(~80°) rim was seen collapsing into the pool while plumes of fine sediment were ejected by vigorous bubble discharges. Despite apparent concentrations of methane, neither feature had been colonized by mussels. Either the unstable substrata on the rims impeded settlement of mussels, or the features were so recent that colonization had not yet occurred.

Doming of the sea floor under pressure from upwardly migrating gas, followed by eruption, will excavate a pockmark with raised rims (*1, 2*). If hypersaline pore fluids are present, as they are in the vicinity of brine pool NR-1 (*10*), lateral seepage through the pockmark walls will fill the depression with brine. Sediments suspended at the bottom of the pool may continue to be excavated by bubble discharge after the initial eruption. Anoxic conditions may also inhibit microbial oxidation of methane and prevent the formation of authigenic carbonates, which are common at North Sea pockmarks (*5*) and Gulf of Mexico gas seeps (*10, 11*). Although these features may be relatively small, a careful examination of seismic records should reveal additional brine-filled pockmarks on the continental slope of the northern Gulf of Mexico. Their potential as habitats for chemosynthetic communities is evident.

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18. The site was discovered in May 1989 by scientists on board the U.S. Navy Submarine *NR-1*, which acquired video and emulsion photographs, side-scan sonar records, and 25-kHz subbottom profiles. It was revisited in September 1989 with the submersible Johnson *Sea-Link I*, which took additional photographs and collected samples of the brine, escaping gas, and over 1000 living mussels.
19. The mechanical arm of the Johnson *Sea Link I* was lowered about 1 m below the density interface. Brine was collected at 1 atm inside the dive chamber via a hose that ran from the end of the arm. Temperature was measured by a Sea Bird conductivity and temperature detector (CTD) held by the arm. Salinity was measured with a conductive salinometer after weight dilution to near seawater conductance. Dissolved oxygen was measured by Winkler titration. Gaseous hydrocarbons were determined by flame-ionized gas chromatography. Carbon isotope ratios are reported as per mil deviations from a PDB standard: $\delta^{13}C$ (per mil) = $10^3 \times (^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{PDB} - 1)$.
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21. Mussels were collected at discrete locations with a mechanical scoop and placed in a closed container. Water and sediment from the container were sieved through a 1-mm mesh, and all recovered specimens were measured with a calipers. Some of these specimens were maintained alive for up to 5 months with methane gas as their sole source of food.
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25. Near 27°26′30″N, 91°40′30″W at 662-m depth, and near 27°35′59″N, 92°55′58″W at 590-m depth.

26. We thank the captains and crews of the U.S. Navy Submarine *NR-1* and the Johnson *Sea-Link I* for enabling our submersible operations. R. A. Burke, Jr., supervised gaseous hydrocarbon analyses. Ship time was funded by Office of Naval Research and National Oceanic and Atmospheric Administration–National Undersea Research Program. Additional support was from the Texas A&M University and Louisiana State University Sea Grant Programs. We thank Exxon Corporation, U.S.A., and Ensearch Exploration, Inc., for support and suggesting dive sites for study.

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Upper Eocene Gilled Mushroom from the Dominican Republic

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***Coprinites dominicana* gen.nov., spec.nov. (Coprinaceae) found in amber from the Dominican Republic is the earliest known gilled mushroom, the first fossil fleshy agaric determinable, and the only known fossil "mushroom" (Agaricales) from the tropics. This find is of significance with respect to the evolutionary development of the Basidiomycetes. Because the appearance of this fossil is quite modern, it suggests that mushrooms as a group probably arose much earlier than the early to mid-Tertiary.**

THE RELATIVELY RECENT EXTRACTION of fossiliferous amber in the Dominican Republic has led to the discovery of many new taxa of invertebrates as well as vertebrates (*1*). Up to the present, however, there has been no report on the occurrence of higher fungi in Dominican amber. In fact, fossil Agaricales (Polyporaceae, Paxillaceae, Gomphidiaceae, Boletaceae, and "Agaricaceae") in general are extremely rare.

A nearly complete fossil representative of the "Agaricaceae" recently found in amber from the Dominican Republic consisted of a complete pileus (cap) containing spore-bearing lamellae and the top portion of the stipe. Part of the broken bottom portion of the stipe lay adjacent to the pileus.

The fossil specimen originated from the La Toca mine, located between Santiago and Puerto Plata in the Cordillera Septentrional of the northern portion of the Dominican Republic. The mine is in the Altimira facies of the El Mamey Formation (upper Eocene), which is shale-sandstone interspersed with a conglomerate of well-rounded pebbles (*2*). Differences in the magnitudes of absorption peaks in nuclear magnetic resonance spectra of the exo-methylene group of amber (*3*) from different mines in the Dominican Republic were used to calibrate the ages of the various mines, with the age (20 million to 23 million years) (based on foraminifera counts) of the Palo Alto mine used as a standard (*4*). The ages of various specimens of Dominican amber ranged from 15 million to 40 million years; that from the La Toca mine was the oldest, some 35 million to 40 million years old (lower Oligocene to upper Eocene).

The amber containing the fossil has all the visual characteristics of natural Dominican amber. A series of chemical and physical tests (*5*) performed on a small portion of the amber piece verified that it was authentic. The piece of yellow transparent amber containing the mushroom weighed 0.5 g and

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was elliptical in shape, 9 mm long and 6 mm wide.

A microscopical examination of the fossil mushroom indicates that it has affinities with the present-day genus *Coprinus*. The presence of relatively small spores, the absence of clamp connections and the absence of autodeliquescence, together with the tropical habitat, suggest that the fossil is related to an assemblage of species originally placed in the subgenus *Conioagaricus* of the genus *Agaricus* (*6*). Because we could not place our fossil species with assurance into any of the existing genera of Agaricales (*7*), we describe it as *Coprinites dominicana* gen. nov., spec.nov. in the family Coprinaceae (Figs. 1 through 5). The brownish-pink pileus is 3.5 mm in largest diameter, and its shape is convex with a small depression in the middle. The margin is grooved to pleated and incurved. The pileus is thin-fleshed with a squamulose-pectinate surface. The lamellae are distinct and 28 are visible, only 15 of which extend from the margin to the stipe. The lamellae are nondecurrent and the hymenium bears basidia with spores. The light brown spores are smooth, ellipsoid to oblong, 6 to 7 μm long, and have what appears to be a germ pore. The pileus hyphae are thin-walled, lack clamp connections and sclerified elements, and are broad and elongated at the pileus surface. The stipe diameter is 0.4 mm. The habitat was apparently on wood (some present-day members of this family do grow on wood and it is unlikely that ground-dwelling forms would have been preserved in amber).

The relatively small spores, the absence of clamp connections, the absence of autodeliquescence, the tropical habitat, and the above described characteristics separate this fossil species from present-day forms. We were unable to section the lamellae to observe their shape or to determine the solubility of the spore wall pigment or the dimorphism of the basidia, characters that would help separate the fossil from *Coprinus* and related genera. The specimen (AF-9-11) is now in the Poinar collection of Dominican amber maintained at the University of California, Berkeley. *Coprinites dominicana* is the earliest known gilled "mushroom" (Agaricales), the first fossil fleshy agaric determin-

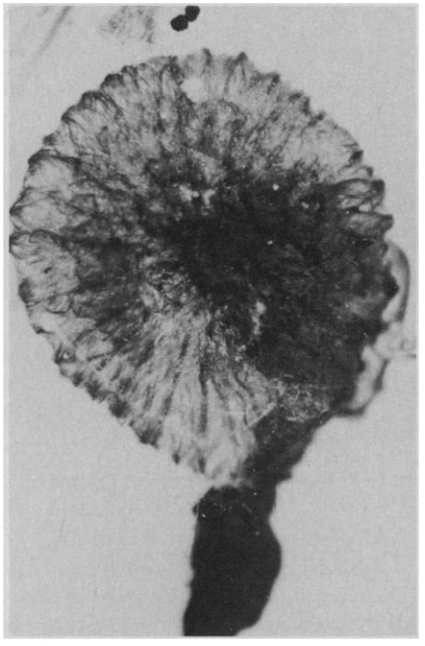

Fig. 1 (left). Dorsal view of the pileus of *Coprinites dominicana*.

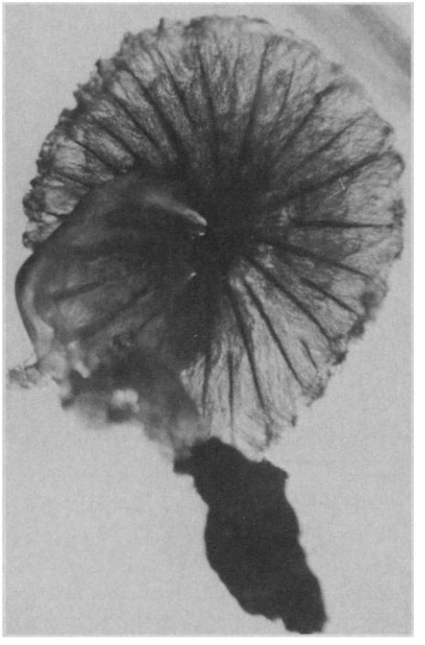

Fig. 2 (right). Ventral view of the pileus of *C. dominicana* showing the lamellae (gills).

Fig. 3. Mycelium (showing simple septa) from the stipe of *C. dominicana*.

Fig. 4. Uppermost layer of the pileus of *C. dominicana*.

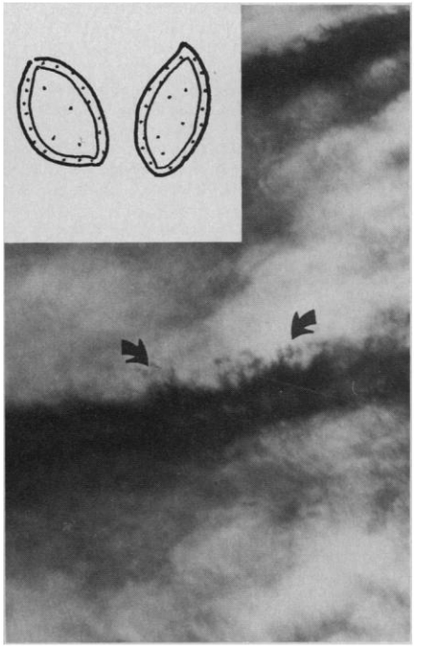

Fig. 5. Basidia and spores (arrows) on portions of the hymenium of *C. dominicana*. (Inset shows two spores in detail.)

able, and the only known fossil "mushroom" from the tropics. The record of plants and animals in Dominican amber, as well as the type of tree that produced the amber, clearly indicates that, during the resin production period, the environment was a tropical one (*8*). The present find does not detract from that conclusion. Other fungi that have been described as "Agaricaceae" are either clearly not members of the Agaricales (the four species of *Archagaricon*) or are much more recent (Pleistocene to Miocene) (*9*).

Considering the rarity of fossil agarics, explainable by the difficulties involved in conserving a soft fruiting body composed of thin-walled hyphal cells, the discovery of *C. dominica* is of importance with respect to the evolutionary development of the Basidiomycetes. It has been shown that *Palaeosclerotium* from the Pennsylvanian (*10*) is probably an ascomycete with clamp connections (*11*). However, other Pennsylvanian wood fungi with clamp connections may represent early Basidiomycetes. The oldest confirmed basidiomycete is the Jurassic pore fungus *Phellinites*, a member of the Polyporaceae associated with *Araucaria* trunks in Patagonia (*12*). Considering the "normal" appearance of the fossil mushroom described here, it is likely that the "Agaricaceae" arose much earlier than the early to mid-Tertiary.

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Activation of *ras* Oncogenes Preceding the Onset of Neoplasia

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The identification of *ras* oncogenes in human and animal cancers including precancerous lesions indicates that these genes participate in the early stages of neoplastic development. Yet, these observations do not define the timing of *ras* oncogene activation in the multistep process of carcinogenesis. To ascertain the timing of *ras* oncogene activation, an animal model system was devised that involves the induction of mammary carcinomas in rats exposed at birth to the carcinogen nitrosomethylurea. High-resolution restriction fragment length polymorphism analysis of polymerase chain reaction–amplified *ras* sequences revealed the presence of both H-*ras* and K-*ras* oncogenes in normal mammary glands 2 weeks after carcinogen treatment and at least 2 months before the onset of neoplasia. These *ras* oncogenes can remain latent within the mammary gland until exposure to estrogens, demonstrating that activation of *ras* oncogenes can precede the onset of neoplasia and suggesting that normal physiological proliferative processes such as estrogen-induced mammary gland development may lead to neoplasia if the targeted cells harbor latent *ras* oncogenes.

ACTIVATED *ras* ONCOGENES HAVE been identified in some common forms of human cancer including carcinomas of the lung, colon, and pancreas (*1*). In many instances, these oncogenes are already present in well-defined precancerous lesions (for example, adenomas and myelodysplasias), indicating that they play a role in the early stages of carcinogenesis (*1*). Progression into the more malignant stages of the disease requires additional events often involving either activation of other oncogenes or deletion of growth suppressor genes (*2*).

Animal model systems have been used to elucidate the role of *ras* oncogenes in carcinogenesis (*3, 4*). Indeed, the first indication that *ras* oncogene activation was involved in the early stages of carcinogenesis was provided by study of premalignant mouse skin papillomas (*5*). Moreover, *ras* oncogenes present in tumors induced by carcinogens of known mutagenic specificity often exhibit the type of mutation induced by the initiating carcinogen (*3, 4*). These results have been interpreted as evidence for a direct interaction between the initiating carcinogen and critical *ras* DNA sequences, a proposal that implies that *ras* oncogene activation must be at least concomitant with initiation of neoplasia (*6*). However, since cancer does not develop immediately after carcinogenic exposure, *ras* oncogenes remain latent and require the cooperation of additional factors to trigger neoplastic development. We have investigated the validity of these hypotheses by determining the timing of *ras* oncogene activation and the nature of some of the additional events required for the onset of carcinogenesis.

Carcinogenic exposure during sexual development leads to mammary carcinogenesis in female rats (*7*). Molecular analysis of these tumors has revealed the presence of activated H-*ras* oncogenes, particularly in those mammary carcinomas induced by the direct-acting carcinogen nitrosomethylurea (NMU) (*8–10*). In order to address the temporal relationship between *ras* oncogene activation and the onset of neoplasia, we separated the timing of the carcinogenic insult from sexual development. For this purpose, neonatal (2-day-old) Sprague-Dawley rats were administered a single subcutaneous injection of NMU (50 mg/kg of body weight). These animals developed mammary tumors with an incidence of 80% (35 animals out of 44 treated) and a mean latency of 3.7 months, about 2 months after reaching sexual maturity (Table 1). Most of these tumors were diagnosed as carcinomas (80%), although some benign fibroadenomas (20%) were also observed. Next, we investigated whether these neonatally induced tumors harbored *ras* oncogenes by utilizing a combination of polymerase chain reaction (PCR) amplification (*11*) and allele-specific oligonucleotide hybridization techniques (*12, 13*) to detect *ras* oncogenes activated by G to A transitions, the mutation previously found in all *ras* oncogenes of NMU-induced mammary tumors of rats (*8–10*). Fifteen of the 28 mammary carcinomas (54%) contained G to A mutated H-*ras* oncogenes (Table 1). A similar incidence (three activated oncogenes in seven tumors) was observed in the benign fibroadenomas. In addition to H-*ras* oncogenes, G to A mutated K-*ras* oncogenes were observed in five carcinomas and one fibroadenoma (17% overall incidence). These results indi-

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