Taphonomic bias in exceptionally preserved biotas

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Keywords: Exceptional preservation, taphonomy, Cambrian, Ordovician

ABSTRACT

Exceptionally preserved fossil biotas provide crucial data on early animal evolution. Fossil anatomy allows for reconstruction of the animal stem lineages, informing the stepwise process of crown group character acquisition. However, a confounding factor to these evolutionary analyses is information loss during fossil formation. Here we identify that the Ordovician Fezouata Shale has a clear taphonomic difference when compared to the Cambrian Burgess Shale and Chengjiang Biota. In the Fezouata Shale, soft cellular structures are most commonly associated with partially mineralized and sclerotized tissues, which may be protecting the soft tissue. Also, entirely soft non-cuticularized organisms are absent from the Fezouata Shale. Conversely, the Cambrian sites commonly preserve entirely soft cellular bodies and a higher diversity of tissue types per genus. The Burgess and Chengjiang biotas are remarkably similar, preserving near identical proportions of average tissue types per genus. However, the Burgess shale has almost double the proportion of genera that are entirely soft as compared to the Chengjiang Biota, indicating that the classic Burgess Shale was the acme for soft tissue preservation. Constraining these biases aids the differentiation of evolutionary and taphonomic absences, which is vital to incorporating anatomical data into a coherent framework of character acquisition during the earliest evolution of animals.

1. INTRODUCTION

Exceptionally preserved biotas have revolutionized our understanding of animal origins and evolution owing to the preservation in these deposits of soft-bodied and lightly sclerotized organisms, which under normal circumstances have little to no fossilization potential (Butterfield, 1995). Burgess Shale-type (BST) preservation deposits including the Burgess Shale (Wuliuan, Miaolingian; ~505 Ma, Canada) and the Chengjiang Biota (Stage 3, Cambrian Series 2; ~530 Ma, China) are particularly famous *Lagerstätten,* yielding hundreds of exceptionally preserved Cambrian taxa (Fig. 1a-c) critical to our understanding of the earliest metazoan-dominated communities and evolutionary events such as the Cambrian Explosion (Daley et al., 2018). The youngest of these deposits, the Fezouata Shale, is the only Ordovician (Tremadocian; ~479-478 Ma, Morocco) *Lagerstätte* to yield a diverse exceptionally preserved fauna (Fig. 1d-f). With over 185 taxa of marine invertebrates (Van Roy et al., 2015a) recovered from specific intervals in the Zagora area (Lefebvre et al., 2018; Saleh et al., 2018, 2019), this formation offers new insights into the diversification of metazoans, at a key interval between the Cambrian Explosion and the Ordovician Radiation (Van Roy et al., 2010, 2015b; Lefebvre et al., 2019). Despite being anatomically and biologically informative, even these spectacular fossil localities inevitably have taphonomic biases, because no fossil site can ever be a perfect replication of all the anatomical and ecological information of a living community (Butterfield, 2003; Brasier et al., 2010; Landing et al., 2018). Gathering "complete" data is impossible even in studies on modern living communities. It is therefore essential to understand what factors may be affecting the fossil preservation at a community level in order to properly reconstruct ancient ecosystems and biodiversity fluctuations over geological time.

The aim of this study is to examine the taphonomic signal of these deposits, allowing a solid understanding of the preservation bias at play in each locality. For this reason, a taphonomic classification of all eumetazoan genera from the Fezouata Shale (N= 178) was established, and compared with the preservation of genera from the Burgess Shale (N=103) and the Chengjiang Biota (N=133) based on the presence / absence of different types of anatomical structures: (A) biomineralized skeletons, (B) sclerotized parts (i.e. possessing an organically strengthened part or organ) (C) soft with an unsclerotized cuticle (i.e. a non-cellular outer body surface that is either collagenous or formed by polymerized polysaccharides), (D) soft cellular outer layer defining at least a part of the body (e.g. tentacles of hyoliths), and (E) soft internal cellular organ/tissue (e.g. digestive or nervous systems) (Fig.1).

2. MATERIAL AND METHODS

In order to define the preservation pattern in all three exceptionally preserved biotas, the various possible co-occurrences of characters A (biomineralized), B (sclerotized), C (unsclerotized, cuticularized), D (cellular body walls), and E (internal tissues) were tallied (e.g. AB, AC, CDE, and ABCDE) (Tab. 1). To avoid any overlap between categories, the data were analyzed on a five-fold Venn diagram per site. In order to see if there is any difference between sites, the total number of genera having just one character regardless of its nature (e.g. A, or B, or C, or D, or E) was plotted against the number of genera that have pairs (e.g. AB), threes (e.g. ABC) or fours (e.g. ABCD) for all exceptionally preserved biotas (Fig. 2). Afterward, the average number of tissue types per genus, as derived from the dataset, was calculated by adding the probability of the occurrence of all classes of structures A, B, C, D, and E (Tab. 2). In order to constrain the categories causing the biggest variations in preservation between sites, plots were made to show the proportion of paired and triple categories in localities (Fig. 3).

The association of soft internal organs (E) with other structures, in all three localities was also investigated. For this, the probabilities of discovering two classes of structures together having already found one of them were calculated (Tab. 3). For example, $p(E|A)$ is the probability of E occurring if A has occurred. The reverse conditional approach was also made and the probability of finding A given that E has been found $p(A|E)$ was also calculated (Tab. 3). Then, the likelihood of producing the distribution of combinations of structures found in the Burgess Shale and the Chengjiang Biota assuming that the Fezouata Shale has the “true” preservation regime was investigated using the following parametrized binomial P(x≥n) | Bi(n, p):

$$P(x) = \qquad \frac{\quad}{\quad}$$

In this equation, p = p(E|A) for the Fezouata Shale, q = 1-p, n is the number of genera preserving an A in the Burgess Shale or the Chengjinag Biota, and x is the number of desired success which is, in this case, at least the actual number n of genera preserving both A and E in the Burgess Shale/Chengjiang Biota. All calculated probabilities are added up and the probability P(x≥n) | Bi(n, p), of producing the actual Burgess Shale/Chengjinag Biota AE category, considering that the Fezouata Shale regime is “true”, is then obtained (Tab. 4). This was then performed for other tissues combinations (i.e. BE, CE, and DE) (Tab. 4). This approach was then extended to the assumption that the Burgess Shale preservation distribution is “true” and finally assuming that the Chengjiang Biota preservation distribution is the “true” preservation model (Tab. 5).

Finally, the probability of finding organisms with only soft cellular tissues (both internal and external to the exclusion of everything else with A’ for instance indicating the set that is defined as not containing and members of A) p(A’∩B’∩C’∩D∩E|E) for all three *Lagerstätten* was calculated.

3. RESULTS

All three *Lagerstätten* preserve numerous biomineralized skeletons (A), sclerotized parts (B), unsclerotized, soft cuticular parts (C), and internal soft parts (E) (Tab. 1). However, genera having cellular body walls defining the entire body (i.e. D, DE), with or without internal organs (E) are absent in the Fezouata Shale. In comparison the Chengjiang Biota (9 genera) and the Burgess Shale (13 genera) have a considerable number of entirely soft organisms preserved (Tab. 1). Further, numerous biomineralized and sclerotized genera in the

Burgess Shale and the Chengjiang Biota preserve external soft tissues defining a part of the body (i.e. AD, BD, BDE, ACDE) (Tab. 1). These genera are absent from the Fezouata Shale, with the exception of two specimens of aculiferan molluscs (both, however, densely covered by sclerites). The Burgess Shale and the Chengjiang Biota preserve almost twice as many tissues per genus as the Fezouata Shale (Fig. 2), with the mean number of tissue types per genus in the Cambrian sites being 2.2 (Burgess = 2.206; Chengjiang = 2.185) whilst it is 1.316 for the Fezouata Shale (Tab. 2). The overall distribution of tissue frequency by genus are similar for the Burgess Shale and the Chengjiang Biota, with mean and variance suggesting they are drawn from comparable if not identical populations (variance Burgess Shale = 0.026; Chengjiang Biota = 0.030; t = -0.45, p(same mean) = 0.6532; F = 1.154, p(same variance) = 0.454). However, the distribution for the Fezouata Shale is very different (variance = 0.08034), with both t and F-tests reporting significance for the mean and variance respectively when compared to Burgess Shale (t = 29.53, p(same mean) = 1.035×10^{-87}; F = 3.0685, p(same variance) = 3.195×10^{-9}) and the Chengjiang Biota (t = 32.34, p(same mean) = 3.414×10^{-101}; F = 2.5591, p(same variance) = 1.718×10^{-8}).

The three studied localities show a dominance of both BCE and ACE categories (Fig. 3). This is at least partly linked to the high number of arthropods found at all localities, with their external anatomy often consisting of ventral unsclerotized cuticle (C) and a reinforced dorsal area consisting of a biomineralized exoskeleton (A) or sclerotized cuticle (B), found in conjunction with internal soft parts (E). However, when the preservation of two tissue types occurs in the Fezouata Shale, it consists mostly of the association of biomineralized skeletons and internal soft tissues (AE is 9 of the 21 pairs that consist of the possible sets AB, AC, AD, AE, BC, BD, BE, CD, CE, DE), sclerotized tissue and internal soft tissue (7 of the 21 pairs), and biominerals and sclerotized tissue (3 of 21 pairs). All other tissue associations are rare or absent. In the Burgess Shale, the dominant association is between cellular soft bodied tissues and internal organs (13 of 36 pairs), with sclerotized and cuticularized tissues also commonly associated (7 of 36 pairs). In the Chengjiang Biota, the dominant association is between sclerotized and cuticularized tissues (16 of 57 pairs), with additional common associations between cuticularized tissues and internal organs (12 of 57 pairs), cellular soft bodied tissues and internal organs (9 of 57 pairs), and biominerals and sclerotized tissues (8 of 57 pairs) (Fig. 3). The probabilities of finding internal soft tissues in a given fossil genus, in co-occurrence with any of the other types of structures, show that the distribution of tissues in the Burgess Shale and the Chengjiang Biota are much more similar to each other (Tab. 3) and are significantly different from the Fezouata Shale (Tab. 4). In the Fezouata Shale, only a small proportion of all biomineralized genera also preserve internal organs ($p(E|A) = 0.162$) (Tab. 3), but of the genera that do have internal organs the majority are associated with biominerals ($(A|E) = 0.667$) (Tab. 3). This means that although a biomineral does not guarantee the preservation of internal anatomies, it could still be seen as a very helpful pre-requisite in the Fezouata Shale. Conversely, biominerals in paleoenvironments such as the Burgess Shale and the Chengjiang Biota do not seem to have any role in soft tissue preservation ($p(A|E) = 0.183$ and $p(A|E) = 0.273$ for the Burgess Shale and the Chengjiang Biota respectively, which are not significantly different to chance association (Tab. 3). The result of probabilistic modelling (Tab. 4) shows that the distributions of tissue associations found at the Fezouata Shale cannot be generated by randomly sampling a biota with a similar composition to that of either the Chengjiang Biota or the Burgess Shale, and in all possible soft tissue combinations the Fezouata Shale is statistically significantly different to both of the Cambrian biotas studied (Tab. 4). Finally, it is worth noting that the absence of entirely soft bodied organisms at the Fezouata Shale is not just a striking observation, but it is also statistically significant from the proportions found at the Cambrian sites. The absence of entirely soft bodied organisms at the Fezouata Shale cannot be generated by randomly sampling a population like that found in the

Cambrian sites with any confidence (with p-values of 0.00137 and 0.03819 for Burgess Shale and Chengjiang Biota models respectively). Therefore, the Burgess Shale ($p(D \cap E|E) = 0.2167$) and the Chengjiang Biota ($p(D \cap E|E) = 0.113$) both show significantly higher probabilities of recovering entirely soft bodied genera. The preservation of entirely soft bodied genera is also different between the Chengjiang Biota and the Burgess Shale (Tab. 3), with the higher incidence being found in the Burgess Shale. This difference is significant and could not be generated by chance or subsampling (Tab. 5).

4. DISCUSSION

Soft part preservation in the Fezouata Shale is strikingly different from the preservation in the Chengjiang Biota and the Burgess Shale. This difference in the occurrences of soft tissues cannot result from a collection bias, because all three localities were subjected to collecting efforts that actively focused on finding and sampling fossils with labile soft part. Instead, the observed pattern of preservation suggests that the presence of non-cellular layers covering internal anatomies in the Fezouata Shale was essential for exceptional preservation, unlike at the Burgess Shale and Chengjiang Biota. The near complete absence of preserved external soft tissues is possibly related to them being less decay-resistant than mineralized, sclerotized or even cuticularized structures. Under most circumstances, even unsclerotized soft cuticle is more decay resistant than cellular tissue, because cuticular structures are not subject to autolysis, and the composition of complex polymerized polysaccharides means cuticle is more difficult to break down than cellular tissues (Briggs and Kear, 1993). The decay-resistance of complex biopolymers found in the cuticle was also recently invoked to explain the rare but selective preservation of cuticularized organisms in coarse clastic sediments (MacGabhann et al., 2019).

In the Fezouata Shale, there was a pathway of preservation in place that systematically failed to preserve (i) almost all soft-bodied organisms lacking a cuticular cover in particular, and (ii) external soft cellular tissues in general. In this deposit, dead individuals experienced harsh decay prior to their preservation owing to a relative burial tardiness (Saleh et al., 2018) in comparison with the Burgess Shale and the Chengjiang Biota in which fossils were killed and preserved directly during an obrution event (Gaines, 2014). This decay may also have been retarded by berthierine, a mineral that can slow down microbial activity through the oxidative damage of bacterial cells (McMahon et al., 2016; Anderson et al., 2018; Saleh et al., 2019). Therefore, in contrast to the Burgess Shale and the Chengjiang Biota, the external conditions at the Fezouata Shale were generally less permissive for the preservation of external soft tissues. However, resistant skeletal parts and cuticular external surfaces created isolated environments within the carcasses that maintained a chemical equilibrium conducive to the preservation of internal organs.

The systematic taphonomic bias described here for the Fezouata Shale has implications for understanding the original faunal community assemblage, specifically in regard to the proportions of genera preserved in the fossil record. The systematic removal of all soft-bodied organisms, lacking a non-cellular external envelope (cuticle), and external cellular soft tissues leads to an underestimation of the original diversity at the Cambro-Ordovician transition and distorts faunal composition to a greater extent than in the Burgess Shale or the Chengjiang Biota. Many animal groups could have lived in the Fezouata Shale environment but left little to no trace behind, such as chordates (e.g. *Pikaia*, *Metaspriggina*). A corollary of this finding is that it is now possible to differentiate between ecological and taphonomic absences of numerous genera. For example, the absence of priapulids such as *Ottoia* in the Fezouata Shale (Van Roy et al., 2015a) is likely a real aspect of the fauna, since these cuticle-bearing soft-bodied animals would not have been affected by the same taphonomic bias responsible for the removal of the majority of soft-bodied genera lacking a cuticle.

Now that a source of systematic taphonomic bias operating in the Fezouata Shale has been identified (Fig. 4), and most importantly, compared to the biases in play in the Burgess Shale and the Chengjiang Biota (Fig. 4), it can be accounted for in future paleoecological and evolutionary analyses. This will facilitate more accurate comparisons of faunal community compositions between these biotas in particular, and when comparing exceptionally preserved faunas in general, as similar restrictive mechanisms are likely active to a varying extent at other localities.

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TABLES AND FIGURES

Table 1. Number of genera in different categories in all exceptionally preserved biotas.

Table 2. Proportion of each type of tissue in all categories combined in the Fezouata Shale, the Burgess Shale and the Chengjiang Biota. The probability of preserving cuticularized and cellular tissues, in addition to the number of tissue per genus in the Fezouata Shale are lower than in the Chengjiang Biota and the Burgess Shale.

Table 3. Probabilities of finding internal soft tissues in a fossil given that another tissue was found and vice versa. The obtained numbers for the Burgess Shale and the Chengjiang Biota are more similar to each other than to the Fezouata Shale.

Table 4. Probabilities of reproducing patterns of preservation of the Burgess Shale and the Chengjiang Biota assuming that the Fezouata Shale preservation regime is true. All probabilities are smaller than 0.05 showing that the preservation regime in the Fezouata Shale is different from both the Chengjiang Biota and the Burgess Shale.

Table 5. A: Probabilities of reproducing patterns of preservation of the Burgess Shale assuming that the Chengjiang biota preservation regime is true. B: Probabilities of reproducing patterns of preservation of the Chengjiang Biota assuming that the Burgess Shale preservation regime is true. Some tissue associations are not reproducible in both models (i.e. marked as “No” in the “Pass” column), showing that the pattern of preservation between the Burgess Shale and the Chengjiang Biota is not exactly the same.

Figure 1. Fossils from the three studied exceptionally preserved biotas showing examples of tissue associations. (a) Burgess Shale *Eldonia* USNM57540b preserving soft cellular body walls and internal organs (i.e. DE). (b) *Branchiocaris pretiosa* from the Burgess Shale USNM189028nc showing the association of sclerotized and cuticularized parts in addition to internal organs (BCE). (c) *Anomalocaris saron* ELRC20001a from the Chengjiang Biota belonging as well to the BCE category. (d) Marrellid arthropod from the Fezouata Shale AA-

BIZ31-OI-39 preserving both sclerotized and cuticularized structures (BC). (e) Fezouata Shale stylophoran echinoderm AA.BIZ.15.OI.259 showing the association of biominerals and internal organs (AE). (f) Solutan echinoderm from the Fezouata Shale CASG72938 belonging also to the AE category.

Figure 2. Differences in proportions of genera (Y axis) between single, paired, triple and quadruple character categories (marked as 1, 2, 3, and 4 on the X axis) between the Fezouata Shale, the Burgess Shale and the Chengjiang Biota. The Fezouata Shale shows a dominance of genera preserving only one tissue when compared to the Burgess Shale and Chengjiang Biota.

Figure 3. Pie charts showing the differences in paired and triple character categories between the Fezouata Shale, the Burgess Shale, and the Chengjiang Biota.

Figure 4. Preservation differences between exceptionally preserved biotas and one non-*Lagerstätte* (i.e. preservation of only mineralized genera). The Chengjiang biota and the Burgess Shale preserve more tissue-types than the Fezouata Shale in which soft tissues in direct contact with sea water are not preserved.

Highlights :

- Exceptionally preserved biotas are not subject to the same fossilization bias
- Fossilization bias is constrained in three iconic Burgess Shale type biotas using a new statistical method
- Cambrian localities preserved with high fidelity snapshots of early animal life

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Exceptionally preserved biotas have revolutionized our understanding of animal origins and evolution owing to the preservation in these deposits of soft-bodied and lightly sclerotized organisms, which under normal circumstances have little to no fossilization potential (Butterfield, 1995). Burgess Shale-type (BST) preservation deposits including the Burgess Shale (Wuliuan, Miaolingian; ~505 Ma, Canada) and the Chengjiang Biota (Stage 3, Cambrian Series 2; ~530 Ma, China) are particularly famous *Lagerstätten,* yielding hundreds of exceptionally preserved Cambrian taxa (Fig. 1a-c) critical to our understanding of the earliest metazoan-dominated communities and evolutionary events such as the Cambrian Explosion (Daley et al., 2018). The youngest of these deposits, the Fezouata Shale, is the only Ordovician (Tremadocian; ~479-478 Ma, Morocco) *Lagerstätte* to yield a diverse exceptionally preserved fauna (Fig. 1d-f). With over 185 taxa of marine invertebrates (Van Roy et al., 2015a) recovered from specific intervals in the Zagora area (Lefebvre et al., 2018; Saleh et al., 2018, 2019), this formation offers new insights into the diversification of metazoans, at a key interval between the Cambrian Explosion and the Ordovician Radiation (Van Roy et al., 2010, 2015b; Lefebvre et al., 2019). Despite being anatomically and biologically informative, even these spectacular fossil localities inevitably have taphonomic biases, because no fossil site can ever be a perfect replication of all the anatomical and ecological information of a living community (Butterfield, 2003; Brasier et al., 2010; Landing et al., 2018). Gathering "complete" data is impossible even in studies on modern living communities. It is therefore essential to understand what factors may be affecting the fossil preservation at a community level in order to properly reconstruct ancient ecosystems and biodiversity fluctuations over geological time.

The aim of this study is to examine the taphonomic signal of these deposits, allowing a solid understanding of the preservation bias at play in each locality. For this reason, a taphonomic classification of all eumetazoan genera from the Fezouata Shale (N= 178) was established, and compared with the preservation of genera from the Burgess Shale (N=103) and the Chengjiang Biota (N=133) based on the presence / absence of different types of anatomical structures: (A) biomineralized skeletons, (B) sclerotized parts (i.e. possessing an organically strengthened part or organ) (C) soft with an unsclerotized cuticle (i.e. a non-cellular outer body surface that is either collagenous or formed by polymerized polysaccharides), (D) soft cellular outer layer defining at least a part of the body (e.g. tentacles of hyoliths), and (E) soft internal cellular organ/tissue (e.g. digestive or nervous systems) (Fig.1).

2. MATERIAL AND METHODS

In order to define the preservation pattern in all three exceptionally preserved biotas, the various possible co-occurrences of characters A (biomineralized), B (sclerotized), C (unsclerotized, cuticularized), D (cellular body walls), and E (internal tissues) were tallied (e.g. AB, AC, CDE, and ABCDE) (Tab. 1). To avoid any overlap between categories, the data were analyzed on a five-fold Venn diagram per site. In order to see if there is any difference between sites, the total number of genera having just one character regardless of its nature (e.g. A, or B, or C, or D, or E) was plotted against the number of genera that have pairs (e.g. AB), threes (e.g. ABC) or fours (e.g. ABCD) for all exceptionally preserved biotas (Fig. 2). Afterward, the average number of tissue types per genus, as derived from the dataset, was calculated by adding the probability of the occurrence of all classes of structures A, B, C, D, and E (Tab. 2). In order to constrain the categories causing the biggest variations in preservation between sites, plots were made to show the proportion of paired and triple categories in localities (Fig. 3).

The association of soft internal organs (E) with other structures, in all three localities was also investigated. For this, the probabilities of discovering two classes of structures together having already found one of them were calculated (Tab. 3). For example, $p(E|A)$ is the probability of E occurring if A has occurred. The reverse conditional approach was also made and the probability of finding A given that E has been found $p(A|E)$ was also calculated (Tab. 3). Then, the likelihood of producing the distribution of combinations of structures found in the Burgess Shale and the Chengjiang Biota assuming that the Fezouata Shale has the "true" preservation regime was investigated using the following parametrized binomial P(x≥n) | Bi(n, p):

$$P(x) = \binom{n}{x} p^x q^{n-x} = \frac{n!}{(n-x)!\,x!} p^x q^{n-x}$$

In this equation, p = p(E|A) for the Fezouata Shale, q = 1-p, n is the number of genera preserving an A in the Burgess Shale or the Chengjinag Biota, and x is the number of desired success which is, in this case, at least the actual number n of genera preserving both A and E in the Burgess Shale/Chengjiang Biota. All calculated probabilities are added up and the probability P(x≥n) | Bi(n, p), of producing the actual Burgess Shale/Chengjinag Biota AE category, considering that the Fezouata Shale regime is "true", is then obtained (Tab. 4). This was then performed for other tissues combinations (i.e. BE, CE, and DE) (Tab. 4). This approach was then extended to the assumption that the Burgess Shale preservation distribution is "true" and finally assuming that the Chengjiang Biota preservation distribution is the "true" preservation model (Tab. 5).

Finally, the probability of finding organisms with only soft cellular tissues (both internal and external to the exclusion of everything else with A' for instance indicating the set that is defined as not containing and members of A) p(A'∩B'∩C'∩D∩E|E) for all three *Lagerstätten* was calculated.

3. RESULTS

All three *Lagerstätten* preserve numerous biomineralized skeletons (A), sclerotized parts (B), unsclerotized, soft cuticular parts (C), and internal soft parts (E) (Tab. 1). However, genera having cellular body walls defining the entire body (i.e. D, DE), with or without internal organs (E) are absent in the Fezouata Shale. In comparison the Chengjiang Biota (9 genera) and the Burgess Shale (13 genera) have a considerable number of entirely soft organisms preserved (Tab. 1). Further, numerous biomineralized and sclerotized genera in the

Burgess Shale and the Chengjiang Biota preserve external soft tissues defining a part of the body (i.e. AD, BD, BDE, ACDE) (Tab. 1). These genera are absent from the Fezouata Shale, with the exception of two specimens of aculiferan molluscs (both, however, densely covered by sclerites). The Burgess Shale and the Chengjiang Biota preserve almost twice as many tissues per genus as the Fezouata Shale (Fig. 2), with the mean number of tissue types per genus in the Cambrian sites being 2.2 (Burgess = 2.206; Chengjiang = 2.185) whilst it is 1.316 for the Fezouata Shale (Tab. 2). The overall distribution of tissue frequency by genus are similar for the Burgess Shale and the Chengjiang Biota, with mean and variance suggesting they are drawn from comparable if not identical populations (variance Burgess Shale = 0.026; Chengjiang Biota = 0.030; t = -0.45, p(same mean) = 0.6532; F = 1.154, p(same variance) = 0.454). However, the distribution for the Fezouata Shale is very different (variance = 0.08034), with both t and F-tests reporting significance for the mean and variance respectively when compared to Burgess Shale (t = 29.53, p(same mean) = 1.035×10^{-87}; F = 3.0685, p(same variance) = 3.195×10^{-9}) and the Chengjiang Biota (t = 32.34, p(same mean) = 3.414×10^{-101}; F = 2.5591, p(same variance) = 1.718×10^{-8}).

The three studied localities show a dominance of both BCE and ACE categories (Fig. 3). This is at least partly linked to the high number of arthropods found at all localities, with their external anatomy often consisting of ventral unsclerotized cuticle (C) and a reinforced dorsal area consisting of a biomineralized exoskeleton (A) or sclerotized cuticle (B), found in conjunction with internal soft parts (E). However, when the preservation of two tissue types occurs in the Fezouata Shale, it consists mostly of the association of biomineralized skeletons and internal soft tissues (AE is 9 of the 21 pairs that consist of the possible sets AB, AC, AD, AE, BC, BD, BE, CD, CE, DE), sclerotized tissue and internal soft tissue (7 of the 21 pairs), and biominerals and sclerotized tissue (3 of 21 pairs). All other tissue associations are rare or absent. In the Burgess Shale, the dominant association is between cellular soft bodied tissues and internal organs (13 of 36 pairs), with sclerotized and cuticularized tissues also commonly associated (7 of 36 pairs). In the Chengjiang Biota, the dominant association is between sclerotized and cuticularized tissues (16 of 57 pairs), with additional common associations between cuticularized tissues and internal organs (12 of 57 pairs), cellular soft bodied tissues and internal organs (9 of 57 pairs), and biominerals and sclerotized tissues (8 of 57 pairs) (Fig. 3). The probabilities of finding internal soft tissues in a given fossil genus, in co-occurrence with any of the other types of structures, show that the distribution of tissues in the Burgess Shale and the Chengjiang Biota are much more similar to each other (Tab. 3) and are significantly different from the Fezouata Shale (Tab. 4). In the Fezouata Shale, only a small proportion of all biomineralized genera also preserve internal organs ($p(E|A) = 0.162$) (Tab. 3), but of the genera that do have internal organs the majority are associated with biominerals ($(A|E) = 0.667$) (Tab. 3). This means that although a biomineral does not guarantee the preservation of internal anatomies, it could still be seen as a very helpful pre-requisite in the Fezouata Shale. Conversely, biominerals in paleoenvironments such as the Burgess Shale and the Chengjiang Biota do not seem to have any role in soft tissue preservation ($p(A|E) = 0.183$ and $p(A|E) = 0.273$ for the Burgess Shale and the Chengjiang Biota respectively, which are not significantly different to chance association (Tab. 3). The result of probabilistic modelling (Tab. 4) shows that the distributions of tissue associations found at the Fezouata Shale cannot be generated by randomly sampling a biota with a similar composition to that of either the Chengjiang Biota or the Burgess Shale, and in all possible soft tissue combinations the Fezouata Shale is statistically significantly different to both of the Cambrian biotas studied (Tab. 4). Finally, it is worth noting that the absence of entirely soft bodied organisms at the Fezouata Shale is not just a striking observation, but it is also statistically significant from the proportions found at the Cambrian sites. The absence of entirely soft bodied organisms at the Fezouata Shale cannot be generated by randomly sampling a population like that found in the

Cambrian sites with any confidence (with p-values of 0.00137 and 0.03819 for Burgess Shale and Chengjiang Biota models respectively). Therefore, the Burgess Shale ($p(D \cap E|E)$ = 0.2167) and the Chengjiang Biota ($p(D \cap E|E)$ = 0.113) both show significantly higher probabilities of recovering entirely soft bodied genera. The preservation of entirely soft bodied genera is also different between the Chengjiang Biota and the Burgess Shale (Tab. 3), with the higher incidence being found in the Burgess Shale. This difference is significant and could not be generated by chance or subsampling (Tab. 5).

4. DISCUSSION

Soft part preservation in the Fezouata Shale is strikingly different from the preservation in the Chengjiang Biota and the Burgess Shale. This difference in the occurrences of soft tissues cannot result from a collection bias, because all three localities were subjected to collecting efforts that actively focused on finding and sampling fossils with labile soft part. Instead, the observed pattern of preservation suggests that the presence of non-cellular layers covering internal anatomies in the Fezouata Shale was essential for exceptional preservation, unlike at the Burgess Shale and Chengjiang Biota. The near complete absence of preserved external soft tissues is possibly related to them being less decay-resistant than mineralized, sclerotized or even cuticularized structures. Under most circumstances, even unsclerotized soft cuticle is more decay resistant than cellular tissue, because cuticular structures are not subject to autolysis, and the composition of complex polymerized polysaccharides means cuticle is more difficult to break down than cellular tissues (Briggs and Kear, 1993). The decay-resistance of complex biopolymers found in the cuticle was also recently invoked to explain the rare but selective preservation of cuticularized organisms in coarse clastic sediments (MacGabhann et al., 2019).

In the Fezouata Shale, there was a pathway of preservation in place that systematically failed to preserve (i) almost all soft-bodied organisms lacking a cuticular cover in particular, and (ii) external soft cellular tissues in general. In this deposit, dead individuals experienced harsh decay prior to their preservation owing to a relative burial tardiness (Saleh et al., 2018) in comparison with the Burgess Shale and the Chengjiang Biota in which fossils were killed and preserved directly during an obrution event (Gaines, 2014). This decay may also have been retarded by berthierine, a mineral that can slow down microbial activity through the oxidative damage of bacterial cells (McMahon et al., 2016; Anderson et al., 2018; Saleh et al., 2019). Therefore, in contrast to the Burgess Shale and the Chengjiang Biota, the external conditions at the Fezouata Shale were generally less permissive for the preservation of external soft tissues. However, resistant skeletal parts and cuticular external surfaces created isolated environments within the carcasses that maintained a chemical equilibrium conducive to the preservation of internal organs.

The systematic taphonomic bias described here for the Fezouata Shale has implications for understanding the original faunal community assemblage, specifically in regard to the proportions of genera preserved in the fossil record. The systematic removal of all soft-bodied organisms, lacking a non-cellular external envelope (cuticle), and external cellular soft tissues leads to an underestimation of the original diversity at the Cambro-Ordovician transition and distorts faunal composition to a greater extent than in the Burgess Shale or the Chengjiang Biota. Many animal groups could have lived in the Fezouata Shale environment but left little to no trace behind, such as chordates (e.g. *Pikaia*, *Metaspriggina*). A corollary of this finding is that it is now possible to differentiate between ecological and taphonomic absences of numerous genera. For example, the absence of priapulids such as *Ottoia* in the Fezouata Shale (Van Roy et al., 2015a) is likely a real aspect of the fauna, since these cuticle-bearing soft-bodied animals would not have been affected by the same taphonomic bias responsible for the removal of the majority of soft-bodied genera lacking a cuticle.

Now that a source of systematic taphonomic bias operating in the Fezouata Shale has been identified (Fig. 4), and most importantly, compared to the biases in play in the Burgess Shale and the Chengjiang Biota (Fig. 4), it can be accounted for in future paleoecological and evolutionary analyses. This will facilitate more accurate comparisons of faunal community compositions between these biotas in particular, and when comparing exceptionally preserved faunas in general, as similar restrictive mechanisms are likely active to a varying extent at other localities.

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TABLES AND FIGURES

Table 1. Number of genera in different categories in all exceptionally preserved biotas.

Table 2. Proportion of each type of tissue in all categories combined in the Fezouata Shale, the Burgess Shale and the Chengjiang Biota. The probability of preserving cuticularized and cellular tissues, in addition to the number of tissue per genus in the Fezouata Shale are lower than in the Chengjiang Biota and the Burgess Shale.

Table 3. Probabilities of finding internal soft tissues in a fossil given that another tissue was found and vice versa. The obtained numbers for the Burgess Shale and the Chengjiang Biota are more similar to each other than to the Fezouata Shale.

Table 4. Probabilities of reproducing patterns of preservation of the Burgess Shale and the Chengjiang Biota assuming that the Fezouata Shale preservation regime is true. All probabilities are smaller than 0.05 showing that the preservation regime in the Fezouata Shale is different from both the Chengjiang Biota and the Burgess Shale.

Table 5. A: Probabilities of reproducing patterns of preservation of the Burgess Shale assuming that the Chengjiang biota preservation regime is true. B: Probabilities of reproducing patterns of preservation of the Chengjiang Biota assuming that the Burgess Shale preservation regime is true. Some tissue associations are not reproducible in both models (i.e. marked as “No” in the “Pass” column), showing that the pattern of preservation between the Burgess Shale and the Chengjiang Biota is not exactly the same.

Figure 1. Fossils from the three studied exceptionally preserved biotas showing examples of tissue associations. (a) Burgess Shale *Eldonia* USNM57540b preserving soft cellular body walls and internal organs (i.e. DE). (b) *Branchiocaris pretiosa* from the Burgess Shale USNM189028nc showing the association of sclerotized and cuticularized parts in addition to internal organs (BCE). (c) *Anomalocaris saron* ELRC20001a from the Chengjiang Biota belonging as well to the BCE category. (d) Marrellid arthropod from the Fezouata Shale AA-

BIZ31-OI-39 preserving both sclerotized and cuticularized structures (BC). (e) Fezouata Shale stylophoran echinoderm AA.BIZ.15.OI.259 showing the association of biominerals and internal organs (AE). (f) Solutan echinoderm from the Fezouata Shale CASG72938 belonging also to the AE category.

Figure 2. Differences in proportions of genera (Y axis) between single, paired, triple and quadruple character categories (marked as 1, 2, 3, and 4 on the X axis) between the Fezouata Shale, the Burgess Shale and the Chengjiang Biota. The Fezouata Shale shows a dominance of genera preserving only one tissue when compared to the Burgess Shale and Chengjiang Biota.

Figure 3. Pie charts showing the differences in paired and triple character categories between the Fezouata Shale, the Burgess Shale, and the Chengjiang Biota.

Figure 4. Preservation differences between exceptionally preserved biotas and one non-*Lagerstätte* (i.e. preservation of only mineralized genera). The Chengjiang biota and the Burgess Shale preserve more tissue-types than the Fezouata Shale in which soft tissues in direct contact with sea water are not preserved.

Figure 1
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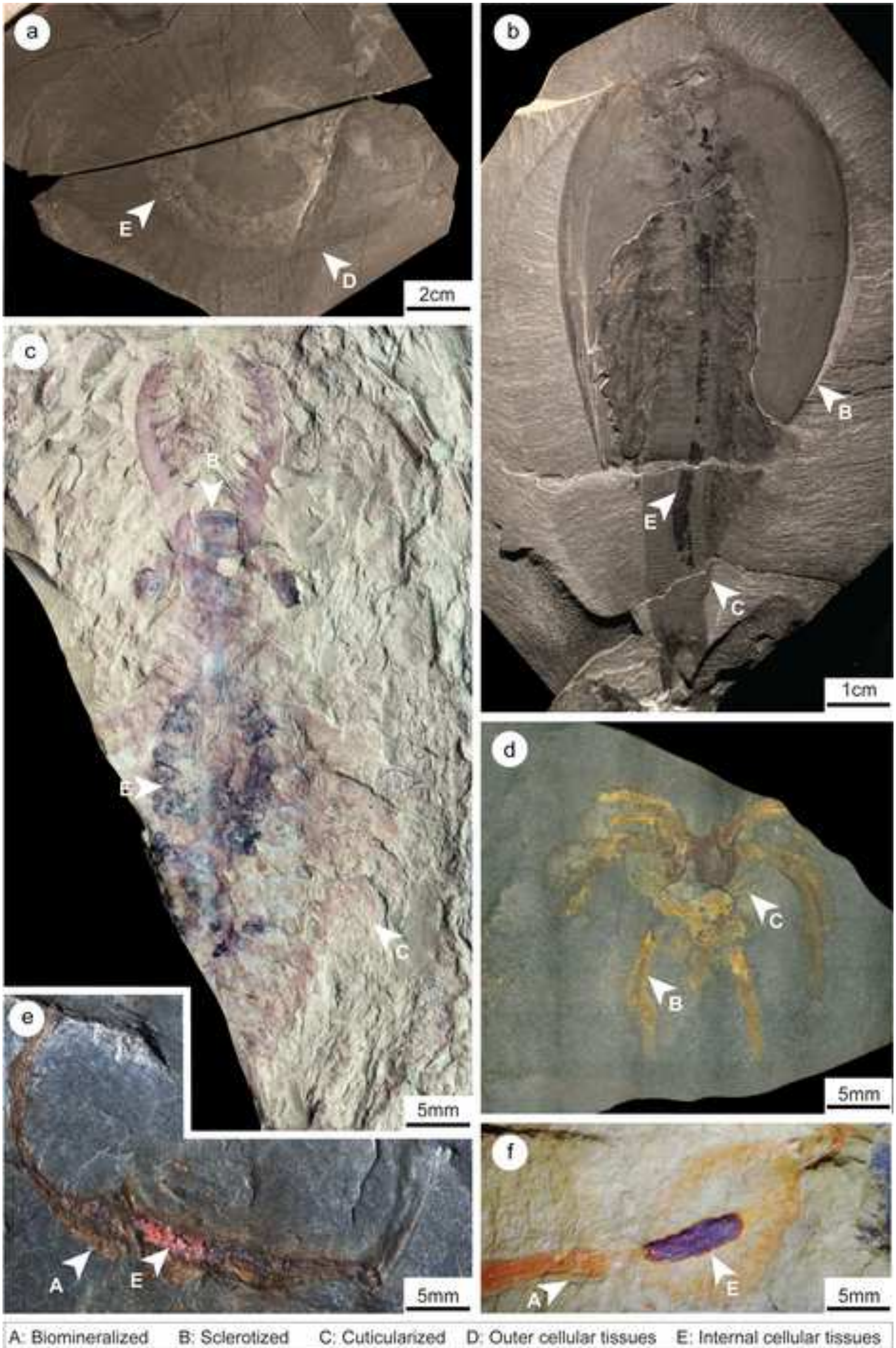

Figure 2

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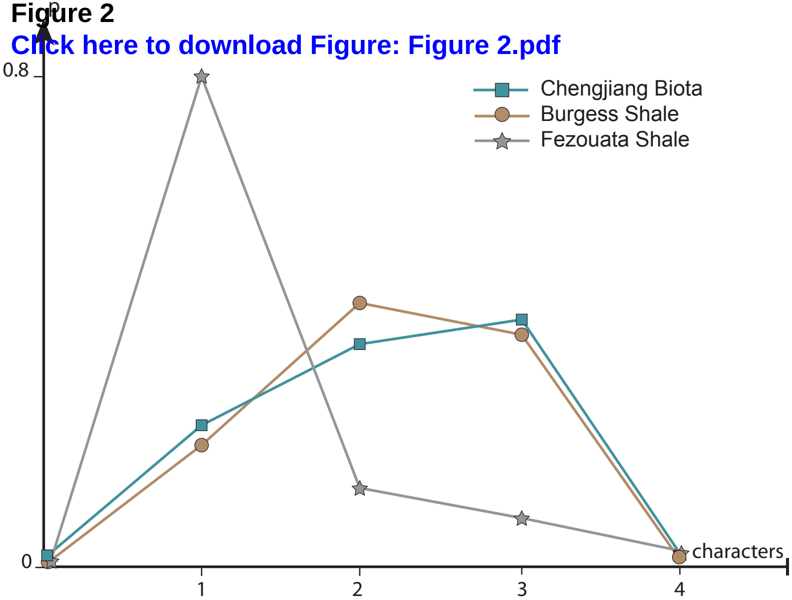

Figure 3

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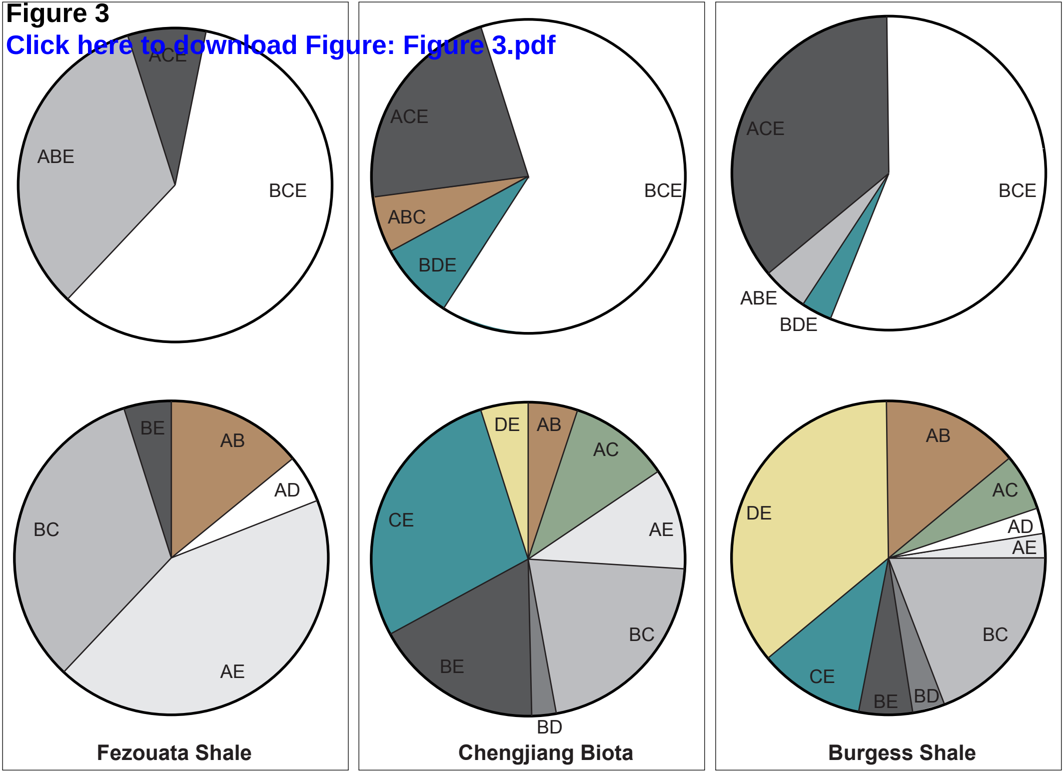

A: Biomineralized B: Sclerotized C: Cuticularized D: Outer cellular tissues E: Internal cellular tissues

Figure 4

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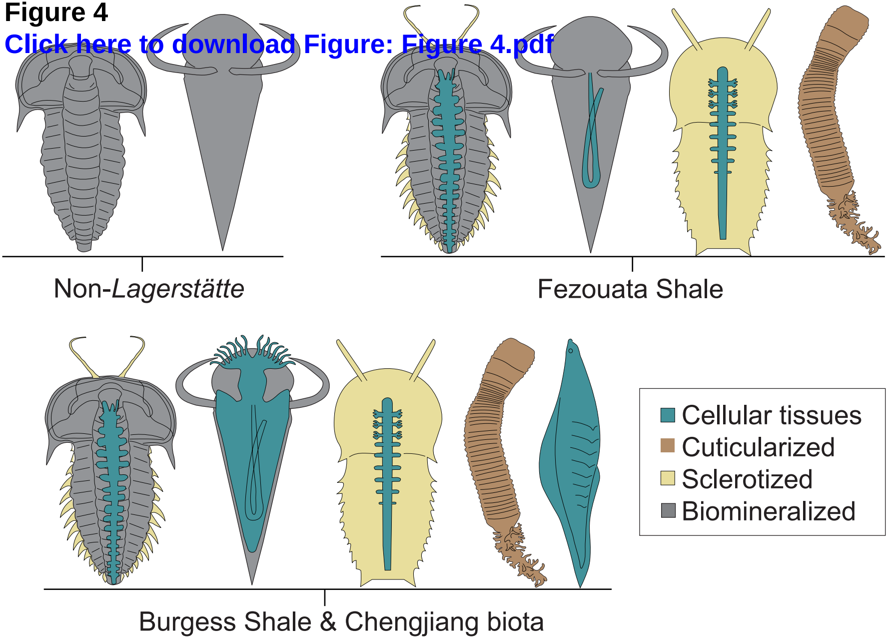

Table 1
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	Fezouata Shale	Burgess Shale	Chengjiang Biota
A	90	15	4
B	41	7	9
C	3	0	6
D	0	1	4
E	1	0	0
AB	3	5	8
AC	0	2	2
AD	1	1	0
AE	9	1	0
BC	7	7	16
BD	0	1	4
BE	1	2	6
CD	0	0	0
CE	0	4	12
DE	0	13	9
ABC	0	2	0
ABD	0	0	0
ABE	5	0	2
ACD	0	0	0
ACE	1	8	19
ADE	0	0	0
BCD	0	0	0
BCE	7	28	28
BDE	0	2	1
CDE	0	0	0
ABCD	0	0	0
ABCE	3	1	1
ACDE	0	1	0
ACDE	0	0	0
BCDE	0	0	0
ABCDE	0	0	0

Table 1

Table 2
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	Fezouata Shale N(total)=173	Burgess Shale N(total)=101	Chengjiang Biota N(total)=133
A	N(A)=112 p(A)=0.647	N(A)= 36 p(A)=0.356	N(A)= 36 p(A) = 0.270
B	N(B)=67 p(B) = 0.387	N(B)=55 p(B)=0.544	N(B)=75 p(B)=0.563
C	N(C)=21 p(C) = 0.121	N(C)=53 p(C)=0.524	N(C)=84 p(C)=0.631
D	N(D)=1 p(D)=0.005	N(D)=19 p(D)=0.188	N(D)=18 p(D)=0.135
E	N(E)=27 p(E)=0.156	N(E)=60 p(E)=0.594	N(E)=78 p(E)=0.586
Total = tissue/genus	1.316	2.206	2.185

Table 2

Table 3
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	Fezouata Shale	Burgess Shale	Chengjiang Biota
p(E|A)	0.162	0.306	0.611
p(E|B)	0.239	0.607	0.507
p(E|C)	0.524	0.789	0.714
p(E|D)	0	0.842	0.556
p(A|E)	0.667	0.183	0.278
p(B|E)	0.593	0.567	0.481
p(C|E)	0.407	0.683	0.759
p(D|E)	0	0.267	0.127

Table 3

Table 4
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	Burgess Shale	Chengjiang Biota
p(E|A)	P(X≥11) | Bi(36, 0.162) = 0.0235	P(X≥22) | Bi(36, 0.162) < 0.000001
p(E|B)	P(X≥34) | Bi(56, 0.239) < 0.000001	P(X≥38) | Bi(75, 0.239) < 0.000001
p(E|C)	P(X≥41) | Bi(52, 0.524) = 0.0000738	P(X≥60) | Bi(84, 0.524) = 0.000291
p(E|D)	0	0

Table 4

Table 5
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	A: Burgess given a Chengjiang Biota model	B: Chengjiang given the Burgess Shale model	Pass?
p(E|A)	P(X≤11)|Bi(36, 0.611) = 0.000201	P(X≥22)|Bi(36, 0.306) = 0.000149	No
p(E|B)	P(X≥34)|Bi(56, 0.507) = 0.0857	P(X≤38)|Bi(75, 0.607) = 0.292	Yes
p(E|C)	P(X≥41)|Bi(52, 0.714) = 0.150	P(X≤60)|Bi(84, 0.789) = 0.0649	Yes
p(E|D)	P(X≥16)|Bi(19, 0.556) = 0.00887	P(X≤10)|Bi(18, 0.842) = 0.000758	No

Table 5