Large and robust lenticular microorganisms on the young Earth

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ABSTRACT

In recent years, remarkable organic microfossils have been reported from Archean deposits in the Pilbara craton of Australia. The structures are set apart from other ancient microfossils by their complex lenticular morphology combined with their large size and robust, unusually thick walls. Potentially similar forms were reported in 1992 from the ∼3.4 Ga Kromberg Formation (KF) of the Kaapvaal craton, South Africa, but their origin has remained uncertain. Here we report the first determination of *in situ* carbon isotopic composition ($\delta^{13}C$) of the lenticular structures in the KF (obtained with Secondary Ion Mass Spectrometry [SIMS]) as well as the first comparison of these structures to those from the Pilbara, using morphological, isotopic, and sedimentological criteria.

Our results support interpretations that the KF forms are *bona fide*, organic Archean microfossils and represent some of the oldest morphologically preserved organisms on Earth. The combination of morphology, occurrence, and $\delta^{13}C$ values argues that the lenticular forms represent microbes that had planktonic stages to their life cycles. The similarity in morphology, $\delta^{13}C$, and facies associations among specimens from Australia and South Africa suggests that the lenticular microfossils on the two continents represent related organisms. The biological success of these organisms is demonstrated by their abundance, widespread distribution, and the fact that, as a group, they appear to have been present at least 400 million years. This success may be due in part to their robust structure and planktonic habit, features that may have contributed to survival on a young planet. Isotopic results further suggest that the lenticular organisms were autotrophs, an interpretation supporting the view that autotrophic metabolisms developed early on the young Earth.

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1. Introduction

Archean lenticular, organic structures were first reported by Walsh (1992) from the ∼3.4 Ga Kromberg Formation (KF), in the Barberton Greenstone Belt, from the eastern part of Kaapvaal craton of South Africa (Figs. 1 and 2). Those structures were originally interpreted as "possible microfossils" because they lacked cellular elaboration that could firmly establish a biological origin. In addition, their large size and spindle-like shape set them apart from other Precambrian microfossils (Walsh, 1992; Altermann, 2001).

Similar forms have since been discovered in the ∼3 Ga Farrel Quartzite (FQ) (Sugitani et al., 2007) and the ∼3.4 Ga Strelley Pool Formation (SPF) (Sugitani et al., 2010, 2013), both in the Pilbara craton of Australia (Figs. 1, 3). Detailed analyses have provided sufficient data to confidently interpret the Pilbara examples as *bona fide* Archean microfossils (Oehler et al., 2009, 2010; Grey and Sugitani, 2009; House et al., 2013; Lepot et al., 2013; Schopf et al., 2010; Sugitani et al., 2009a, b, 2010, 2013, 2015a), but the KF lenticular forms have not been chemically analyzed until now. Here we report $\delta^{13}C$ of individual, *in situ* lenticular structures from the KF as well as comparisons of $\delta^{13}C$, morphology, spatial distribution, and facies of the South African and Australian forms, which provide insight into the significance of these unusual structures on the early Earth.

2. Materials and methods

2.1. Sample locations

The location of the Barberton KF sample is indicated in Fig. 2. It is from latitude/longitude of 23.929°S/30.916°E, which is Locality A of Walsh (1992), on the western limb of the Onverwacht Anticline.
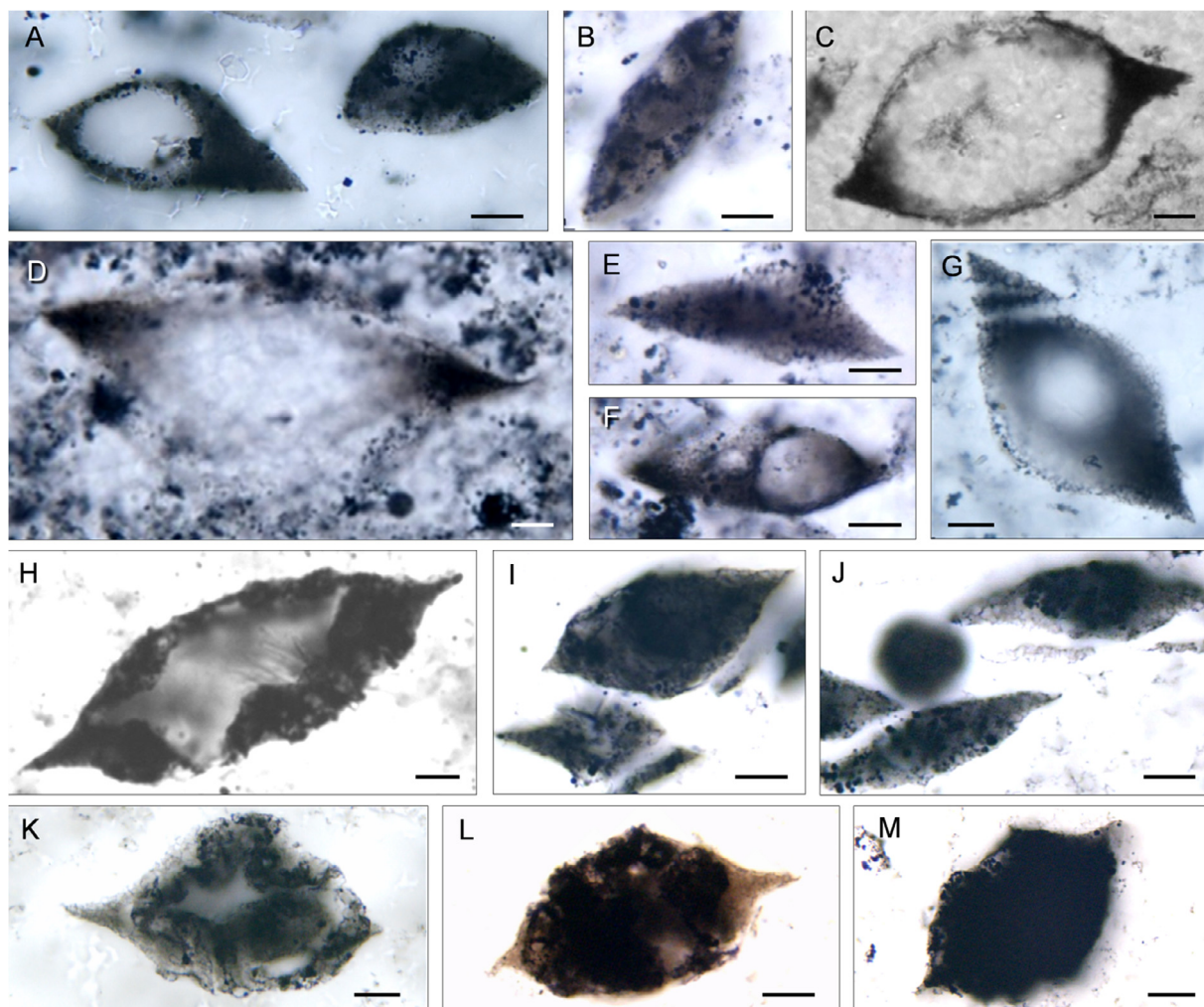
Fig. 1. Lenticular forms; transmitted light photomicrographs of polished thin sections. Kromberg Formation, South Africa (A–G); Strelley Pool Formation, Waterfall locality WFLX2-1c, Australia; (H–J); Farrel Quartzite, Australia (K-M). Scale bars, 10 μm.

The locations of samples from the Pilbara craton are shown in Fig. 3. FQ examples were from the Mt. Grant locality of Sugitani et al. (2007) and the SPF examples were from Waterfall locality WFLX2-1c, Section 2, shown in Sugitani et al. (2015b).

2.2. Methods

Microstructures of interest were located in polished thin sections with optical microscopy. Those near the surface of the section were selected and mapped for SIMS analysis. $\delta^{13}C_{PDB}$ data were acquired in 2013 on KF samples, using the University of California at Los Angeles (UCLA) Cameca 1270 Secondary Ion Mass Spectrometer (SIMS) with a multicollector configuration and $^{12}C_2^-$ detected by an off-axis electron multiplier (EM), $^{12}C^{13}C^-$ measured using the axial EM, and a ~15 μm, 0.01–0.5 nA Cs^+ primary ion beam (House et al., 2013).

During a similar session on the same instrument in 2015, we observed that morphologically similar fossils appeared ~7‰ heavier than those observed in 2013, and that an identical Barberton fossil appeared 10‰ heavier than its corresponding 2013 measurement. We now suspect that incomplete sample charge compensation was the primary issue with the 2015 session, and those data have not been included here for publication. The session, though, illustrated the risk that there could be small quasi-simultaneous arrival (QSA) effects (Slodzian et al., 2004) on carbon-rich

lenticular fossils not observed when analyzing the less carbon-rich natural chert PPRG#215-1 standard.

In part due to concern that the EM-EM configuration used in 2013 might be susceptible to QSA effects, in February of 2016, additional $\delta^{13}C$ compositions were determined with the new UCLA Cameca 1290 SIMS using a multicollector configuration with $^{12}C_2^-$ detected by an off-axis Faraday Cup (FC) and $^{12}C^{13}C^-$ measured using the axial EM. This arrangement is not susceptible to QSA effects. This third SIMS session was used to generate a confident dataset that includes previously analyzed FQ lenticular fossils and spheroids, Barberton lenticular fossils, and SPF lenticular fossils.

Under some SIMS conditions, there appears to be a matrix effect that is correlated with H/C (Sangély et al., 2005; Williford et al., 2015). We do not think that our results were affected by this type of matrix effect, as the H/C of the ancient materials analyzed were similar to that of the PPRG #215-1 standard. Additionally, past work under similar conditions has not suggested a matrix effect correlated with H/C (House et al., 2000), and our current work shows that our three different standards (which had quite different H/C ratios) had similar observed instrumental mass fractionations.

For all SIMS sessions (2013, 2015, and 2016), the molecular ions were used because they produce stronger count rates than rates of atomic ions and the mass difference between $^{12}C^{12}C$ and $^{12}C^{13}C$ permits an on-axis detector to be used, which allows for verification of the targets by ion imaging on the channel plate prior to isotopic
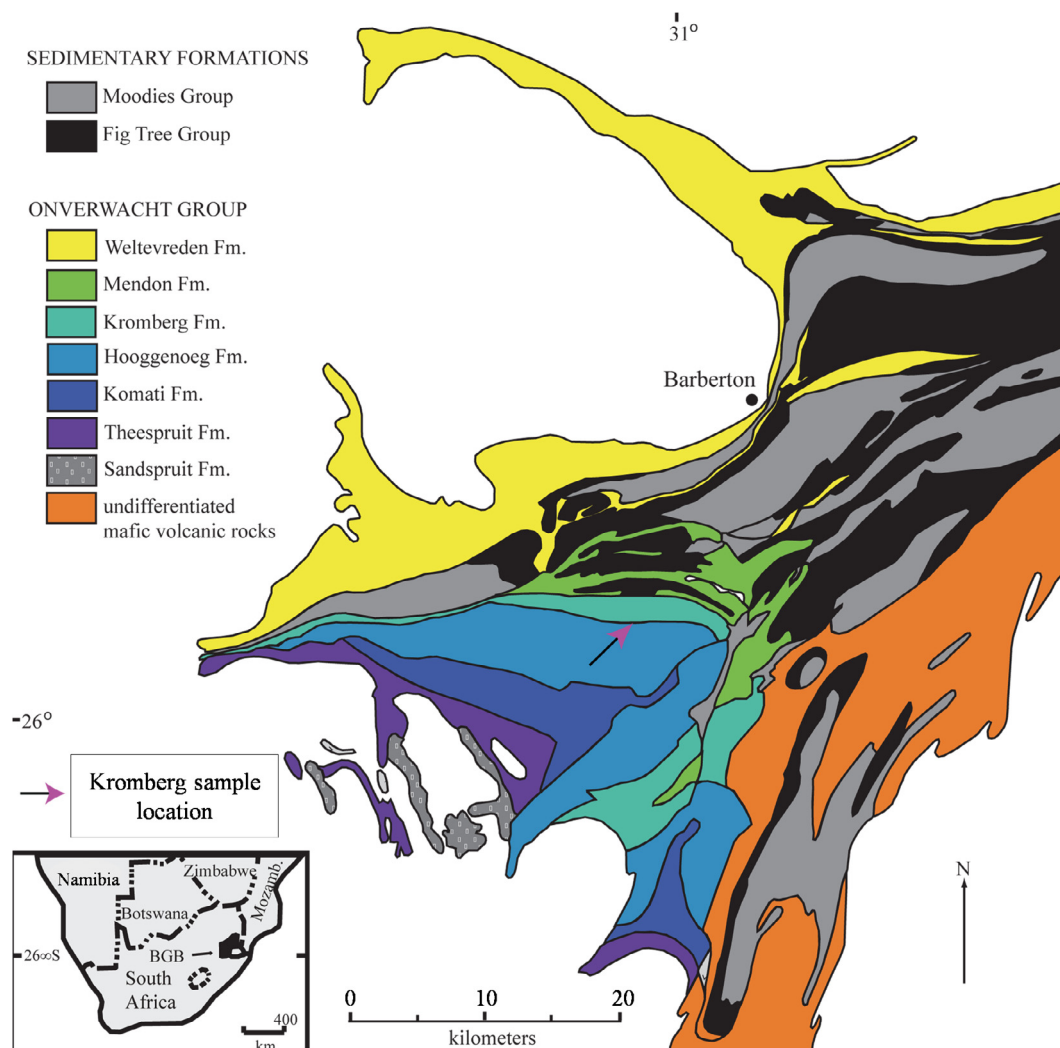
Fig. 2. Map showing Kromberg Formation sample location, Barberton Greenstone Belt (BGB), eastern Kaapvaal craton, South Africa (modified from Lowe et al., 2012).

analysis. Charge compensation was achieved using a normal incident electron gun and a gold coat. The SIMS analyses were calibrated against repeated spot analyses of PPRG#215-1 Precambrian chert following the method previously used for Bitter Springs and Gunflint microfossils (House et al., 2000). The instrumental mass fractionation was found to be similar to past experience with these SIMS conditions and the results from 2016 were very similar to those of 2013 and similar to the results published for FQ (House et al., 2013). For the 2016 session, we also added two additional synthetic carbon standards (C905 and PEEKGF30; House, 2015). These were found to be quite homogeneous giving a good demonstration of spot-to-spot reproducibility, but we also found them to show a small (1-3‰) matrix effect when compared with the natural chert PPRG#215-1 (Supplementary Table S1). After SIMS analysis, the locations of SIMS spots were checked by optical microscopy (Supplementary Figs. S1 and S2). SIMS spots that were discovered to be above the fossils or located primarily off the structure of interest were omitted from the results.

3. Results

3.1. Lenticular forms

The lenticular structures (Fig. 1) are all carbonaceous and preserved in chert. While they were described initially as "spindle-shaped", the broader term, "lenticular", is now preferred and used here. The structures are relatively large (up to 150 µm long and 65 µm wide). They appear to be robust (with little evidence of folding or wrinkling) and to have thick walls comprised of dense accumulations of reticulate and globular organic matter. The Australian forms are 20–70 µm long and 15–35 µm wide, with central portions that are either hollow or have alveolar (spongy) texture (Sugitani et al., 2007, 2010, 2013). Some specimens include an equatorial flange that appears in two-dimensions as 2–30 µm long, tail-like appendages (Sugitani et al., 2009a, b), that extend from the tapered ends. Other specimens are non-flanged. These lenticular structures occur singly, in pairs, chains, or clusters of up to 20 individuals. The KF lenticular structures from South Africa are similar, though with larger size ranges (~10–150 µm long and 5–65 µm wide). Many contain the tapered ends seen in the Australian forms and some contain cavity-like internal structures, ~10–100 µm long by 5–60 µm wide; others have no obvious cavities, while some specimens contain two such features.

3.2. Geologic settings

Each of the deposits in which these forms occur represents a shallow-water setting and is commonly associated with volcanics, evaporites, detrital constituents, and evidence for early silicification and low temperature, hydrothermal input. All examples of the lenticular forms occur within non-stromatolitic layers in the cherts, even though stromatolitic horizons and layers are known.
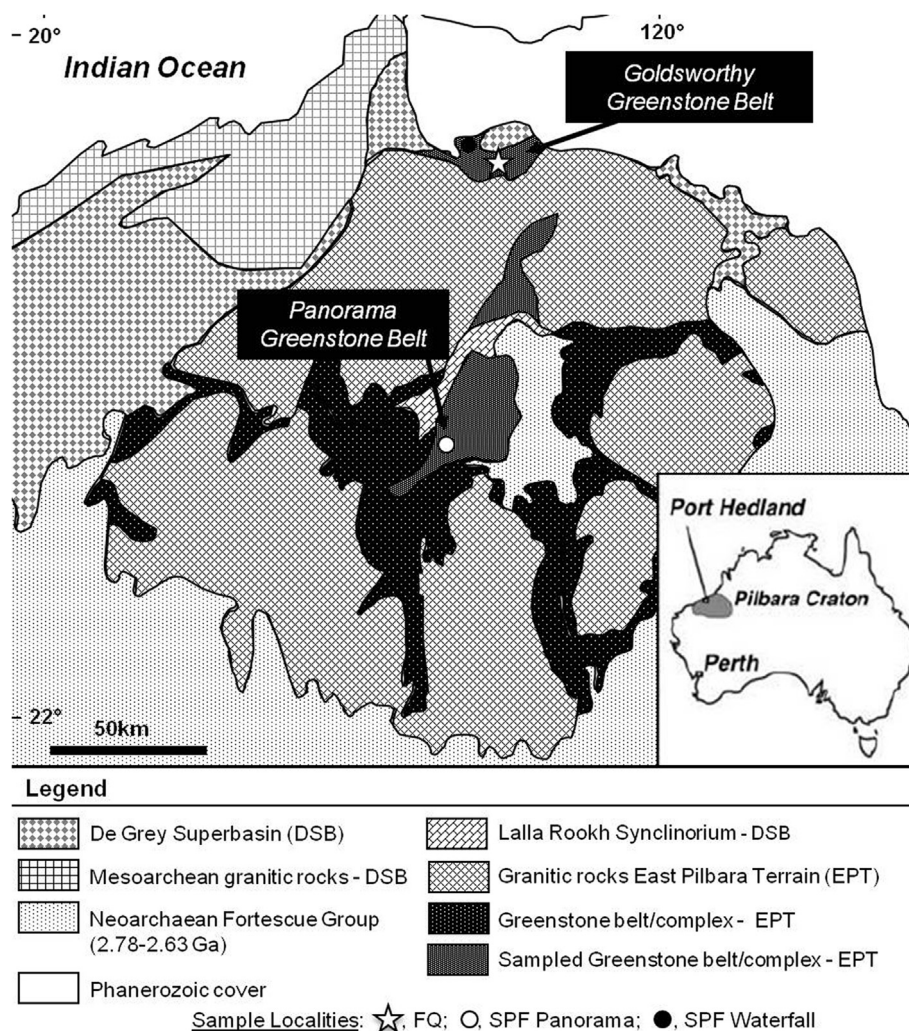
Fig. 3. Map showing locations of the Farrel Quartzite (FQ) and Strelley Pool Formation (SPF) in the Pilbara craton of Australia (after Hickman, 2008).

For example, in the SPF, stromatolitic horizons have been described by Allwood et al. (2006), but those contain no spindle-like forms and they are stratigraphically separated from the horizons with non-stromatolitic cherts containing the lenticular forms (Sugitani et al., 2010).

The KF is part of the 3.55 to 3.30 Ga Onverwacht Group in the Barberton Greenstone Belt (BGB) of the eastern Kaapvaal craton (Fig. 2). Recent study suggests that silica enrichment in the BGB is attributable to low temperature hydrothermal activity on broad areas of an ancient oceanic, plateau-like setting (Hofmann and Harris, 2008). The KF is a sequence of volcanics and volcaniclastics, with its lowest member comprised of banded, black and white cherts and sandstones, detrital layers and a silicified evaporite (Lowe and Worrell, 1998). The lenticular forms occur within detrital layers of the cherts, near the evaporite horizon, in a unit interpreted as a shallow-water deposit that formed adjacent to an alluvial fan (Walsh, 1992).

Among the Australian formations (Fig. 3), the FQ is a succession of sandstones with lithic fragments, chert breccias, and two black chert/evaporite horizons (Sugitani et al., 2007). The lenticular structures occur in black cherts, in facies interpreted as a continental margin deposit with detrital input. The SPF occurs in the Waterfall and Panorama localities, separated by nearly 100 km. The Panorama locality (Sugitani et al., 2013) comprises lower cherty members and upper sandstones/tuffs; lenticular forms are in a black chert interpreted as part of a shallow-water, peritidal to inner

platform deposit. The Waterfall locality comprises a sequence of sandstones interbedded with cherty units, two of which are silicified evaporites (Sugitani et al., 2010, 2015b). Lenticular structures are abundant in a massive, light grey to black chert in the uppermost cherty unit. This locality is interpreted as part of a shallow-water coastal basin, temporarily connected to the open ocean, but intermittently evaporitic, with input from seawater, rivers and low-temperature hydrothermal fluids (Sugitani et al., 2015b).

3.3. SIMS data

SIMS-derived $\delta^{13}C_{PDB}$ data (Fig. 4; Table 1) show that the carbon isotope values of KF fossils (-39.3 to -35.5‰, weighted mean of -37.3 ± 0.4‰) are nearly identical to those from the Australian FQ lenticular fossils, including those measured in 2013 on the UCLA Cameca 1270 SIMS (-35.6 to -40.5‰, weighted mean of -37.0 ± 0.4‰; House et al., 2013) and those reanalyzed in 2016 with the UCLA 1290 SIMS (-37.7 to -36.4‰, weighted mean of -37.0 ± 0.5‰). Two examples of "dense masses" or organic matter in the KF also were analyzed (Supplementary Fig. S1E–G). These masses resemble the lenticular forms in overall shape, dense carbonaceous composition, and $\delta^{13}C$ values (-37.7‰ and -35.3‰), though their sizes are at the upper end of the size range of the lenticular structures. The origin of these masses is still under investigation, but one possibility is that they may be less well preserved remnants of lenticular forms.
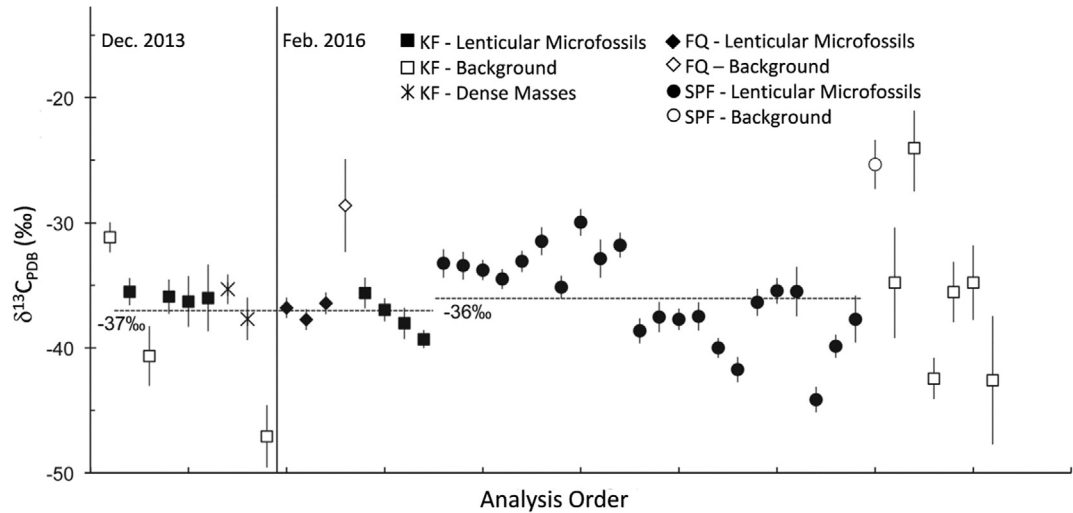
Fig. 4. SIMS $\delta^{13}C_{PDB}$ values. KF, Kromberg Formation; FQ, Farrel Quartzite; SPF, Strelley Pool Formation, Waterfall locality WFLX2-1c. Dotted lines, weighted means for sets of lenticular forms ($\sim$ -37‰ for KF and FQ; $\sim$ -36‰ for SPF). Error bars, ±1σ.

Table 1

Summary of SIMS $\delta^{13}C_{PDB}$ values. KF, Kromberg Fm.; FQ, Farrel Quartzite; SPF, Strelley Pool Fm.; SD, standard deviation; MSWD, mean standard weighted distribution. The weighted means and weighted SD are the means and SD of the values for each group of SIMS measurements weighted by the uncertainty of each analysis. MSWD is the reduced chi-squared statistic used here to test the goodness of fit between the uncertainties of each analysis and the observed variance of the analyses in each group. See Supplementary Table S1 for details.

| Formation (analysis years)	Structure	# of analyses/ # of structures	Range of $\delta^{13}C$ values (‰)	Weighted mean $\delta^{13}C$ values (‰)	MSWD	Weighted SD (‰)
KF (2013, 2016)	Lenticular forms	8/5	-39.3 to -35.5	-37.3 ± 0.4	2.0	1.6
	Dense masses	2/2	-37.7; -35.3			
	Background	9/9	-47.1 to -24.0	-36.4 ± 0.7	8.6	6.6
FQ (2016)	Lenticular forms	3/1	-37.7 to -36.4	-37.0 ± 0.5	0.6	0.6
	Background	1	-28.6			
SPF WFLX2-1c (2016)	Lenticular forms	22/22	-44.1 to -30.0	-36.1 ± 0.2	12.4	3.0
	Background	1	-25.3			
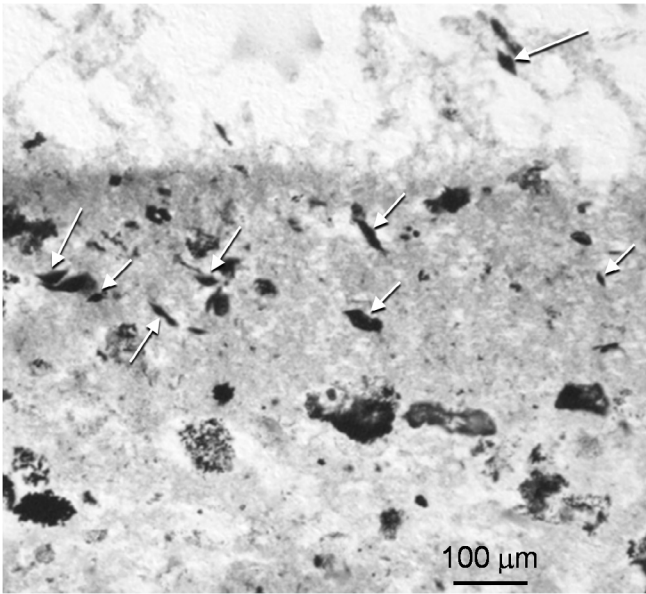
Fig. 5. Spatial distribution of lenticular fossils (arrows) in Waterfall locality WFLX2-1c of the Strelley Pool Formation. Optical photomicrograph in transmitted light.

The KF data are also similar to our analyses of SPF fossils from Waterfall locality WFLX2-1c (-44.1 to -30.0‰, weighted mean of -36.1 ± 0.2‰) and to data reported by Lepot et al. (2013) from locality WF4 of the SPF (-39.6 to -28.5‰, average of -32‰).

$\delta^{13}C$ values of background in all deposits show weighted means somewhat heavier than the weighted means of the lenticular forms, and this is consistent with results of the earlier, detailed study of FQ lenticular forms and background (House et al., 2013). Background analyses are considered to have measured OM, as petrographic examination after SIMS showed that areas selected for analysis contain diffuse and particulate material that resembles organic matter in color and texture but lack any evidence of carbonates (see Supplementary Figs. S1 and S2). Only a single analysis was obtained for FQ background in 2016, as multiple background analyses of the same FQ sample were obtained in 2013 (House et al., 2013), and the objective in 2016 was to simply re-measure FQ material to ascertain that values obtained with the newer UCLA Cameca 1290 SIMS were comparable to values obtained in 2013 with the older Cameca 1270 (Section 2.2 Methods). While the KF background values vary greatly, much of this variance is attributable to the limited counts that were obtained on those samples.

4. Discussion

The isotopic similarities among the KF, SPF and FQ specimens are striking. The weighted mean $\delta^{13}C$ values for the lenticular structures from all three deposits, disparate in both space and time, are identical within stated uncertainties (-36.1 to -37.3‰; Table 1). These $\delta^{13}C$ values additionally are distinctive in that they are more negative (enriched in ^{12}C) than many examples of similarly aged, Archean organic matter. For example, nine different formations reported by Schopf (2006) from South Africa and Australia,

ranging in age from ~3.2 to 3.5 Ga, show mean bulk $\delta^{13}C$ values from -27 to -32‰. These included samples from both hydrothermal and shallow marine settings, most having putative microfossils and many from stromatolites or microbial mats. Similarly, the 3.24 Ga black smoker Sulphur Springs deposit from Western Australia was reported to have a mean bulk $\delta^{13}C$ value of -30.7‰, with a range from -26.8 to -34.0‰ (Duck et al., 2007). And a recent study of SIMS-derived $\delta^{13}C$ values of a variety of individual organic structures (clots, veins, stylolites, layers and laminae) in the ~3.5 Ga Dresser Formation, showed average values that ranged from -25.7 to -33.6‰ (Morag et al., 2016).

The Morag study also summarized data from eight other reports of bulk and SIMS-derived $\delta^{13}C$ values of early Archean samples from the Pilbara. The range of all values was between ~ -35 and -45‰. However, Morag et al. pointed out that two of the studies with the most negative isotopic values (Ueno et al., 2001 of the Dresser Formation; Wacey et al., 2011 of the SPF) used a graphite calibration standard, which differs from the kerogen in ancient chert samples and can create an instrumental mass bias, leading to a systematic shift of ~ -7‰ in reported $\delta^{13}C$ values. Thus, the values reported in those two studies might have been less negative by ~7‰, had they been calibrated to an ancient kerogen standard, such as the natural carbonaceous chert (Archean sample PPRG-215) used as the calibration standard for the SIMS analyses reported here, as well as those reported in the earlier FQ study of House et al. (2013), the SPF study of Lepot et al. (2013), and the Dresser Formation study of Morag et al. (2016). These observations support the conclusion that the Archean lenticular structures are more enriched in ^{12}C (isotopically lighter) than many examples of similarly aged organic matter.

The $\delta^{13}C$ values for the lenticular structures are unlikely to be explained by an abiotic, Fischer-Tropsch type synthesis (FTT). Van Zuilen et al. (2007) assessed carbonaceous material in cherts of the Barberton Greenstone Belt, including samples from the KF, based on geologic relationships, Laser Raman spectroscopy, SIMS, and petrography. They observed that although hydrothermal alteration of mafic/ultramafic basalts had occurred in the Barberton, there was no evidence that the organic material in the KF cherts formed from FTT reactions, as those carbonaceous cherts were neither underlain by swarms of organic-rich feeder dikes, nor were they in direct contact with underlying hydrothermally altered ultramafics or preferentially developed above serpentinized rocks (which could have provided hydrogen for FTT reactions). These relationships argue that the carbonaceous material in cherts of the KF is unlikely to be abiotic. Van Zuilen et al., further stated that the organic matter in the KF samples had all the characteristics of metamorphosed biologic material. Similarly, Lepot et al. (2013) concluded that the SIMS-derived $\delta^{13}C$ values of organic matter in SPF lenticular structures most likely reflect a biological origin, as those values varied with texture in a way that seemed unlikely to be explained by a fully abiotic origin.

FQ lenticular forms have been independently interpreted as biogenic based on their SIMS-derived $\delta^{13}C$ values and the narrow range of those values (House et al., 2013). A great variety of additional morphological and chemical data from both the FQ and SPF supports the conclusion that the lenticular fossils in these Australian deposits are biogenic (Oehler et al., 2010; House et al., 2013; Lepot et al., 2013; Sugitani et al., 2010, 2013, 2015a). In comparison to the Australian lenticular forms, those from the KF have similarly light $\delta^{13}C$ values, an equally narrow range of $\delta^{13}C$ values, comparable morphology, and a similar occurrence in non-stromatolitic cherts. Accordingly, early interpretations of the KF lenticular forms as "possible microfossils" can now be updated to a conclusion that these early Archean structures from South Africa are also "*bona fide* microfossils".

The FQ lenticular forms investigated in both 2016 and 2013 have lighter carbon isotopic compositions than the analyzed samples of background organic matter. This relationship is unlikely to occur if the organisms represented by the lenticular fossils were either fermenting or respiring heterotrophs, as these types of metabolisms would result in cells isotopically heavier than consumed background organic matter (House et al., 2013 and references therein). In contrast, the isotopic relationship where the cells are isotopically lighter than background organic matter could be explained by an autotrophic metabolism in a setting where CO_2 levels were high (resulting in maximal fractionation between cells of the autotrophic organisms and their source CO_2). This conclusion would be consistent with a planktonic lifestyle for the lenticular organisms, where CO_2 would not be limiting (as it can be in be benthic habitats and microbial mats where CO_2 is uncirculated; Kaufman and Xiao, 2003). A planktonic interpretation, for at least some stages in the life cycle of such organisms, is also suggested by 1) the lenticular fossils' near-equant shapes and equatorial flanges (traits that could have been advantageous to floating) and, as seen in Fig. 5, 2) the dispersed spatial distribution of the lenticular forms and 3) the association of the lenticular fossils with amorphous organic matter that lacks the fine laminations common in remnants of microbial mats.

Finally, we note that the SPF structures analyzed here exhibit a wider range of isotopic values than the lenticular structures from the FQ or KF. This range (-30.0 to -44.1‰) is similar to that of FQ spheroidal structures (House et al., 2013) and SPF lenticular forms analyzed by Lepot et al. (2013). While possible explanations of these wide ranges could involve incorporation of products of a variety of metabolisms (e.g., methanogenic and methanotrophic) and varying degrees of heterotrophic processing or diagenesis, the significance of these ranges is still under investigation.

Until a few years ago, evidence of *bona fide* microfossils in Earth's Archean units included mainly simple organic coccoids, filaments, and rods (Schopf, 2006). Many of these are associated with stromatolites, the laminated sedimentary structures formed by interactions between microbial mats and the physical environment. The lenticular microfossils from South Africa and Australia are different from these simple Archean coccoids, filaments, and rods. The lenticular microfossils do not appear to represent mat-forming or stromatolitic organisms, and they exhibit a complexity of form (with their lenticular morphology, internal structures, and equatorial flanges) that is unique among known Archean microfossils (e.g., Altermann, 2001; Schopf, 2006). Their thick-walled, apparent robustness is distinctive as well. Most similarly preserved Archean and Proterozoic microfossils have thinner walls that are comprised of less dense carbonaceous matter and form more delicate-seeming structures, often with folds and sometimes sinuous shapes. A robust architecture of the lenticular forms is also supported by their occurrence along with detrital clasts, a relationship perhaps indicative of their resilience and possibly suggesting that the lenticular structures represent thickened coverings of spores or colonial forms (Walsh, 1992). And though large organic microfossils have been reported from the 3.2 Ga Moodies Group (Javaux et al., 2010), those are spheroidal and apparently delicate (as they are commonly folded and wrinkled), and they show no evidence of a lenticular morphology. Thus, the lenticular microfossils described here appear to be represent highly unusual forms not presently known in other Archean or Proterozoic deposits.

5. Conclusions

The combination of the distinctive $\delta^{13}C$ values and complex morphology of the lenticular forms from both Australia and South Africa sets them apart from other Archean and Proterozoic

microfossils. These characteristics suggest that the structures in the KF from South Africa, like those from the SPF and FQ from Australia, are *bona fide* Archean microfossils, and further, that the populations represented by the lenticular forms on both continents were biologically related.

These microorganisms were highly successful in the Archean world. They were abundant, widespread, and as a group, existed at least 400 million years, from ~3.4 Ga in the KF and SPF to ~3.0 Ga in the FQ. This success may have been due to a combination of their robust architecture (Walsh, 1992) and planktonic habit – characteristics that could have enhanced survival on a young planet experiencing high levels of UV radiation (Catling and Claire, 2005; Cockell and Raven, 2007) or sudden environmental changes (e.g., ocean surface heating, rapid seawater evaporation, and tsunamis) caused by major meteorite/asteroid impacts, such as are recorded in the Kaapvaal and Pilbara cratons (Byerly et al., 2002; Lowe et al., 2014; Lowe and Byerly, 2015). Their thickened walls could have provided a degree of protection to these types of stressors, as do the walls of microbial spores which provide protection from desiccation, toxic materials in the environment, and major temperature changes (Roszak and Colwell, 1987; Horneck et al., 2001; Hoekstra, 2002; Henriques and Moran, 2007; Sella et al., 2014). And a planktonic habit that resulted in widespread distribution could have aided survival in times when potentially lethal, local conditions developed.

In addition, there is some evidence that the continental nuclei of the Pilbara and the eastern Kaapvaal could have formed a single volcanic plateau as early as 3.5 Ga (Van Kranendonk et al., 2015). The geographic/stratigraphic distribution of the lenticular microfossils is consistent with this scenario, and it is possible that the organisms represented by the lenticular forms could have expanded across submerged areas of the platform – their dispersal facilitated by a planktonic lifestyle.

Finally, the carbon isotopic data suggest that the microorganisms represented by the lenticular forms were most likely autotrophs. This conclusion, along with results from the ~3.5 Ga Dresser Formation (Morag et al., 2016), supports the concept that autotrophic metabolism developed early on the young Earth.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.precamres.2017.04.031.

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