**
Antenna	0.043	0.835
Thorax	1.069	0.301
Leg	**8.096**	**0.004**
Wing	1.099	0.294
Abdomen	**4.7**	**0.030**

Statistically significant values are given in bold.

Kerogenization includes a transformation from original organic material by partial or complete chemical alteration of organic matter into cyclic and aliphatic hydrocarbons (Stankiewicz et al., 1997; Stankiewicz et al., 1998; Gupta et al., 2006). According to Schiffbauer et al. (2014), the fossils preserved as carbonaceous compressions with only rare and diffuse pyrite were likely rapidly buried in the methanogenesis zone of the sediment column. On the other hand, pyritization requires degradation of organic matter, reactive iron and sulphate from environmental sources (Berner, 1984). The precipitation of pyrite occurs when the degradation of organic matter by microbial activity produces ionic constituents necessary for mineralization (Raiswell et al., 1993; Briggs, 2003). Schiffbauer et al. (2014) hypothesized that completely pyritized fossils spent more time in the bacterial sulfate reduction (BSR) zone. Degradation in the BSR zone is more efficient than degradation in the methanogenesis zone because BSR is more metabolically efficient than the degradation pathways available in the methanogenesis zone (Elsayed et al., 2015; Mao et al., 2015). Therefore, it is expected that a specimen preserved in the BSR zone will undergo more degradation than a specimen preserved in the methanogenesis zone (Anderson and Smith, 2017).

Based on the argument above, our results do not match this prediction and diverged from those of Anderson and Smith (2017). In their study, there is a significant difference between iron-mineralized and keroginized insects from the Green River Formation, where the preservation quality of mineralized insects was consistently lower than the kerogenized insects.

The distributions of quality scores were significantly different for four morphological categories (head, legs, abdomen and eyes) when comparing all insects between the two preservation types. However, there were no significant differences in the frequency of preservation scores of antennae, thoraces and wings between both the pyritized and kerogenized insects. For thoraces, this is not surprising as they tend to have thick and sclerotized tissues. The proportions of the maximum-score-value for the individual morphological categories show that the preservational quality of the mineralized insects was often higher than that of the kerogenized insects. The head, wings and abdomen were the morphological categories that most frequently presented substancial differences among the individual insect taxa. Four individual taxa showed significant differences for the abdomen (Auchenorrhyncha, Blattodea, Ensifera and Heteroptera), whereas three are significantly different for the wings (Caelifera, Diptera and Neuroptera). For the head, two individual taxa presented significant differences (Auchenorrhyncha and Ensifera). Only one taxonomic group showed differences for the antenna (Neuroptera), thorax (Diptera) and legs (Ensifera) categories. The abdomen was more affected in insects in the kerogenized zone, as only 15.4% of kerogenized insects scored maximum value.

The articulation state of insects is not significant different between the two modes of preservation. The specific articulation states of fully articulated and partially articulated specimens do not vary significantly either. The lack of a significant difference for articulation state could likely be explained by the fact that the fully articulated individuals were the most common in both pyritization and kerogenization zones. Otherwise, when comparing only disarticulated specimens, the articulation state was significantly different between the two modes of preservation, with kerogenized insects having higher disarticulation values. The high preservational fidelity of insect morphological components suggest that these insects likely dropped into water while flying over the lake or were immersed by rising water levels (still alive or after undergoing a short period of decay on land).

The Crato paleolake was most probably a permanent freshwater environment with no evidence of subaerial exposure (Neumann et al., 2003; Assine et al., 2014). Our results strongly corroborate this interpretation. The low number of disarticulated insects supports that the Crato paleolake experienced a low energy deposition. Insect carcasses can require prolonged decay and disturbance to disarticulate before the final burial (Martínez-Delclòs and Martinell, 1993; Smith et al., 2006). Additionally, disarticulation during reworking is an unlikely possibility in the finely laminated limestones of the Crato Formation.

The difference in the taxonomic composition between the two modes of preservation is unlikely to be the source of preservation quality trends in our results, as only the Ephemeroptera is present solely as pyritized specimens (Fig. 3). Ephemeroptera only preserved in the pyritized zone is notable because adult mayflies are known for their extremely short life spans and emergence in large numbers in the summer months. This also suggests that a mass mortality event likely happened during that time of year.

We hypothesize that the higher preservation quality of pyritized insects is because of the shortened period of microbial decay needed to generate Fe^{2+} and HS^{-}, thus leading to the

Table 4
The distribution of the individual morphological categories scoring zero for all kerogenized and pyritized insects from the Crato Formation. Statistically significant values are given in bold.

Morphological Categories	All insects	Pyritized insects	Kerogenized insects	chi-squared tests	
				χ2	*P-value*
Head	79	50	29	0.599	0.428
Eye	185	107	78	**7.653**	**0.005**
Antenna	287	194	93	0.006	0.936
Thorax	32	19	13	0.931	0.334
Leg	114	66	48	**4.660**	**0.030**
Wing	36	27	9	0.952	0.329
Abdomen	67	38	29	3.462	0.062

Statistically significant values are given in bold.

mineralization of organic matter (Raiswell et al., 1993; Sagemann et al., 1999; Schiffbauer et al., 2014). The pyritized insects could have been deposited in the most distal part of the lake reflecting maximum highstand of the lacustrine system (Neumann et al., 2003; Heimhofer et al., 2010). Thus, this deposition would have occurred under lower-energy conditions, bottom water anoxia, and a near absence of bioturbation and carbonate precipitation mediated by microbial communities (Catto et al., 2016; Osés et al., 2016; Warren et al., 2017). At this stage, the Crato paleolake sediments were predominantly calcareous and iron-poor. Sedimentary iron may have reached the lake through pulses of freshwater (Catto et al., 2016). Therefore, the pyritization process was probably iron-limited. Nevertheless, the amount of organic matter was also low in the pyritization zone (Neumann et al., 2003). Thus, this scenario would allow the fixation of sulphide by iron at decay sites and thus pyritization in insects instead of yield widespread pyritization.

These suitable conditions promoted the early mineralization of insects. The pyritized Odonata retain much higher values of discernible eyes (ommatidia) than the kerogenized Odonata, which shows how quickly and early the mineralization of the pyritized insects occurred (Fig. 5K). Osés et al. (2016) hypothesized that predation or diseases could have facilitated the pyritization of partially disarticulated and fragmented fossil insects with fine details. In contrast, areas where kerogenizaton dominated would have existed during the early and late phase of lake-level highstand (Neumann et al., 2003; Heimhofer et al., 2010). The dark color of these strata is likely due to a combination of higher organic matter, clay content and terrigenous influence. During this phase, the water body of the lake was influenced by rising water level but throughflow most probably was limited (Heimhofer et al., 2010). Increased residence time of water in a restricted basin would have promoted stagnant conditions and increased contribution of clay minerals accompanied by decreased carbonate content. The slightly highest values of organic matter in the kerogenization zone (Heimhofer et al., 2010; Catto et al., 2016) yielded widespread pyritization at the bottom of the lake. Osés et al. (2017) proposed that clay content and low microspar porosity contributed to a narrow BSR zone, and it is possible that there may have been a decrease in sulphate percolation in these strata. Thus, the low supply of ions allowed the kerogenous insects to experience a minimum of microbial degradation within the BSR. A short BSR would have decreased the period of microbial decay available to generate the ions that can promote mineralization.

Both pyritized and kerogenized insects come from the same pronounced rhythmically bedded deposit, so it is expected that the biostratinomic processes experienced by insects should be similar. However, the greater degree of disarticulation of insects in the kerogenization areas would have to have taken place during the biostratinomy stage. Nevertheless, it is difficult to estimate the exact source of biostratinomic differences between the pyritized and kerogenized insects from the Crato Formation.

There is a possibility that specimens were initially preserved with finer details, but during later diagenesis that preservation-fidelity has been lost. Insects affected by intense oxidation process can exhibit iron-oxide overgrowths, or even changes in articulation state (Anderson and Smith, 2017). In the case of the pyritized insects, many specimens initially mineralized have been affected by narrow fractures. These breaks in the host rock facilitate the percolation of oxidizing fluids, which could dissolve away the fossil material leaving behind only the outline of former insect (personal observation of FIB, 2019). Specimens bearing these signals were not including in this study.

6. Conclusion

Here, we compared the preservational quality of insects preserved via pyritization and kerogenization from the Crato Formation. The pyritization process requires partial degradation of the carcass leading to a decrease in preservation of their morphological details. But, surprisingly, our results show that the pyritized insects have higher overall preservation quality than fossil insects that have been preserved by kerogenization.

Of all insects studied, the differences in distribution of scores for individual morphological categories were significant in the abdomen, head, legs and eyes. Thorax, antennae and wings did not present statistically significant differences. For all insects, the differences in measures of articulation state between the two modes of preservation were similar. However, when comparing only the proportions of the disarticulated insects, the differences were statistically significant. Our results suggest that these preservation patterns may be controlled by factors such as morphological characteristics, ecology and also abiotic factors such as depositional environment. Overall, in comparisons in which the difference between pyritized and kerogenized insects are statistically significant, the higher preservation-fidelity scores tended to belong to the pyritized specimens. Clearly, Crato Formation was not selective as it has several insect groups (pyritized plus kerogenized) displaying preservation of biomineralized tissues on a micron-scale as well as gross morphological features. Thus, different groups of insects may have different taphonomic processes, but all can be classified as exceptional.

It seems likely that the low degree of disarticulation in pyritized insects suggests a greater concentration of ions (sulfate and ferrous iron) allowing for the rapid mineralization of insects with fine details. In contrast, sediments slightly richer in organic matter in the kerogenization zone yielded widespread pyritization, thus decreasing the contributions of sulphate and iron to fossil mineralization. In summary, the taphonomic bias towards lower quality of kerogenized insects is most likely due to the combination of the processes that occurred during both biostratinomy and early diagenesis stages. Comparing two different preservational pathways is a complex task in and of itself, mainly when two or more different preservational pathways are simultaneously associated with the same fossil deposit. Therefore, it is not always easy to highlight which preservational pathway is the best. We recognize that due to lack of the appropriate storage space to accommodate large collections, paleontologists often focus their search for specimens on the highest quality, while poorly preserved specimens are not often collected. Clearly, taphonomic analysis of biased paleontological collections can lead to biased results. However, statistical analysis and taphonomic studies of assemblages dominated by high-level taxa will allow us to understand the influence of depositional environment on the preservation of insects as a whole as well as the biological factors that may control their taphonomic patterns.

The comparison of very similar representatives preserved by two different pathways showed that mineralized fossils can also be preserved with a high level of fidelity. This study suggests that fossil deposits preserved via pyritization, have as much influence on the overall preservation quality as those preserved by keroginization. Others studies comparing kerogenized and partially or fully mineralized soft-tissues are performed in different periods or even in different depositional environments (Penney and Langan, 2006; Lin and Briggs, 2010). Whereas the Crato Fm. is one of the few lithological units in the world where kerogenized and pyritized insects of the same taxonomic group with similar taphonomic histories may be preserved within the same strata, and even at the same outcrop, and this is where the greatest importance of the present study lies.

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References

Anderson, E.P., Smith, D.M., 2017. The same picture through different lenses: quantifying the effects of two preservation pathways on Green River Formation insects. Paleobiology 43 (2), 224–247.
Assine, M.L., 2007. Bacia do Araripe. Boletim de Geociências da Petrobrás, vol. 15, pp. 371–389.
Assine, M.L., Perinotto, J.A., Neumann, V.H., Custódio, M.A., Varejão, F.G., Mescolotti, P.C., 2014. Sequências deposicionais do Andar Alagoas (Aptiano superior) da Bacia do Araripe, Nordeste do Brasil. Boletim de Geociencias da Petrobras 22, 3–28.
Barling, N., Martill, D.M., Heads, S.W., Gallien, F., 2015. High fidelity preservation of fossil insects from the Crato Formation (Lower Cretaceous) of Brazil. Cretaceous Research 52 (B), 605–622.
Berner, R.A., 1984. Sedimentary pyrite formation: An update. Geochimica et Cosmochimica Acta 48, 605–615.
Bezerra, F.I., Da Silva, J.H., De Paula, A.J., Olveira, N.C., Paschoal, A.R., Freire, P.T.C., Viana, B.C., Mendes, M., 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, F.I., Da Silva, J.H., Miguel, E.C., Paschoal, A.R., Nascimento Jr., D.R., Freire, P.T.C., Viana, B.C., Mendes, M., 2020. Chemical and mineral comparison of fossil insect cuticles from Crato *Konservat Lagerstätte*, Lower Cretaceous of Brazil. Journal of Iberian Geology 46, 61–76.
Briggs, D.E.G., 1999. Molecular taphonomy of animal and plant cuticles: Selective preservation and diagenesis. Philosophical Transactions of the Royal Society of London, ser. B: Biological Sciences 354, 7–16.
Briggs, D.E.G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. Annual Review of Earth and Planetary Sciences 31, 275–301.
Catto, B., Jahnert, R.J., Warren, L.V., Varejao, F.G., Assine, M.L., 2016. The microbial natureof laminated limestones: lessons from the Upper Aptian, vol. 341. Araripe Basin, Brazil, pp. 304–315. Sedimentary Geology.
Delgado, A. de O., Buck, P.V., Osés, G.L., Ghilardi, R.P., Rangel, E.C., Pacheco, M.L.A.F., 2014. Paleometry: a brand new area in Brazilian science. Materials Research 17, 1434–1441.
Edwards, H.G.M., Farwell, D.W., Villar, S.E.J., 2007. Raman microspectroscopic studies of amber resins with insect inclusions. Spectrochimica Acta 68, 1089–1095.
Elsayed, O.F., Maillard, E., Vuilleumier, S., Millet, M., Imfeld, G., 2015. Degradation of chloroacetanilide herbicides and bacterial community composition in lab-scale wetlands. The Science of the Total Environment 520, 222–223.
Franke, T.M., Ho, T., Christe, C.A., 2012. The Chi-Square test: Often used and more often misinterpreted. American Journal of Evaluation 33 (3), 448–458.
Grimaldi, D., Engel, M.S. (Eds.), 2005. Evolution of the insects. Cambridge University Press, p. 755.
Gupta, N.S., Michels, R., Briggs, D.E.G., Evershed, R.P., Pancost, R.D., 2006. The organic preservation of fossil arthropods: an experimental study. Proceedings of the Royal Society B 273, 2777–2783.
Heimhofer, U., Ariztegui, D., Lenniger, M., Hesselbo, S.P., Martill, D.M., Rios-Netto, A.M., 2010. Deciphering the depositional environment of the laminated Crato fossil beds (Early Cretaceous, Araripe Basin, North-eastern Brazil). Sedimentology 57, 677–694.
Henwood, A., 1992. Soft-part preservation of beetles in Tertiary amber from the Dominican Republic. Palaeontology 35, 901–912.
Henwood, A., 1993. Ecology and taphonomy of Dominican Republic amber and its Inclusions. Lethaia 26, 237–245.
Labandeira, C.C., Sepkoski Jr., J.J., 1993. Insect diversity in the fossil record. Science 261, 310–315.
Lin, J.P., Briggs, D.E.G., 2010. Burgess Shale-Type preservation: a comparison of Naraoiids (Arthropoda) from three Cambrian localities. PALAIOS 25 (7), 463–467.
Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews 45, 540–555.
Martill, D.M., Loveridge, R.F., 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.
Martínez-Delclòs, X., Martinell, J., 1993. Insect taphonomy experiments: Their application to the Cretaceous outcrops of lithographic limestones from Spain. Kaupia 2, 133–144.
Martínez-Delclòs, X., Briggs, D.E.G., Peñalver, E., 2004. Taphonomy of insects in carbonates and amber. Palaeogeography, Palaeoclimatology, Palaeoecology 203, 19–64.
McCoy, V.E., Soriano, C., Gabbott, S.E., 2018. A review of preservational variation of fossil inclusions in amber of different chemical groups. Earth and Environmental Science Transactions of the Royal Society of Edinburgh 107 (2–3), 203–211.
McNamara, M.E., 2013. The taphonomy of colour in fossil insects and feathers. Palaeontology 56 (3), 557–575.
Neumann, V.H., 1999. Estratigrafía, sedimentología, geoquímica y diagénesis de los sistemas lacustres Aptienses-Albienses de la Cuenca de Araripe (Noreste de Brasil) (Unpubl. PhD thesis). Universidad de Barcelona, p. 250.
Neumann, V.H., Borrego, A.G., 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.
Osés, G.L., Petri, S., Becker-Kerber, B., Romero, G.R., Rizzutto, M.A., Rodrigues, F., Galante, D., Silva, T.F., Curado, J.F., Rangel, E.C., Ribeiro, R.P., Pacheco, M.L., 2016. Deciphering the preservation of fossil insects: a case study from the Crato Member, Early Cretaceous of Brazil. PeerJ 4, 1–28.
Osés, G.L., Petri, S., Voltani, C.G., Prado, G.M., Douglas, G., Rizzutto, M.A., Rudnitzki, I.D., Silva, D., Rodrigues, F., Rangel, E.C., Sucerquia, P.A., Pacheco, M.L., 2017. Deciphering pyritization-kerogenization gradient for fish soft-tissue preservation. Scientific Reports 7, 1–15.
Penney, D., Langan, A.M., 2006. Comparing amber fossil assemblages across the Cenozoic. Biology Letters 2 (2), 266–270.
Raiswell, R., Whaler, K., Dean, S., Coleman, M.L., Briggs, D.E.G., 1993. A simple three-dimensional model of diffusion-with-precipitation applied to localized pyrite formation in framboids, fossils and detrital iron minerals. Marine Geology 113, 89–100.
Sagemann, J., Bale, S.J., Briggs, D.E.G., Parkes, R.J., 1999. Controls on the formation of authigenic minerals in association with decaying organic matter: an experimental approach. Geochimica et Cosmochimica Acta 63, 1083–1095.
Schiffbauer, J.D., Xiao, S., Cai, Y., Wallace, A.F., Hua, H., Hunter, J., Xu, H., Peng, Y., Kaufman, A.J., 2014. A unifying model for Neoproterozoic–Palaeozoic exceptional fossil preservation through pyritization and carbonaceous compression. Nature Communications 5, 1–12.
Schmidt, A.R., Jancke, S., Lindquist, E.E., Ragazzi, E., Roghi, G., Nascimbene, P.C., Schmidt, K., Wappler, T., Grimaldi, D.A., 2012. Arthropods in amber from the Triassic Period. Proceedings of the National Academy of Sciences 109 (37), 14796–14801.
Seilacher, A., 1970. Begriff und Bedeutung der Fossil-Lagerstätten. Neues Jahrbuch für Geologie und Paläontologie, pp. 34–39. Monatshefte.
Smith, D.M., 2000. Beetle taphonomy in a recent ephemeral lake, southeastern Arizona. PALAIOS 15, 152–160.
Smith, D.M., 2012. Exceptional preservation of insects in lacustrine environments. PALAIOS 27, 346–353.
Smith, D.M., Moe-Hoffman, A.P., 2007. Taphonomy of Diptera in lacustrine environments: a case study from Florissant fossil beds, Colorado. PALAIOS 22, 623–629.
Smith, D.M., Cook, A., Nufio, C.R., 2006. How physical characteristics of beetles affect their fossil preservation. PALAIOS 21 (3), 305–310.
Solórzano-Kraemer, M.M., Kraemer, A.S., Stebner, F., Bickel, D.J., Rust, J., 2015. Entrapment bias of arthropods in Miocene amber revealed by trapping experiments in a tropical forest in Chiapas, Mexico. PloS One 10 (3), e0118820.
Solórzano-Kraemer, M.M., Delclòs, X., Clapham, M.E., Arillo, A., Peris, D., Jäger, P., Stebner, F., Peñalver, E., 2018. Arthropods in modern resins reveal if amber

accurately recorded forest arthropod communities. Proceedings of the National Academy of Sciences of the United States of America 115 (26), 6739–6744.
Stankiewicz, B.A., Briggs, D.E.G., Evershed, R.P., 1997. Chemical composition of Paleozoic and Mesozoic fossil invertebrate cuticles as revealed by pyrolysis-gas Chromatography/Mass Spectrometry. Energy & Fuels 11, 515–521.
Stankiewicz, B.A., Scott, A.C., Collinson, M.E., Finch, P., Mösle, B., Briggs, D.E.G., Evershed, R.P., 1998. Molecular taphonomy of arthropod and plant cuticles from the Carboniferous of North America: implications for the origin of kerogen. Journal of the Geological Society 155, 453–462.
Thoene-Henning, J., Smith, D.M., Nufio, C.R., Meyer, H.W., 2012. Depositional setting and fossil insect preservation: A study of the late Eocene Florissant Formation, Colorado. PALAIOS 27, 481–488.
Wang, B., Zhuang, H., Jarzembowski, E.A., Fang, Y., Zhen, D., 2013. Taphonomic variability of fossil insects: a biostratinomic study of Palaeontinidae and Tettigarctidae (Insecta: Hemiptera) from the Jurassic Daohugou lagerstatten. PALAIOS 28, 233–242.
Warren, L.V., Varejão, F.G., Quaglio, F., Simões, M.G., Fürsich, F.T., Poiré, D.G., Catto, B., Assine, M.L., 2017. Stromatolites from the AptianCrato Formation, a hypersaline lake system in the Araripe Basin, northeastern Brazil. Facies 63 (3), 1–19.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cretres.2020.104631.