

Early Eukaryotes in the Lakhanda Biota (Mesoproterozoic, Southeastern Siberia)—Morphological and Geochemical Evidence

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Abstract—The Mesoproterozoic Lakhanda Group (~1030 Ma) preserves one of the most diverse communities of pre-Ediacaran eukaryotes. More precisely, the Lakhanda Biota includes more than twenty taxa that have been assigned to eukaryotes with different degrees of confidence. Eight of these taxa meet current criteria for the identification of eukaryotic fossils in ancient records. These include previously described fossils such as ornamented acritarchs (*Valeria lophostriata*, *Trachyhystrichosphaera aimika*), filamentous coenocytic organisms (*Aimonema ramosa*, *Palaeovaucheria clavata*), as well as fossils with smooth-walled envelopes and single outgrowth structures (*Caudosphaera expansa*, *Germinosphaera bispinosa*, and *Jacutianema solubila*). In addition to these, we found as yet undescribed fossils which share remarkable similarities with *Ourasphaira giraldae*, a possible higher fungi species known from the (?) Meso- to Neoproterozoic of Arctic Canada. Regardless of the exact systematic affinity, these fossils can confidently be assigned to eukaryotes because of the size and high morphological complexity. Intriguingly, the organic record of the Lakhanda Formation lacks biomarkers indicative of eukaryotes (that is, regular steranes). This finding would be in line with the idea that eukaryotes were present but not significant in Mesoproterozoic marine ecosystems. However, preliminary data from an ongoing study indicate an advanced thermal maturity of the organic matter, emphasizing that this conclusion might not be drawn with absolute confidence.

Keywords: Lakhanda biota, eukaryotes, biomarkers, Mesoproterozoic

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INTRODUCTION

The radiation of early eukaryotes is one of the most critical evolutionary developments in the history of life and chronicled by the Proterozoic fossil record. In this regard, the Mesoproterozoic Era (1600–1000 million years, Ma) is particularly interesting. The first undoubted eukaryotic fossils occur in Paleo-Mesoproterozoic boundary deposits (~1600 Ma) in Australia, China and India [1, 2]. These fossils most likely represent stem-group eukaryotes and usually share characteristics of different, phylogenetically distant

modern lineages or have no recent analogues. By the end of the Mesoproterozoic and at the beginning of the Neoproterozoic (~1000 Ma), eukaryotes developed morphologies that are characteristic to various crown groups in the phylogenetic tree (Fig. 1). Important examples from that time are the iconic multicellular red alga *Bangiomorpha pubescens* Butterfield, 2000 (Rhodophyceae), the multicellular green alga *Proterocladus antiquus* Tang et al., 2020 (Chloroplastida), as well as the fungus *Ourasphaira giraldae* Loron et al., 2019 (Nucleotmycea) [1–4]. By the mid-Neoproterozoic (~800 Ma), eukaryotic fossil communities are taxonomically diverse and included biomineralizing organisms [1, 5].

Despite the constantly expanding archive of early eukaryote evolution, the exact phylogenetic relationships among fossil taxa are as yet unresolved. Furthermore, it is difficult to reconstruct the impact of early eukaryotes on the Mesoproterozoic bio- and geosphere. These problems are mainly due to the limited number of well-preserved fossils and their relatively simple morphology. Several attempts have been made to understand the Proterozoic evolution of eukaryotes

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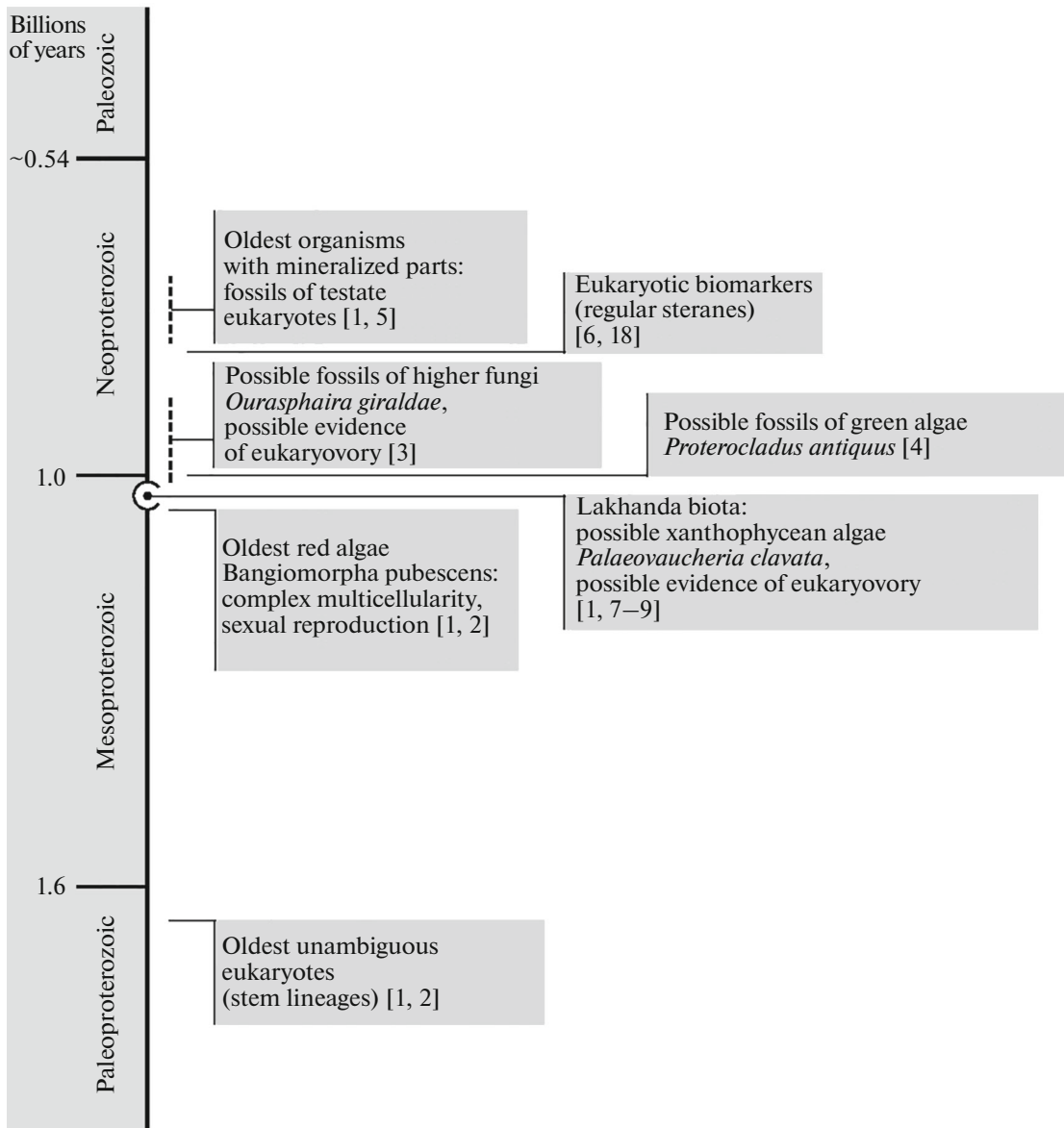


Fig. 1. Major developments in the early evolution of eukaryotes as chronicled in the Proterozoic record. Note that the Lakhanda Biota falls into a time of notable changes.

better by analyzing biomarkers (chemofossils) [6]. Biomarkers are organic molecules that can be unambiguously linked to specific precursor compounds synthesized by organisms. Since all organisms form organic molecules, biomarkers potentially offer additional insights into past ecosystems. Unfortunately, however, ancient biomarker records potentially have been affected by a variety of destructive processes (biodegradation, thermal destruction during burial) and therefore might provide biased views [6]—similarly to paleontological records.

Marine mudstones of the Lakhanda Group (~1030 Ma) preserve one of the most diverse pre-Ediacaran assemblages of organic-walled fossils. The sed-

imentary succession of the Lakhanda Group containing this biota crops out in the middle reaches of the Maya River, Uchur-Maya region, in southeastern Siberia (Fig. 2). After more than half of a century of exploration, almost forty taxonomically and ecologically diverse taxa have been described, half of which have been assigned to eukaryotes with different degrees of confidence [7, 8]. Notably, some of these taxa may represent crown group eukaryotes, and some show possible evidence for eukaryovory [9]. These findings are of great relevance, since it was hypothesized that selective predation might have been one of the main drivers of early eukaryote diversification [3]. Thus, the Lakhanda biota is highly significant to our understanding of the early evolution of eukaryotes.

Here, we briefly review and discuss some of the most important paleontological findings from the Lakhanda group, and also report new taxa that can be assigned to eukaryotes based on morphological characteristics. In addition, we will give a brief update on organic geochemical research on the host rocks of the Lakhanda group that is currently being carried out.

THE LAKHANDA BIOTA—MORPHOLOGY OF FOSSILS

At present, there is no well-established set of criteria for the unambiguous identification of eukaryotic fossils in Proterozoic rocks. However, the identification of diagnostic features and possible synapomorphies in ancient taxa, as well as a careful comparison of these fossils with living eukaryotes, may allow an assignment to crown lineages of the domain [1, 10]. It should be noted, that this approach is mainly restricted to the interpretation of large and morphologically complex forms.

The Lakhanda biota includes various large and morphologically complex fossils that are widely accepted to be of eukaryotic origin. Most noteworthy are *Palaeovaucheria clavata* Hermann, 1981, *Aimonema ramosa* Hermann et Podkovyrov, 2010, *Valeria lophostriata* (Jankauskas, 1979) Jankauskas, 1982, *Trachyhystrichosphaera aimika* Hermann, 1976, *Caudosphaera expansa* Hermann et Timofeev, 1989, *Germinosphaera bispinosa* Mikhailova, 1986, and *Jacutianema solubila* Timofeev et Hermann, 1979 [7, 8]. A brief analysis of the morphology of these taxa will be provided below.

P. clavata exhibits a variety of notable characteristics including occasionally branching thalli (diameter 18–50 μm), sparse septa and circular openings (mostly limited to filaments that terminate in clavate cells), and occasional axial swellings separated by septa (Figs. 3a, 3b). Similar features are known from reproductive structures of the modern yellow-green algae genus *Vaucheria* (Stramenopila), such as zoosporangia, zoospores, antheridia and oogonia [7]. For this reason, *P. clavata* was originally discussed as possible yellow-green algae, which would make it the oldest representative of autotrophic stramenopiles.

Problematically, specimens of *P. clavata* are incompletely articulated, rendering the above discussed systematic placement of this species uncertain. In fact, many more uncertainties surround the interpretation of this species. For instance, it has been proposed that the species *A. ramosa* Hermann, 2010 might represent more complete specimens of *P. clavata* [1]. Indeed, fossils of both species share some morphological features as for example branching filaments of irregular diameter (18–50 μm in *P. clavata* and 30–50 μm in *A. ramosa*) that occasionally show bulbous terminations (Fig. 3c). However, in contrast to *P. clavata*, *A. ramosa* exhibits a reticulate thallus, which is

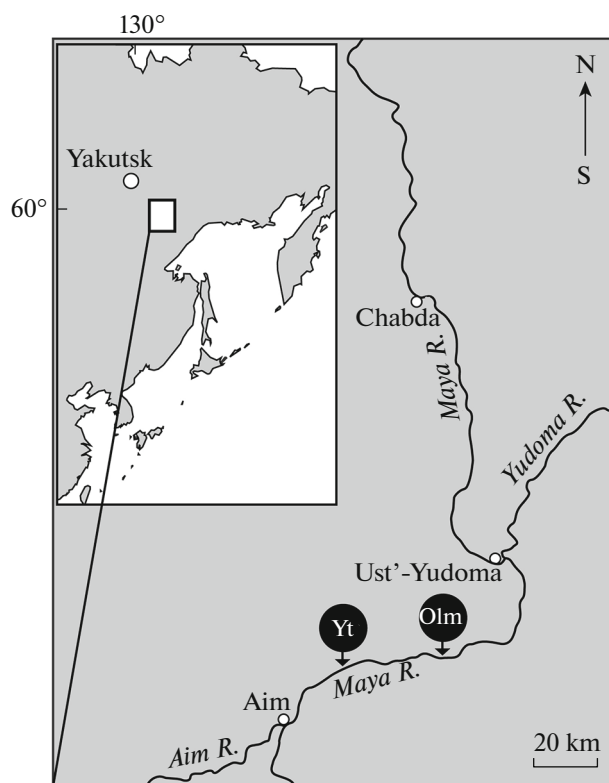


Fig. 2. Location of the studied sections, containing the Lakhanda Biota: Yt—Ytyrynda section, GPS coordinates (WGS84) 58.907510° N, 134.518742° E; Olm—Olemecken section; GPS coordinates (WGS84) 58.931815° N, 134.958032° W.

unknown in yellow-green algae, but a typical feature of nematophagous fungi [8]. In addition, no septa and filament openings occur in *A. ramosa*. For these reasons, *A. ramosa* and *P. clavata* seem to represent different organisms. Regardless of this question, both taxa are likely of eukaryotic origin, since the diameter of hyphae in prokaryotes does not exceed 2 μm [11].

Obviously, *P. clavata* is rather special case. In fact, most Proterozoic fossils lack diagnostic features observed in modern organisms. It is not clear whether this is due to a loss of morphological detail during fossilisation (that is, taphonomy), or if the respective characteristics only evolved later in Earth's history. Regardless of this question, the vast majority of Proterozoic fossils can at best be assigned to the domain Eukaryota based on morphology, while any interpretation beyond that is likely tentative. Important criteria for establishing a eukaryotic origin of fossils include a relatively large body size and morphological complexity (e.g., cell-wall ornamentation, the presence of processes/extensions). These traits are due to a high level of cellular compartmentalization which is not known from prokaryotes and thus can be considered a hallmark of eukaryotic life.

In addition to *P. clavata* and *A. ramosa*, six further eukaryotic taxa can reliably be identified in the

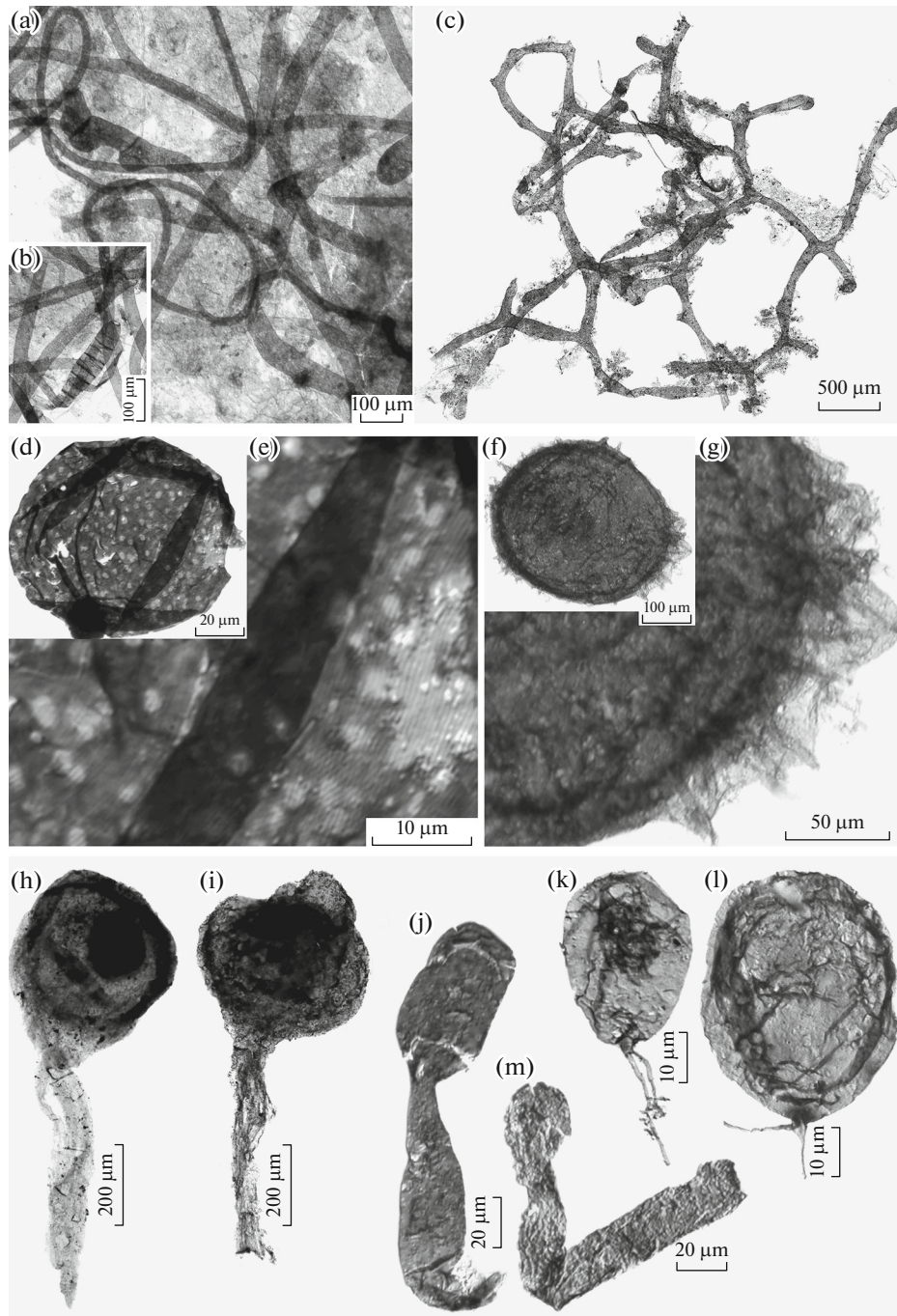


Fig. 3. Eukaryotic fossils of the Lakhanda biota. (a, b) Filamentous coenocytic remains of *Palaeovaucheria clavata*. (a) Occasionally branching thalli and axial swellings separated by septa (possibly antheridium and oogonium); (b) circular opening at the tip of the filament termination (possible zoosporangium); (c) reticulate siphonous thallus of *Aimonema ramosa*; (d, e) ornamented envelope of *Valeria lophostriata*: (d) overview, (e) detail of the wall, showing concentric ridges on the surface; (f, g) acantomorph acritarch *Trachyhystrichosphaera aimika*, 1976: (f) general view, (g) detail of the envelope, showing processes; (h, i) sporangia-like eukaryote *Caudosphaera expansa*; (f, k) – new, as yet undescribed sporangium-like fossil; (l, m) *Jacutianema solubila*, 1976, a species resembling germinating zoospores of some eukaryotes; Fossils stored in the collection of the Institute of Precambrian Geology and Geochronology RAS (Russia): (a) sample no. 28/4, (b) sample no. 27/25, (c) holotype no. 7/5-III.08, (h) holotype no. 26/7-IV.69; fossils stored in the collection of the Paleontological Institute of the Russian Academy of Sciences (Russia): (j) sample no. 5805/3001, (l) sample no. 5805/3002, (m) sample no. 5805/3003; fossils stored in the collection of the Geochron Center for Collective Use, Institute of Petroleum Geology and Geophysics, Siberian Branch of the Russian Academy of Sciences (Russia): (d, e) sample no. 396-14, (f, g) sample no. MH-8-2-1; (j) sample no. 395-9; (k) sample no. 395-10; sample locality and stratigraphy of fossils shown in (a–i, l, m) southeastern Siberia, Uchur-Maya region, Maya River, Ytyrynda section, Lakhanda Group, Kumakha Formation; sample locality and stratigraphy of fossils shown in (j, k) southeastern Siberia, Uchur-Maya region, Maya River, Olemeken section, Lakhanda group, Nel'kan formation.

Lakhanda biota. These include acritarchs with distinctly ornamented (concentrically “striated”) surface patterns (*V. lophostriata*), acantomorph acritarchs (*T. aimika*), sporangia-like fossils (*C. expansa* and a new, as yet undescribed form), as well as taxa which resemble germinating spores of lower fungi, stramenopiles and green algae (*G. bispinosa* and *J. solubila*).

The acritarch *V. lophostriata* exhibits a spheroidal shape (diameter >60 µm). The interpretation as eukaryote is mainly based on the size of concentric ridges on the surface, which are ~1 µm in width (Figs. 3d, 3e) and thus much wider as comparable structures in prokaryotes (nanoscale). The acritarch *T. aimika* exhibits a distinctly large body (>200 µm in diameter) that is covered with sparsely arranged, hollow processes that have diameters of ~5 µm and lengths >40 µm (Figs. 3f, 3g). Bacterial cells and macroscopic colonial envelopes of cyanobacteria can have comparable sizes, but are typically not ornamented. Some bacterial sporangia-like structures may also possess protrusions, but the diameter of these structures is significantly smaller than that of the processes present in *T. aimika* (nanometer vs micrometer scale) [10]. Traditionally, most acritarchs are interpreted as unicellular protists, mainly phytoplankton, but some forms resemble zoospore fungi. Unfortunately, any further interpretation of their systematics would be speculative due to the lack of diagnostic features.

Forms characterized by smooth spheroidal envelopes with solitary processes constitute another important group of fossils found in the Lakhanda biota. Morphologically most distinctive is *C. expansa*. This species exhibits large spheres (>200 µm in diameter) with tail-like extensions that are composed of thin filaments in places (~1 µm in diameter) (Figs. 3h, 3i). Another, as yet not described sporangia-like fossil shows spheres (30–70 µm in diameter) with a short protrusion (<1 µm length) attached to a long filament (~1 µm in diameter) (Figs. 3j, 3k). Morphologically, this fossil strongly resembles *O. giraldae* from the (?) Meso-Neoproterozoic of Arctic Canada, which is interpreted as fungi [3]. At the moment, we do not yet have data on the biochemistry of the new Lakhanda fossil, which complicates its systematic interpretation. Fossils of the species *G. bispinosa* exhibit spheroidal envelopes (30–40 µm in diameter) and also possess a solitary extension. However, their extension is connected to the envelope interior and gradually tapers towards the free end. *J. solubila* is represented by envelopes of the similar diameter (30–40 µm in diameter) but can be distinguished from *G. bispinosa* by the extension consistent in diameter (Figs. 3l, 3m). Germinating spores of some bacteria are also characterized by tail-like processes, but these are significantly smaller in size [10]. Another potential problem are bacteria that develop complex branching structures which host macroscale (1000 µm) fruiting-bodies [12]. These fruiting-bodies might resemble sporangia in eukaryotic organisms but are more fragile and thus

unlikely to fossilize [12]. Furthermore, the surface of such fruiting bodies is never smooth, but traces the contours of the embedded cells, which is very different to eukaryotes [e.g., 13]. Hence, these fossils most likely represent eukaryotes such as primitive fungi, stramenopiles, and/or green algae [e.g., 7, 14–16].

THE LAKHANDA BIOTA— BIOGEOCHEMISTRY OF THE HOST ROCKS

As discussed above, the Lakhanda Group obviously contains a wealth of eukaryotic fossils. This raises the question whether the record also preserves molecular traces of early eukaryotes, especially since biomarkers that are specific to this domain (that is, regular steranes) are typically scarce in Paleoproterozoic to Mesoproterozoic rocks [6]. An absence of steranes was also reported for a single sample from the Lakhanda Group [17], for which, unfortunately, data integrity has not been verified (thermal maturity, biodegradation, hydrocarbon syngenicity). The verification of data integrity, however, is absolutely essential in biomarker studies on ancient rocks to ensure reliability of the results [6]. In order to fill this gap in knowledge, we are currently assessing organic matter preservation on an extensive sample set from the Lakhanda Group. First results indicate that steranes are absent in the Lakhanda Group, while bacterial biomarkers seem to be present in traces. This is in line with previous results [17] and would support the idea that eukaryotes were present but not significant in Mesoproterozoic ecosystems as suspected earlier [e.g., 1, 6, 18]. However, at the same time it seems that the organic matter is of higher thermal maturity, emphasizing that this conclusion might not be drawn with absolute confidence. Thus, comparative morphological analysis offers the most reliable tool for assessing the diversity of the Proterozoic Lakhanda biota.

CONCLUSION

Based on current criteria for the identification of eukaryotic fossils in ancient records, eight taxa of the Lakhanda biota can confidently be assigned to this domain. In addition to previously described ornamented acritarchs (*V. lophostriata*, *T. aimika*), these include filamentous coenocytic organisms (*A. ramosa*, *P. clavata*) as well as fossil with smooth-walled envelopes and single outgrowth structures (*C. expansa*, *G. bispinosa*, and *J. solubila*). Furthermore, we have identified an as yet undescribed fossil of likely eukaryotic origin, which resembles the recently described fungi species *O. giraldae* from the (?) Meso- to Neoproterozoic of Arctic Canada. All these fossils can confidently be assigned to eukaryotes because of their size and morphological complexity. Surprisingly, it seems that the Lakhanda formation lacks biomarkers indicative of eukaryotes (that is, regular steranes). In combination with the paleontological record, this may

indicate the presence but relative insignificance of eukaryotic organisms in the Lakhanda ecosystem. However, data obtained in an ongoing study indicate an advanced thermal maturity of the organic matter, emphasizing that this conclusion might not be drawn with absolute confidence.

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REFERENCES

1. Butterfield, N.J., *Palaeontology*, 2015, vol. 58, pp. 5–17.
2. Javaux, E.J. and Lepot, K., *Earth-Sci. Rev.*, 2018, vol. 176, pp. 68–86.
3. Loron, C.C., Francois, C., Rainbird, R.H., et al., *Nature*, 2019, vol. 570, no. 7760, pp. 232–235.
4. Tang, Q., Pang, K., Yuan, X.L., and Xiao, S.H., *Nature Ecol. Evol.*, 2020, vol. 4, no. 4, pp. 543–559.
5. Wood, R., *Emerg. Top. Life Sci.*, 2018, vol. 2, no. 2, pp. 201–212.
6. Love, G.D. and Zumberge, J.A., in *Proterozoic Lipid Biomarker Records*, Cambridge: Cambridge University Press, 2021, pp. 1–95.
7. Yankauskas, T.V., Mikhailova, N.S., German, T.N., et al., *Mikrofossilii dokembriya SSSR* (Microfossils of the Precambrian of the Soviet Union), Leningrad: Nauka, 1989.
8. German, T.N. and Podkovyrov, V.N., *Paleontol. Zh.*, 2010, no. 4. S, pp. 15–23.
9. Shuvalova, Yu.V., Nagovitsin, K.E., and Parkhaev, P.Yu., *Dokl. Biol. Sci.*, 2021, vol. 496, pp. 34–40.
10. Javaux, E.J., Knoll, A.H., and Walter, M.R., *Orig. Life. Evol. Biosph.*, 2003, vol. 33, no. 1, pp. 75–94.
11. Kampfer, P., Parkes, L., van Keulen, G., and Dyson, P., in *The Prokaryotes: Actinobacteria*, Berlin: Springer, 2014, pp. 889–1010.
12. Shimkets, L.J., Dworkin, M., and Reichenbach, H., in *The Prokaryotes*, vol. 7: *Proteobacteria: Delta, Epsilon Subclass*, New York: Springer, 2006, pp. 31–115.
13. Grilione, P.L. and Pangborn, J., *J. Bacteriol.*, 1975, vol. 124, no. 3, pp. 1558–1565.
14. Tell, G., *Darwiniana*, 1970, vol. 16, nos. 1–2, pp. 139–143.
15. Longcore, J., *Mycologia*, 1995, vol. 87, pp. 25–33.
16. An, J.W., Kang, P.J., and Nam, K.W., *J. Appl. Phycol.*, 2020, vol. 32, no. 4, pp. 2689–2696.
17. Pawlowska, M.M., Butterfield, N.J., and Brocks, J.J., *Geology*, 2013, vol. 41, no. 2, pp. 103–106.
18. Knoll, A.H., Summons, R.E., Waldbauer, J.R., and Zumberge, J.E., in *Evolution of Primary Producers in the Sea*, Cambridge: Academic Press, 2007, pp. 133–163.