Evaluating Evidence of Ancient Animals

A. Seilacher *et al.* (*1*) studied the Chorhat Sandstone (Semri Group of Vindhyan Supergroup) and concluded that burrows in the form of meandering linear markings were made by peristaltic movements of some worm-like animals more than a billion years ago. But these markings are more likely pseudo–trace fossils or casts of some megascopic algae for several reasons: (i) the explanation by Seilacher *et al.* for the absence of any backfill (spreite) structure in the burrow tunnel is not supported by evidence in the report and is dubiously assumed to have collapsed; (ii) there is an absence of any algal-mat impression on the bedding surface; (iii) the rippled nature of the bedding indicates a consistent supply of silt and sand under agitated energy conditions and suggests a noncongenial environment for the delicate triploblastic animals (worm-like) to survive and to mine underneath the algal-mat; (iv) there is an absence of any faecal structures (coprolite), as is produced by extant worms. Moreover, the use of the term "triploblastic" is inappropriate because there is a lack of conclusive evidence to support the triploblastic nature of their purported animals.

An earlier study (*2*), which reported these trace-fossil like structures in these horizons, demonstrated that many of these putative trace-bearing rippled horizons represent terrestrial environment with sufficient aeolian influence, contrary to what Seilacher *et al.* (*1*) present in their study. Even the associated supratidal-aeolian setting (*2*) would not be suitable to sustain any delicate worm-like animal and algal-mat system because of high aridity.

Last, the Vindhyan succession has long been reported to contain similar trace fossil occurrences (*3*, and others) that include worm-burrows as well, but these studies were not mentioned by Seilacher *et al*. Our literature survey (*4*) revealed that the earliest record of Proterozoic mega-fossils were noticed from the same Vindhyan Supergroup in 1823. Almost 50 records of different fossil findings have been published since then, including many of the exceptionally well preserved megafossils. If at all these purported trace-fossils are to be attributed to a biologically formed structure, they might best be considered to represent the cast of some large megascopic filamentous algae. One such form with a close dimensional range (*Grypania* sp.) has been recorded by us (*4*) from the beds a little higher up in the succession (Rohtasgarh Limestone formation) within the Semri Group itself. Recently, similar organic structures with curved to curvilinear shape have been considered to be trace fossils made by some benthic eumeta zoan organisms from the 1400-Ma Gaoyuzhuang Formation of Jixian, China (*5*), which probably represents a cluster of *Grypania* specimens.

Vibhuti Rai
Rajita Gautam
Department of Geology,
University of Lucknow,
Uttar Pradesh 226007, India
E-mail: vibhutirai@hotmail.com

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Response: We agree with our colleagues Rai and Gautam that pseudofossils are a major problem in Precambrian paleontology. Many previously published "trace fossils" from the Vindhyans fall in this category. Therefore, and because of space limitations, we did not mention or discuss some of the earlier studies in our report (*1*). Several supposed trace fossils (*2*) are now considered not interpretable because of inadequate preservation or documentation (*3*). In other cases, careful inspection strongly suggests a physical origin of the structures. Particularly common (*4*) are evenly curved or sinuous mudcrack fillings. Such structures ("Manchuriophycus") commonly develop in ripple troughs, and have been reported from sediments of all ages (*5*). Depending on the nature of the crack infill, the structure may be preserved in positive as well as negative epirelief, and often has a striking resemblance to sinusoidal or branched, or even paired, trails (*6*). Sometimes, the crack infills develop transverse corrugations (*7*), that make them even more resemble annelid trails ("Rhysonetron," *8*). Supposed traces reported from the Upper Vindhyans (*9*) are significantly better preserved.

We did mention the occurrence of segmented impressions in the Koldaha Shale (*10*), which might well be segmented algae (*11*). However, the structures described in our report (*1*) do not fall into the morphospace and taphofacies of macroalgae such as *Grypania*, or of shrinkage cracks, or of any other known physical structures. To answer particular points raised in the comment: (i) it is correct that terminal backfill (not spreite) would strengthen the burrow interpretation. But undermat miners (*12*) produce mostly open galleries that later collapsed. (ii) In sandstones, biomats can only be inferred from diagnostic sedimentary structures on rippled surfaces, but not on flat, unsculptured ones (*13*). (iii) Palimpsest ripples in the Chorhat Sandstone reflect rare storm events. They became fixed by microbial mats during the long fair-weather periods, during which turbulence was too low to move the sand. (iv) Fecal pellets cannot be preserved in sandstones, whose grain size is similar to that of such pellets.

During our joint fieldwork we could not find convincing evidence of aeolian deposition. All sedimentary structures speak for environments close to storm wave base.

We think that the most critical issue now is not the nature, but rather the age, of the Chorhat burrows. Therefore, we look forward to new radiometric ages from ash beds, currently being determined by other groups.

A. Seilacher
P. K. Bose
F. Pflüger*
Department of Geology and Geophysics,
Yale University,
New Haven CT 06520, USA
E-mail: friedrich.pflueger@schwaben.de

*Present address: Kantstrasse 34, D - 72762 Reutlingen, Germany

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Role of Ceruloplasmin in Cellular Iron Uptake: Addendum

We recently reported that ceruloplasmin (Cp) stimulates iron uptake by HepG2 cells by a transferrin-independent mechanism (*1*). We are grateful to Ian Trowbridge, at the Salk Institute, for a comment about our use of the anti-transferrin receptor monoclonal antibody H68.4 in this study. He correctly points out that H68.4 (originally produced in his laboratory) binds to an epitope of the transferrin receptor cytoplasmic tail inaccessible to antibody in intact cells, and is thus not the preferred reagent for determining the role of the receptor in iron uptake by intact cells. We further thank Trowbridge for his generous gift of anti-transferrin receptor monoclonal antibody 42/6, which blocks transferrin binding to its receptor (*2*), and transferrin-mediated iron uptake in intact cells (*3*). We have repeated our original studies and have obtained the same results with the 42/6 antibody as before, namely, that 42/6 does not inhibit Cp-mediated ^{55}Fe-nitrilotriacetate uptake by HepG2 cells. In a positive control experiment, 42/6 antibody effectively blocks $^{55}Fe_2$-transferrin uptake. In view of Trowbridge's comment, we have reexamined our studies with the original antibody. H68.4, in fact, does block $^{55}Fe_2$-transferrin uptake in HepG2 and K562 cells, but only under conditions of iron deficiency, the condition used in our studies. The mechanism underlying this observation is not clear.

In complementary studies, we have shown that Cp-stimulated iron uptake occurs in K562 cells, which do not secrete transferrin, and in the presence of endocytosis inhibitors, which block transferrin uptake (*1, 4*). Furthermore, our recent results indicate that Cp stimulates iron uptake through a trivalent cation transporter, a mechanism completely distinct from that used by transferrin (*4*). Together, these studies provide compelling evidence that Cp-stimulated iron uptake is transferrin- and transferrin receptor–independent.

Paul L. Fox
Chinmay K. Mukhopadhyay
Zouhair K. Attieh
Department of Cell Biology,
Lerner Research Institute,
Cleveland Clinic Foundation,
Cleveland, OH 44195, USA
E-mail: foxp@ccf.org

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