

nevertheless seems notable that the Chuar microorganisms are comparable in their limited degree of complexity to known stromatolitic microfossils of a similar age (3, 8). This apparently primitive level of organization and, in particular, the absence from the microflora of features characteristic of even moderately advanced microscopic thallophytes (such as true branching and polarity of organization) suggest that the Chuar organisms could predate, perhaps substantially, the emergence of megascopic, multicellular life.

2) The occurrence in the Chuar pisolite and in cherty oolite from Greenland of filaments essentially indistinguishable from *Eomycetopsis* of the Bitter Springs cherts, and the fact that comparable forms are apparently unknown in the Phanerozoic, suggest the possible use of this thallophyte as a late Precambrian "index fossil." In a broader context, it now seems possible that such fossils as *Eomycetopsis* and *Chuarina* may eventually provide a reliable basis for biostratigraphic zonation of the late Precambrian.

3) Despite its abundance in the overlying and underlying Chuar shales, *Chuarina* is absent from the pisolite bed; the converse is true of the *Eomycetopsis*-like filaments which, although prevalent in the cherty pisolite, have not been detected in the associated shales (10). These observations indicate that the distribution of at least some Precambrian fossils is probably facies dependent. Recognition of such dependence has obvious bearing on evolutionary and biostratigraphic interpretations and will become increasingly important as additional paleobiologic data become available from the Precambrian.

4) Although the mode of origin of pisolitic sediments has been a subject of considerable controversy (7), the evidence here presented indicates that some ancient pisolites are demonstrably biogenic. Cherty pisolitic units appear to represent a promising, largely untapped source of evidence regarding the diversity and evolution of Precambrian life.

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29 December 1972

## Fossil Lepidopterous Leaf Mines Demonstrate the Age of Some Insect-Plant Relationships

**Abstract.** *The discovery of impressions of 11 kinds of lepidopterous leaf mines, together with a reevaluation of previous reports, contributes significantly to the fossil record for that insect order. It is also concluded that some specific insect-plant associations have persisted from the Miocene to the present.*

The discovery of fossil leaf mines of 11 species of Lepidoptera from mid-Tertiary deposits in western North America significantly increases the minimum age of the families involved and demonstrates the persistence of some specific insect-plant relationships. Furthermore, three previous reports of fossil mines (1-3) appear to represent incorrect interpretations.

Most primitive families of Lepidoptera consist entirely of leaf-mining species (4). The ability to detect such leaf mines and to identify the insects responsible for each characteristic mine form provides the potential to further our knowledge of the early history of

the order (5). The only evidence indicating the presence of Lepidoptera before the Tertiary period is provided by the head capsule of an advanced ditrysian larva discovered in Canadian amber of the Late Cretaceous (6). Various reports of fossil Lepidoptera from the Tertiary indicate that most modern families had evolved at least by the Oligocene (7).

One of the "healed wounds" on a fossil leaf of *Proteoides wilcoxensis* Berry (? Proteaceae) from the Wilcox deposits of Tennessee (lower Eocene) (1) is a clear impression of a leaf mine made by a member of the Nepticulidae, whereas the previous report (2) of a

Table 1. Additions to the fossil record for leaf-mining Lepidoptera.

Fossil locality	Time	Host	Miners
Temblor Range, California	Middle Miocene	cf. <i>Quercus virginiana</i>	<i>Nepticula</i>
Upper Goldyke, Cedar Mountains, Nevada	Late middle Miocene	<i>Quercus hanibalii</i> Dorf	<i>Nepticula</i> <i>Lithocolletis</i>
Thorn Creek, Idaho	Middle to upper Miocene	<i>Lithocarpus</i> <i>Quercus simulata</i> Knowlt.	<i>Nepticula</i> <i>Eriocraniella</i> <i>Nepticula</i> <i>Cameraria</i>
Buffalo Canyon, Nevada	Upper Miocene	<i>Quercus hanibalii</i>	<i>Nepticula</i> <i>Bucculatrix</i>
Stewart Valley, Nevada	Upper Miocene	<i>Populus trichocarpa</i> var. <i>ingrata</i> (Jeps.) Parish	<i>Lithocolletis</i>
Stinking Water, Columbia Plateau, Washington	Upper Miocene	<i>Quercus pseudolyrata</i> (Lesq.)	? <i>Nepticula</i>
Trout Creek, Oregon	Upper Miocene	<i>Quercus consimilis</i> Newb.	? <i>Acrocercops</i>
Aldritch Station, Nevada	Mio-Pliocene	<i>Quercus hanibalii</i>	<i>Nepticula</i> ? <i>Evippe</i>

fossil nepticulid mine from brown coal deposits in Germany (upper Oligocene, Chattian Stage) is not diagnostic and may represent the mine of a dipteran. Thus, the earliest record for a lepidopteran of known familial lineage is represented by the nepticulid mine from the Eocene Wilcox deposits.

The first report of a fossil mine from western North America represents the genus *Lithocolletis* (Gracillariidae); the unidentified fossil leaf on which the mine occurs is from the White Lake basin of British Columbia (upper Eocene) (8). Subsequently, a fossil mine

of *Caloptilia* (Gracillariidae) from the Latah Formation of Idaho (Miocene) was illustrated; however, the author attributed the mine to feeding by a nepticulid (3).

An examination of fossil leaf material from mid-Tertiary deposits of western North America in the collections of the University of California at Berkeley and Davis resulted in the discovery of traces or impressions of 21 mines representing 11 species (7 genera) of leaf-mining Lepidoptera (Eriocraniidae, Nepticulidae, Lyonetiidae, and Gracillariidae) (Table 1 and

Fig. 1). The impressions of a cocoon (Lyonetiidae) and an apparent feeding shelter (? *Evippe*: Gelechiidae) were also found. The first appearance in the fossil record for Eriocraniidae is represented by these findings.

The leaves on which these mines occurred have been studied by paleobotanists, most notably D. I. Axelrod, R. W. Chaney, and H. D. McGinitie, and several are considered by them to represent equivalents of species extant in the flora of western North America. With one exception, all the fossils are members of the Fagaceae. The exception is a fossilized leaf of *Populus trichocarpa* var. *ingrata* (Jeps.) Parish, on which the traces of four *Lithocolletis* mines occur.

An essentially complete familiarity with the extant leaf-mining fauna of Californian *Quercus* permits some significant correlations with the evidence provided by the fossil mines on leaves of Fagaceae equivalent to living species. A fossil *Nepticula* mine on a leaf of *Quercus wislizenoides* Axelrod, an equivalent of extant *Q. wislizenii* A. DC., is indistinguishable from mines made by living *Nepticula variella* Braun (9).

The fossil record for *Quercus hanibalii* Dorf, the fossil equivalent of *Quercus chrysolepis* Liebm., is lengthier and more extensive than that of any other western oak. Fossil impressions of this equivalent were found to bear several mines representing one species each of *Nepticula*, *Bucculatrix*, and *Lithocolletis* (Table 1). These mines are indistinguishable from those formed by monophagous species which now feed on *Q. chrysolepis* (10). Other evidence shows that a species of *Evippe* also fed on *Q. hanibalii*. This is the first indication of the persistence of specific insect-plant relationships through geologic time, and suggests that at least the community of Lepidoptera which feed on this host has had a coevolutionary relationship which began as early as the middle Miocene (11).

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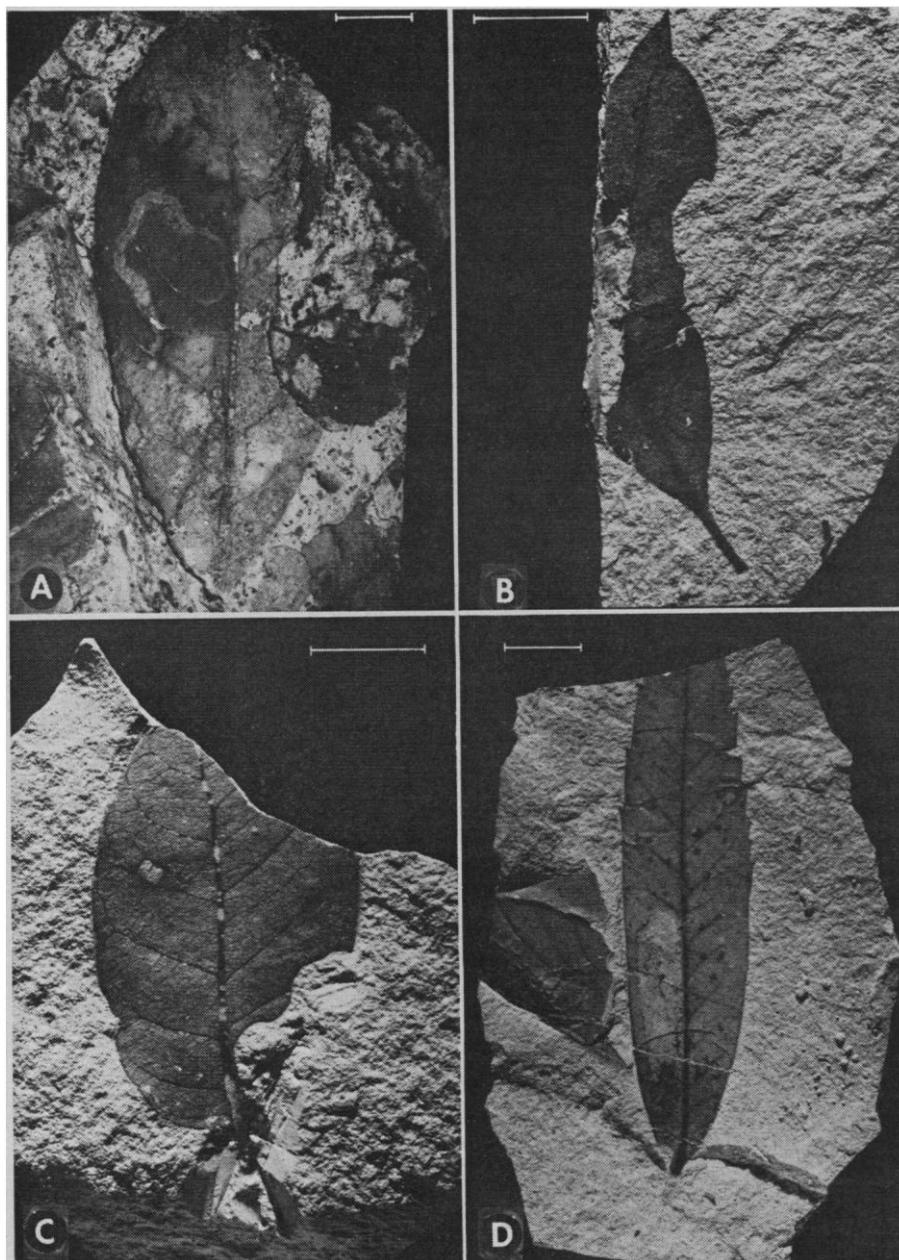


Fig. 1. Lepidopterous leaf mines on fossil leaves (scale bars, 1 cm). (A) *Nepticula* on cf. *Quercus virginiana*, serpentine mine on center left; (B) *Eriocraniella* on *Q. simulata*, inception of mine at lower right, terminal blotch missing (center right); (C) *Bucculatrix* on *Q. hanibalii*, mine on center left; (D) *Lithocolletis* on *Q. simulata*, lower left adjacent to mid-rib. [Photographs by A. Blaker, Scientific Photography Laboratory, University of California, Berkeley]

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  11. Potassium/argon (K/A) dating gives an age of  $18 \times 10^6$  years for Buffalo Canyon and  $16 \times 10^6$  years for Upper Goldyke (D. I. Axelrod, personal communication).
  12. Supported by NSF grants GB 4014 and GB 6813X (principal investigator, J. A. Powell). Examination of fossil leaf material in the collections of the University of California was made possible through the courtesy of D. I. Axelrod and H. E. Schorn. The manuscript was reviewed by J. T. Doyen, J. A. Powell, E. I. Schlinger, and H. E. Schorn.
- 10 October 1972

## Bacterial Origin of Sulfuric Acid in Geothermal Habitats

**Abstract.** *Natural populations of Sulfolobus, a new genus of bacteria occurring in sulfur-rich, acid hot springs and soils, were found to oxidize large amounts of sulfur to sulfuric acid at temperatures up to 85°C. These bacteria are important high-temperature geochemical agents in solfataras soils.*

Solfataras are regions in geothermally active areas characterized by hot acid soils and occasional hot springs that discharge limited amounts of acid water. Solfataras soils are heated by steam rising to the ground surface and contain large amounts of elemental sulfur that arises from the spontaneous oxidation of  $H_2S$  present in the steam (1). The high acidity of solfataras is due to the large quantities of sulfuric acid produced from the biological oxidation of the elemental sulfur (1). Bacteria of the *Thiobacillus thiooxidans* type have been thought to be responsible for this acid production because they could be isolated from solfataras soils, but since they occur only at sites with temperature less than 50°C, movement of acid water from low-temperature sites had to be postulated in order to explain the presence of acid at higher-temperature sites (2). Recently, a new genus of sulfur-oxidizing bacteria, designated *Sulfolobus*, has been isolated from sites with temperatures of 65° to 90°C in solfataras areas (3), and studies with  $^{14}CO_2$  have shown that *Sulfolobus*

is metabolically active in soils up to 85°C (4). The presence of these bacteria in sulfur-rich habitats suggested that they may play an important role in sulfuric acid production. Using  $^{35}S$ -labeled elemental sulfur ( $S^0$ ), we have measured directly the oxidation of  $S^0$  in sulfur-rich hot springs, and the results indicate that *Sulfolobus* does oxidize large quantities of  $S^0$  to sulfuric acid.

Twelve hot, acid, sulfur-containing pools in Yellowstone National Park, Wyoming, were studied, but data are given here for only two, Sulfur Caldron and Moose Pool, both located in the Mud Volcano area of the park (5). Some properties of the two pools are given in Table 1. Large numbers of *Sulfolobus* were present in most of the pools, but other kinds of bacteria could not be detected microscopically. *Sulfolobus* cultures were sought and obtained from seven of the pools. Oxidation of sulfur was measured by using the  $^{35}S^0$  technique described for laboratory cultures of *Sulfolobus* (6). Samples were transported in thermally insulated containers to the field labora-

tory where they were distributed into bottles containing  $^{35}S^0$  and incubated at several temperatures (7). Portions were removed periodically and filtered through 0.45- $\mu m$  filters, and the  $^{35}SO_4^{2-}$  in the filtrates was determined by liquid scintillation counting (8). The amount of  $S^0$  oxidized to  $SO_4^{2-}$  was calculated on the basis of the specific radioactivity of the  $^{35}S^0$  added and the amount of natural  $S^0$  present in the sample. It was assumed that the bacteria oxidized the natural  $S^0$  and added  $S^0$  at the same rates (9).

Elemental sulfur was readily oxidized by samples from most of the sources tested. In Moose Pool, the optimum temperature for oxidation was 80°C, but oxidation occurred at a substantial rate at 85°C also (Fig. 1). At 90°C, some oxidation occurred but eventually ceased, probably due to thermal injury or death of *Sulfolobus*. Moose Pool samples were incubated at 95°C in a separate experiment, and no oxidation occurred at this temperature. No oxidation occurred in bottles to which 4 percent formaldehyde had been added at the start of incubation (Fig. 1); this, together with the lack of oxidation at 95°C, indicated that spontaneous, or nonbiological, oxidation of  $S^0$  did not occur. The absence of a lag in oxidation at the beginning of incubation suggests that the bacteria were actively oxidizing  $S^0$  when the samples were taken from nature. The optimum temperature for  $S^0$  oxidation was reasonably close to measured environmental temperatures (Table 1). Similar results were obtained with samples from Sulfur Caldron, but in this case the optimum temperature for  $S^0$  oxidation, also 80°C, was about 13 degrees higher than the mean environmental temperature (Table 1). These results directly confirm that sulfur oxidation in solfataras is a biological process, a conclusion previously based on an analysis of  $^{34}S/^{32}S$  isotopic ratios (1).

Table 1. Properties of Moose Pool and Sulfur Caldron and rates of  $S^0$  oxidation. Data on sulfuric acid production (10) were used to compute  $S^0$  oxidation rates in the Yellowstone solfataras and the Japanese crater lakes.

Site	Temperature (°C)	pH	$S^0$ (mg/ml)	<i>Sulfolobus</i> (cell/ml)	Surface area (m <sup>2</sup> )	Volume (m <sup>3</sup> )	$S^0$ oxidation	
							g/m <sup>2</sup> /day	kg/day
Moose Pool	72-80.5	1.59	1.02	$2.5 \times 10^7$	300	300	67	20
Sulfur Caldron	65-67.5	1.53	1.09	$7.4 \times 10^7$	100	400	190	19
Solfataras areas								
Roaring Mountain					128,000		3.1	
Amphitheater Springs					72,000		5.0	
Norris Ranger Station					6,700		3.5	
Norris Junction					11,000		2.5	
Crater lakes								
Okunoyu	80				1,000		16	
Oyumuma	50				16,000		25	