

Aquatic organisms as amber inclusions and examples from a modern swamp forest

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To find aquatic organisms in tree resin may seem to be highly unlikely, but the fossil record provides numerous amber-preserved limnetic arthropods (e.g., water beetles, water striders, and crustaceans) and microorganisms (e.g., bacteria, algae, ciliates, testate amoebae, and rotifers). Here we explain the frequently discussed process of embedding aquatic organisms in tree resin based on field studies in a Florida swamp forest. Different aquatic arthropods and all major groups of limnetic microorganisms were found embedded in resin that had contact with swamp water. The taphonomy of aquatic organisms differs from that of terrestrial plants and animals that get stuck on resin surfaces and are enclosed by successive resin outflows. Large and highly motile arthropods are predestined for embedding. The number of microbial inclusions is increased when tiny drops of water with aquatic organisms become enclosed in resin while it is flowing in an aquatic environment. Bacteria and fungi may grow inside the resin as long as it has not solidified and therefore become secondarily accumulated. In contact with air, even resin that had initially been flowing into water may solidify and potentially form amber.

microorganisms | paleoecology | protista | taphonomy

Although tree resin is a very hydrophobic medium, the best-preserved fossils of limnetic insects and microorganisms are found in amber (e.g., refs. 1–21). This taphonomic phenomenon has been widely discussed (2, 4, 6–10, 12–18, 20–23), but there are still just speculations on the taphonomy of aquatic organisms in amber.

The occurrence of aquatic amber fossils is difficult to explain assuming that resin dried exclusively on the bark of trees in amber forests. Previous theories favor the following possibilities of embedding: (i) aquatic microorganisms and insects were trapped when resin was flowing into water-filled tree-holes (phytotelmata) (6, 15, 24); (ii) animals such as water beetles, water striders, larvae of dragonflies, and stoneflies were trapped during dispersal over land (9, 21); (iii) aquatic crustaceans and obligate aquatic larvae of caddisflies, mayflies, and dipterans were trapped when leaving drying ponds on the forest floor (20, 25, 26); and (iv) dead organisms and cuticle, e.g., aquatic crustaceans, were blown by wind onto resin outflows (2, 4, 22, 23).

The following facts stand in contrast to these theories. Phytotelmata are very rare in conifer trees, which makes frequent preservation of their inhabitants unlikely. Finds of obligate aquatic larvae of dipterans and caddisflies, which pupate and emerge exclusively under water, and finds of larvae of mayflies and water bugs, which usually never leave the water, cannot be explained by these theories (21). Furthermore, finds of several limnetic specimens within a single piece of amber, e.g., those of up to eight specimens of amphipods (crustaceans) (2, 22), make the theory of wind-blown dead organisms very unlikely.

Water bodies such as ponds and creeks were generally abundant in amber forests. This is indicated by frequent finds of insect imagoes with an obligate aquatic larval stage such as caddisflies and stoneflies in several famous Lower Cretaceous to Late Cenozoic ambers (e.g., refs. 10, 20, 21, and 27). Based on studies

with modern resin Henwood (28) remarked that $\approx 50\%$ of tree resin solidifies in the soil, not on the bark. This is also supported by frequent finds of litter-dwelling organisms in amber (e.g., refs. 7, 20, and 29). It can therefore be assumed that at least some resin of amber forests had contact with water bodies or solidified close to or in association with ponds or riparian environments.

To reveal the mystery of embedding aquatic organisms into hydrophobic tree resin, we investigated how modern resin in a swamp forest can be a trap for limnetic organisms. Florida's wet forests are predestined for actualistic–paleontological studies on the taphonomy of limnetic organisms in resin because resinous trees are often found standing in shallow water (Fig. 1A). After inducing artificial resin outflows from pine trees standing in swamp water, we were able to investigate the embedding of different aquatic organisms.

Here we provide evidence that none of the four theories mentioned above is necessary to get aquatic organisms entrapped in amber: organisms of different ecological groups, life forms, and size may become enclosed and well preserved in liquid resin when it is in contact with aquatic habitats of the forest floor.

Results and Discussion

Resin in Aquatic Environments. Resin that flowed down the tree trunks and into water split up into three fractions: (i) a resinous exudate that spread as a thin film at the water surface (Fig. 1B), (ii) small elongate resin pieces of up to 4 cm in length with a flat upper and a convex lower surface, hanging at the water surface but still attached to the tree trunk (Fig. 1C and D), (iii) more extensive resin outflows flowed down the trunk to the swamp floor, forming large subaquatic pillow-like to elongate resin pieces of up to $\approx 20 \times 10 \times 3$ cm (Fig. 1E–H). These resin outflows were too heavy to remain at the water surface.

Within the first day or two after initial contact with water, all three resin fractions were traps for limnetic organisms. The resinous film at the surface then dried out and broke into small centimeter-sized fragments, so no long-term preservation can be expected for this exudate. The pieces of resin hanging at the water surface became more and more solid within a few days because of air contact at their upper surface. After 1 week, these pieces were nearly solidified (Fig. 1D). Because this resin flowed slowly from the bark to the surface of the water, no turbulence took place, and trapped organisms were exclusively found attached to the surface of these pieces or just slightly inside the resin. Terrestrial insects (e.g., springtails, bugs, and ants), spiders, water striders, pine pollen, anthers of angiosperm flowers, and stellate hairs of oaks became attached to the upper side or sometimes enclosed within the resin (Fig. 1D), and limnetic microorganisms (e.g., algae and testate amoebae), nematodes, and arthropods were found at its lower side.

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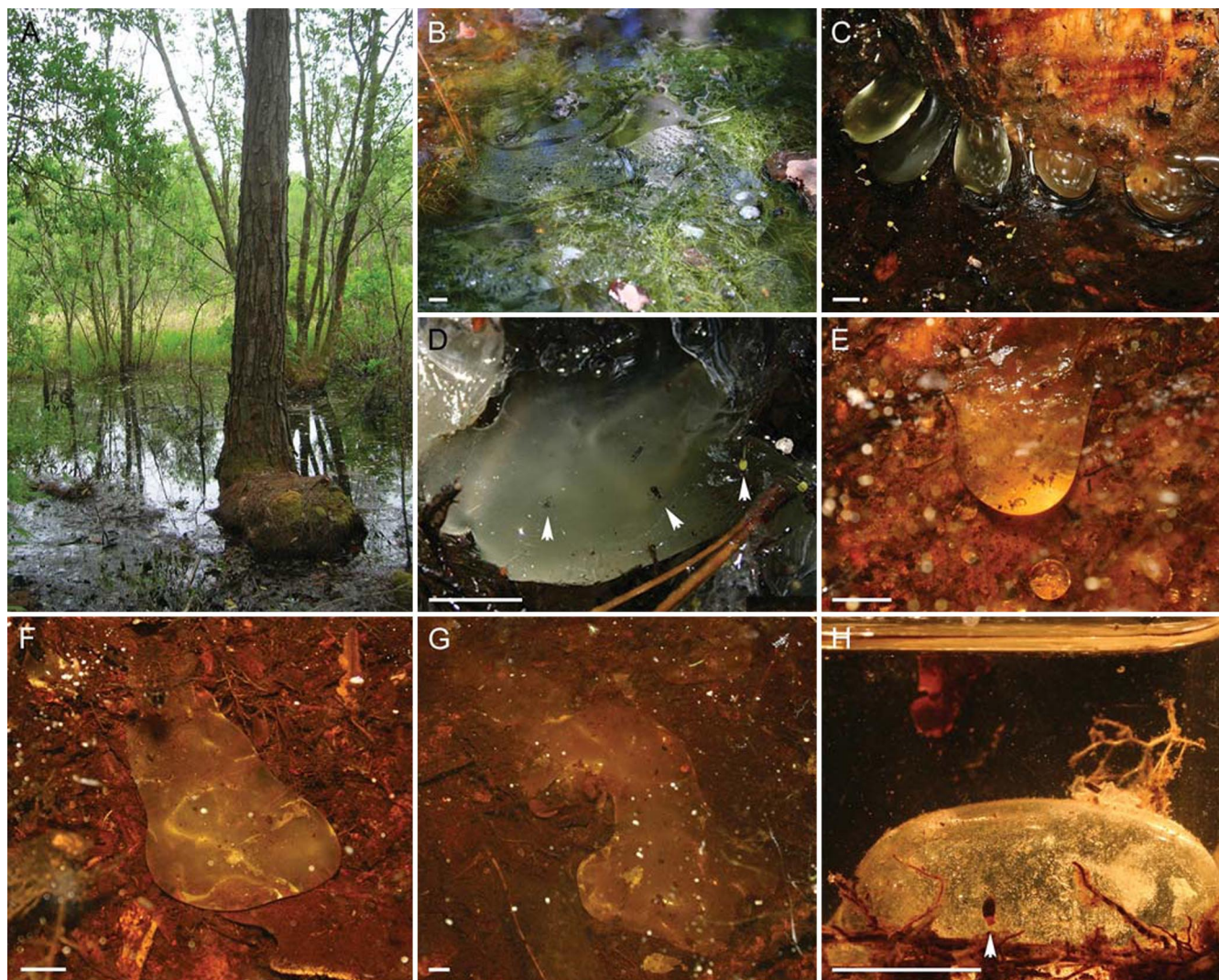


Fig. 1. Resin in Dilcher's swamp forest east of Gainesville (Florida). (A) *Pinus elliotii* and other trees standing in the water. (B) Resinous exudate spreading at the water surface. (C) Small elongate resin pieces of up to 4 cm in length hanging at the water surface attached to the tree trunk. (D) Solidified resin piece at the water surface with trapped insects and anthers (arrows). (E) Subaqueous resin with enclosed detritus flowing downward on the tree trunk. (F) Subaqueous pillow-like resin piece of ≈ 5 cm flowing on the swamp floor. (G) Extensive subaqueous resin flow of ≈ 20 -cm length on the swamp floor. (H) Pillow-like resin piece of ≈ 2.5 cm width in lateral view with trapped water beetle (arrow). (Scale bars: 1 cm.)

In contrast, the large resin bodies on the ground did not solidify as long as they were covered with water. But this resin was also initially a trap for microorganisms before a thin hardened skin developed at the resin surface after 1 or 2 days in the water, which prevented tiny organisms from getting stuck. Nonetheless, the resin remained liquid inside, and therefore large arthropods that were able to break through this skin could become entrapped over a couple of weeks. Flowing under water, over bark, roots, or litter, this resin would frequently cover and enclose detritus (Fig. 1E) and microorganisms of biofilms such as bacteria, algae, and testate amoebae. When flowing over a rough surface, millimeter-sized drops of water were enclosed, which often contained several species of microorganisms (Fig. 2A–C). The resin solidified when exposed to the air in the laboratory or by decreasing water level in the swamp during the summer. In late summer, solidified subaqueous resin was found at the base of the tree trunks and among litter on the dried floor of the swamp.

Trapped Organisms. Single-celled autotrophic eukaryotes such as unicellular green algae, pennate diatoms (*Fragilaria* and *Synedra*),

desmids (*Xanthidium*), chrysophytes (*Synura*), and euglenoid cells were frequently attached to the sticky resin surface and enclosed in resin flowing under water (Fig. 2D and E). Flagellate taxa such as the euglenoid genus *Trachelomonas* are clearly more predestined to become attached and trapped. Filiform algae, represented by the genera *Desmidium* and *Spirogyra* and parts of their filaments, were sometimes extending into the resin, whereas short chains of cells were entirely enclosed (Fig. 3C and D). Branches of the floating higher aquatic plant *Utricularia gibba* L. were rarely found enclosed in resin at the water surface.

Testate amoebae of the genera *Arcella*, *Centropyxis*, and *Gromia* often became attached to the resin surface or enclosed when subaqueous resin flowed over the ground (Figs. 2A and C and 3A). Ciliates such as *Aspidisca* commonly became embedded in the resin when they were initially enclosed within a tiny water drop (Fig. 2F).

Small motile multicellular organisms such as rotifers, nematodes, and mites were frequently attached to the surface and struggled deeper into the resin when moving (Fig. 2G–I). Crustaceans such as ostracods and daphnia rarely became enclosed and were sometimes found in a tiny drop of water in the resin (Fig. 2J).

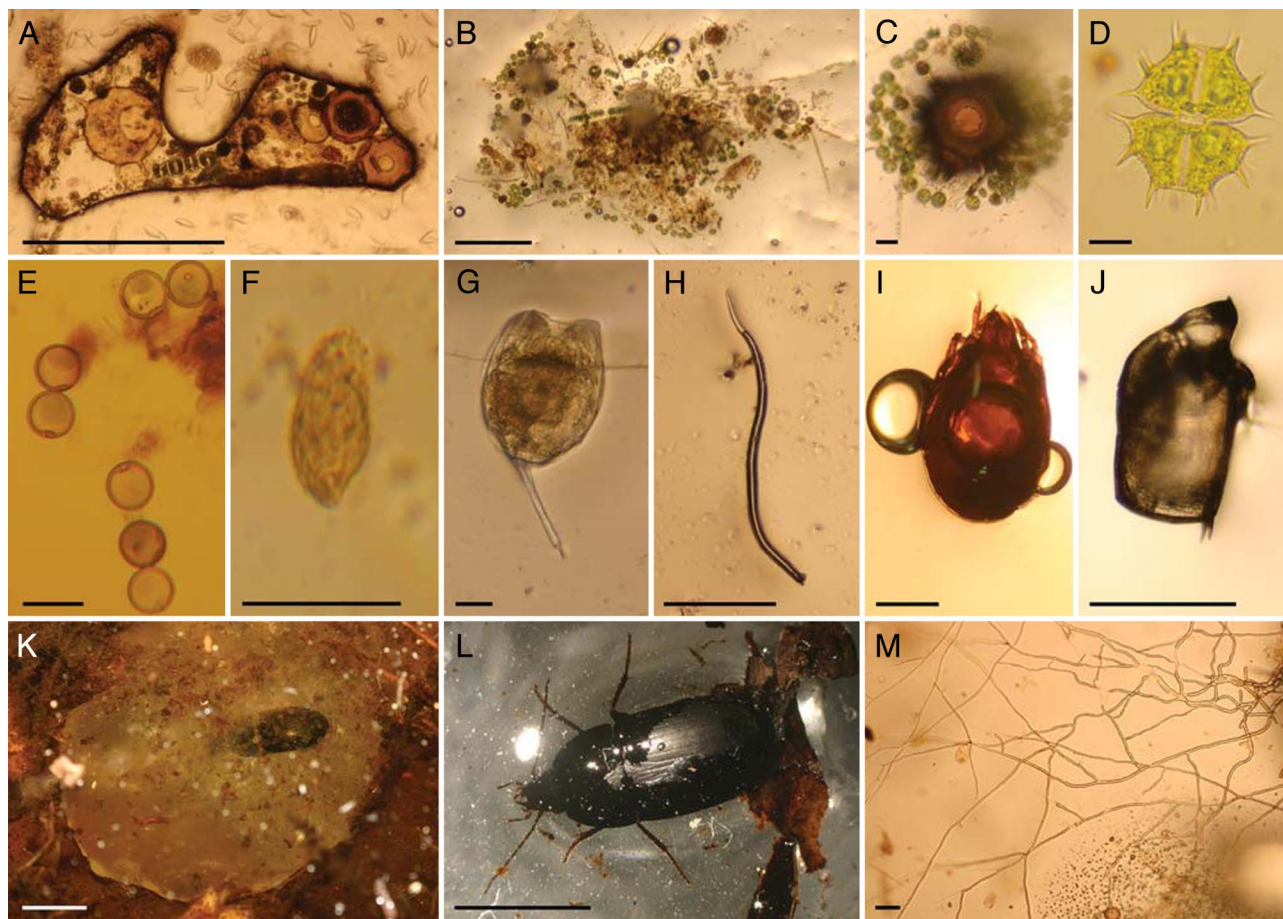


Fig. 2. Aquatic organisms in tree resin. (A) Tiny water drop in resin containing testate amoebae, green algae, and desmids. (B) Assemblage of green algae, diatoms, and desmids at the place of a former tiny drop of water in the resin. (C) Testate amoeba of the genus *Arcella* surrounded by green algae at the place of a former tiny drop of water in the resin. (D) Desmid genus *Xanthidium* well preserved in resin. (E) Euglenoid cells of the genus *Trachelomonas*. Note that flagella are not visible in the resin. (F) Ciliate. (G) Rotifer. (H) Nematode. (I) Mite. (J) Ostracod. (K) Water beetle entirely trapped in subaquatic resin. (L) Water beetle slightly exposed at the resin surface. (M) Fungal hyphae growing in liquid resin. [Scale bars: 5 mm (K and L), 200 μ m (A, B, and H–J), and 30 μ m (C–G and M).]

Fast-swimming water beetles became stuck when colliding with liquid resin bodies, and often several specimens were found entrapped in one piece of resin (Figs. 1H and 2K and L). When trying to escape, their struggles drove them deeper into the resin. For a period of weeks these large arthropods continued to be trapped in large subaquatic pieces of resin. These arthropods were predestined for embedding because they became irreversibly attached to the resin because of their high motility and speed.

Organisms Growing in Resin. Growth of filiform bacteria and fungal hyphae was observed in liquid resin lying in water (Fig. 2M). Patches of filaments were visible after 2 or 3 days. These bacteria and fungi were able to grow in random orientation as long as the resin was liquid. Growth stopped when the resin solidified, and the filiform inclusions were well preserved in the resin. Thus, a secondary accumulation of organisms that are able to grow in liquid resin took place.

Taphonomy and Taphocenoses. Our study reveals that resin, which flowed in aquatic environments, is a potential trap for limnetic organisms and that the hydrophoby of resin does not avoid embedding of various aquatic life forms. Their taphonomy, however, is different from that of terrestrial organisms in amber such as insects and higher plant remains. Attached to fresh resin, terrestrial organisms were entirely covered by further resin outflow. Terrestrial inclusions are therefore usually found at the surfaces of

successive resin outflows in amber (30). The resin flow in water is different because successive resin outflows do not become attached to each other but form separate resin bodies. We observed three possibilities for embedding of aquatic organisms: (i) attaching to resin surface and struggling deeper, (ii) overflowing by subaquatic resin, or (iii) enclosing in tiny water drops in resin. Turbulence of resin in water plays an important role for embedding microbes, and resin that flows slowly into the water may be free from any microbial inclusion. Microorganisms are often arranged in clusters located at the former location of water drops in the resin (Fig. 2A–C). In this situation, unicellular and filiform algae, amoebae, and ciliates occur close to each other. Aquatic and terrestrial fossils may occur as syninclusions in a single piece of amber when the resin comes into contact with water and air while in a liquid stage, e.g., hanging at the water surface.

All life forms of limnetic microbes and arthropods can potentially be present in the resin, and associations of trapped limnetic organisms may represent a majority of abundant organisms of the aquatic habitat. All trophic levels of a microbiocenosis may be found in a single piece of resin: bacteria, algae as producers, ciliates, testate amoebae and rotifers as consumers, and fungi as decomposers. Although all members of a microbiocenosis can potentially become embedded in resin, selective embedding has been observed. Motile and large organisms are more likely to become embedded. Because of their motility they have a higher probability of encountering and becoming attached to the resin. Bacteria and

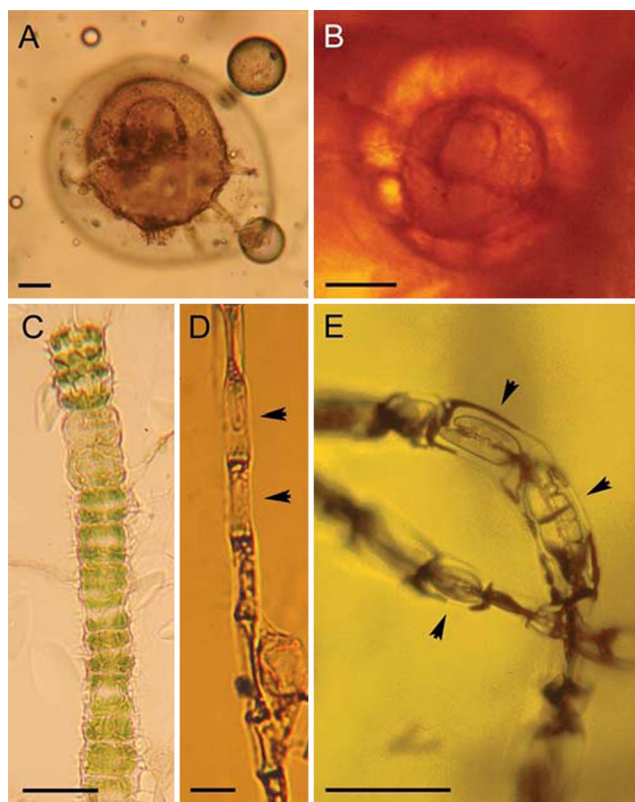


Fig. 3. Comparison of testate amoebae and filiform algae enclosed in modern resin (A, C, and D) and in 100-million-year-old Cretaceous amber from Schliersee, Germany (B and E). (A) Shell of *Centropyxis aculeata* (Ehrenberg) Stein surrounded by a tiny drop of water in modern resin. (B) Fossil *Centropyxis delicatula* Penard surrounded by a circular structure that probably indicates a former water drop. (C) Well preserved filament of the genus *Desmidium* in modern resin. (D) Filament of the genus *Spirogyra* with well preserved (arrows) and badly preserved cells in modern resin. (E) Filaments of the Cretaceous conjugatophyte *Palaeozygnema spiralis* Dörfelt with well preserved (arrows) and badly preserved cells. (Scale bars: 30 μm .)

fungi can be found in almost every fossil resin, and their ability to grow in liquid resin (31–33) leads to an overestimation of their abundance in amber. Therefore, the amount of amber inclusions of a species is not correlated with its abundance in the ancient ecosystem.

Mode of Preservation. All organisms found in the swamp resin were generally well preserved, and cell organelles such as chloroplasts of algae were visible. Some particular modes of preservation resemble those found in amber. Shells of testate amoebae were sometimes found in tiny drops of water in the resin (Fig. 3A). This resembles the circular structure surrounding some testate amoebae in Cretaceous amber (Fig. 3B), which were probably initially enclosed in a tiny drop of water. As in amber, pseudopodia of testate amoebae, cilia of ciliates, and flagella of motile algae were rarely visible after solidification of the modern resin. Probably these organelles were usually either broken off or became attached to the cell surface. In both modern resin and amber, cells of filiform algae show different modes of preservation because well preserved and collapsed cells may occur in one filament (Fig. 3C–E).

Long-Term Conservation and Fossilization. Many ancient amber forests were located close to the sea (e.g., refs. 7, 27, and 34). Indeed, wet forests of coastal plains provide potential long-term preservation of resin and genesis of amber in geological time. Changing water levels are required for solidification of subaquatic resin. Resins that solidify on the forest floor may easily be redeposited into marine deposits or covered by sediment layers stopping processes of oxidation and weathering.

Conclusions

The embedding of aquatic organisms in resin can be explained more easily than previously supposed. Limnetic life forms, from single-celled organisms to large arthropods, may become amber fossils, according to the following steps of embedding and long-term preservation:

1. Tree resin flowing down the tree to the forest floor comes in contact with water bodies or flows into water.
2. Limnetic organisms become attached to the surface of the liquid resin and struggle deeper into the resin, or are covered by new resin, or are enclosed in small water drops in resin. Motile organisms are more likely to become embedded deep in the resin.
3. Bacteria and fungi that are able to use the resin as a source for a while and grow in the liquid resin are secondarily accumulated.
4. Changing water levels allow solidification of the subaquatic resin.
5. Long-term conservation needs a sediment cover or redeposition from coastal plains to the sea.

Materials and Methods

The study site was a swamp in a small depression of several thousand square meters, which is situated in a warm-temperate mixed forest 8.8 km east of the city of Gainesville (Main Street and University Avenue), Florida (29° 38' 41" N latitude, 82° 15' 10" W longitude). Trees of *Pinus elliottii* Engelmann (Pinaceae) were used as study species. In contrast to trees of *Taxodium distichum* (L.) Richard (Cupressaceae) and *Gordonia lasianthus* (L.) J. Ellis (Theaceae), which are also found standing in the water, trees of this pine have abundant natural resin flow and also produce resin at artificial cuts of the trunk. The water level of the swamp can increase immediately after heavy rainfall (≈ 5 cm in 1 day observed) and decreases or even dries up during weeks without rain. The dystrophic waters (pH 4.3–4.5) yield a rich limnetic life.

By using a hand saw, the bark of five pine trees, standing in shallow swamp water, was removed in 20×20 cm sections, ≈ 10 cm above the water surface. One centimeter of sapwood was also cut away. Resin samples were collected at 2-day intervals over the whole month of April 2006. Additional samples were collected in September 2006 when the swamp dried out so that the trees were no longer standing in the water. Resin was collected by using microscopic slides or by putting pieces of resin with the surrounding water in transparent plastic boxes.

The samples were examined under incident and transmitted light microscopes immediately after collection and observed at 1-day intervals until solidification occurred. Resin pillows collected in plastic boxes with associated surrounding water were observed for 1 week in the laboratory.

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