Sci. U.S.A. **81**, 6476 (1981); G. H. W. Wong, P. F. Bartlett, I. Clark-Lewis, F. Battye, J. W. Schrader, *Nature (London)* **310**, 688 (1984).
3. A. Suzumura and D. H. Silberberg, *Brain Res.* **336**, 171 (1985).
4. M. R. Hirsch, J. Wietzerbin, M. Pierres, C. Goridis, *Neurosci. Lett.* **41**, 199 (1983); A. Fontana, W. Fierz, H. Wekerle, *Nature (London)* **307**, 273 (1984); A. Suzumura, D. H. Silberberg, R. P. Lisak, *J. Neuroimmunol.*, in press.
5. R. M. Zinkernagel and P. C. Doherty, *Nature (London)* **248**, 701 (1974); R. M. Zinkernagel and M. B. A. Oldstone, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3666 (1976); S. Jacobson *et al.*, *J. Immunol.* **133**, 754 (1984).
6. A. Helenius *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3846 (1978); T. Inada and C. A. Mims, *Nature (London)* **309**, 59 (1984).
7. J. O. Fleming and L. P. Weiner, in *Immunology of Nervous System Infections*, P. O. Beham, V. ter Meulen, F. Clifford Rose, Eds. (Elsevier, Amsterdam, 1983), pp. 91–96.
8. E. Lavi, D. H. Gilden, Z. Wroblewska, L. B. Rorke, S. R. Weiss, *Neurology* **34**, 597 (1984); E. Lavi, D. H. Gilden, M. K. Highkin, S. R. Weiss, *J. Virol.* **51**, 563 (1984).
9. A. Suzumura, S. Bhat, P. A. Eccleston, R. P. Lisak, D. H. Silberberg, *Brain Res.* **324**, 379 (1984).
10. K. D. McCarthy and J. de Vellis, *J. Cell Biol.* **85**, 890 (1980).
11. The stock MHV-A59 was originally obtained from J. L. Leibowitz [J. A. Robb, C. W. Bond, J. L. Leibowitz, *Virology* **94**, 385 (1979)].
12. E. Lavi *et al.*, in preparation.
13. A. A. Rahman, M. Treschner, K. K. Sethi, H. Brandis, *J. Immunol.* **117**, 253 (1976); J. Costa, A. S. Rabson, C. Yee, T. S. Tralka, *Nature (London)* **269**, 251 (1977).
14. J. E. Merrill *et al.*, *Science* **224**, 1428 (1984).
15. A. R. Collins *et al.*, *Infect. Immun.* **40**, 1192 (1983).
16. Anti-IFN-α/β was provided by D. M. Murasko, Medical College of Pennsylvania, Philadelphia, PA, and was characterized by R. Korngold, K. J. Blank, and D. M. Murasko [*J. Immunol.* **130**, 2236 (1983)].
17. P. I. Schrier, R. Bernards, R. T. M. J. Vaessen, A. Houweling, A. J. van der Eb, *Nature (London)* **305**, 771 (1983).
18. A. Rosenthal *et al.*, *ibid.* **315**, 579 (1985).
19. M. Hirayama, D. H. Silberberg, R. P. Lisak, D. E. Pleasure, *J. Neuropathol. Exp. Neurol.* **42**, 16 (1983).
20. Supported by NIH grants NS11037 and NS21954, and grant RG1421 from the National Multiple Sclerosis Society. We thank D. M. Murasko for the use of antibody to IFN; L. A. Lampson, R. P. Lisak, and D. E. Pleasure for critically reading the manuscript; and L. Buell for typing the manuscript.

30 September 1985; accepted 24 January 1986

Hyperphoretic Dispersal of a *Pyxidiophora* Anamorph

MEREDITH BLACKWELL, J. ROBERT BRIDGES, JOHN C. MOSER, THELMA J. PERRY

It has been suggested that *Thaxteriola* species and other minute, nonmycelial fungi associated with arthropods have phylogenetic relationships with the Laboulbeniales. However, direct development of the thallus of *Thaxteriola* from an ascospore of *Pyxidiophora* has now been discovered. *Thaxteriola* is specialized for dispersal by mites carried on pine bark beetles; other fungi dispersed by arthropods in this symbiotic assemblage rely primarily on arthropod specializations.

A PREVIOUSLY UNKNOWN METHOD of fungal anamorph (asexual state) development with extreme specialization for arthropod dispersal has been discovered. The new information provides the only evidence of the phylogenetic affinity of any of the minute, nonmycelial, entomogenous fungi first reported early in this century (*1*).

The perithecial ascomycete *Pyxidiophora* had been known from dung and fungal substrates (*2, 3*). We report here an undescribed species that is associated with the southern pine beetle symbiotic assemblage. Lundqvist (*2*) first noticed the similarity between ascospores of *Pyxidiophora* and the presumed hyphomycetes, *Thaxteriola* species and *Acariniola* species, from mites in bark beetle habitats in Poland and Louisiana (*1, 2*). However, conidia were not produced in the specimens, and they were regarded merely as ascospores (*2*).

The anamorph is characterized by a nonmycelial thallus consisting of two cells and a darkened holdfast in linear arrangement (*1*). Although *Thaxteriola* was first reported in 1914 (*1*), its development has not been observed until now. We found that the ascospores of *Pyxidiophora* differentiate into a *Thaxteriola* state while still within the ascus and ascocarp (Fig. 1A). Morphological differentiation of the ascospore begins with early development of a septum to divide the spore into two unequal cells, followed by gradual loss of the gelatinous membrane surrounding the ascospore, formation of a darkened holdfast (Figs. 1, A and B) at the end of the distal cell as it is positioned within the ascocarp, and attenuation of the proximal cell tip into a spine (Fig. 1C); an additional septum may be formed in the distal cell (*4*). The ascal products are released to the perithecial ostiole, holdfast-first, in a mucilaginous mass. Mites have been observed crawling over the perithecial necks, where they apparently acquire often multiple infestations of *Thaxteriola* thalli that adhere by the holdfast (Fig. 1C); endoconidium formation occurs later in the terminal cell (Fig. 1C). Presumably, endoconidia germinate by a germ tube to produce the teleomorphic (sexual) thallus (Fig. 2).

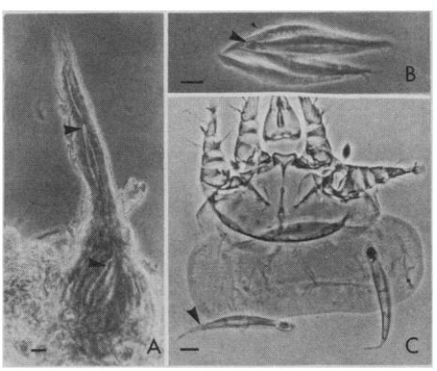

Fig. 1. *Pyxidiophora* and its *Thaxteriola* anamorph. (A) Perithecium containing anamorph that has developed from ascospores. Thalli are oriented so that the holdfast emerges first (arrows). (B) Contents of an ascus showing holdfast that has already developed (large arrow) and remnant of spore membrane (small arrow). (C) Two thalli of *Thaxteriola* attached to *Tarsonemus krantzi*. Endospores are present at arrow. Scale bars, 10 μm

Thus the mystery of the phylogenetic position of *Thaxteriola* has been elucidated in a most unexpected way. A *Pyxidiophora* teleomorph could not have been predicted for *Thaxteriola* because this manner of anamorph production by direct development of the ascospore has not been observed before. Previously described ascomycete anamorphs are single-celled (yeasts) or mycelial. In mycelial forms the anamorph is derived from an ascospore or conidium that germinates by a germ tube. Ascospore germ tube formation has been suppressed, and the *Thaxteriola* anamorph is differentiated from the ascospore itself. Now that the possibility of nonmycelial anamorph production in this manner is recognized, additional species in six genera of minute entomogenous fungi should be reexamined. *Acariniola*, *Amphoromorpha*, *Amphoropsis*, *Endosporella*, *Entomocosma*, and *Myriapodophila* (*1*) have characteristics in common with *Thaxteriola*. All have a small number of cells arranged linearly with a darkened holdfast at one end, lack haustoria, lack a mycelium, form nonwalled or thin-walled endospores within one to several terminal cells, and are associated with arthropods, primarily insects. It has been suggested that these organisms, including *Thaxteriola*, might be related to the Laboulbeniales as reduced (*1*) or even asexual forms (*5*). However, Thaxter (*1*) and Benjamin (*6*) did not believe that there was evidence for a

M. Blackwell, Department of Botany, Louisiana State University, Baton Rouge, LA 70803.
J. R. Bridges, J. C. Moser, T. J. Perry, Southern Forest Experiment Station, U.S. Department of Agriculture, Forest Service, Pineville, LA 71360.

laboulbenialean connection. The possibility that the other six genera may also have ascomycete teleomorphs should be investigated. These might not necessarily all be related to *Pyxidiophora*, but could be examples of convergence selected for anamorph dispersal. The teleomorphs, if they exist, would most likely be found associated with the host arthropod habitat, particularly that habitat from which the next host generation emerges.

The southern pine beetle (*Dendroctonus frontalis* Zimmermann) is the most destructive pest of southern pine forests (*7*). The evolutionary success of bark beetles has been attributed to their symbiotic association with fungi and to their well-developed system of chemical communication with aggregating pheromones (*8*). In addition to fungi, other insects and phoretic mites are closely associated with the southern pine beetle.

Many specializations of insects and mites for dispersal of fungal symbionts have been described. Highly developed mycangia with gland cells are present in females of the southern pine beetle (*9*). Two species of fungi can be carried in the mycangia (*9, 10*). Other fungal species, such as *Ceratocystis minor* (Hedgcock) Hunt, are also involved in a mutualistic association with *D. frontalis*. In this case, inoculum is provided by phoretic mites. Because *D. frontalis* pupates in the outer bark and *C. minor* occurs in the inner bark, emerging individuals do not usually carry ascospores of *C. minor*. However, two species of mites (*Tarsonemus ips* Linquist and *Tarsonemus krantzi* Smiley & Moser) feed on *C. minor*, acquire the ascospores, and transport them to the bark surface in an exoskeletal structure, the sporotheca (*11*). Here the mites attach to *D. frontalis* (*12*) and provide the inoculum for the beetle (*13*). Phoretic mites themselves have dispersal features ranging from relatively unspecialized to highly specialized. An extreme example of specialization for phoresy is known in association with the southern pine beetle in which a pygmephorid mite has dimorphic females, one of which is a phoretomorph. The two forms are so different morphologically that each was originally placed in a separate genus (*14*).

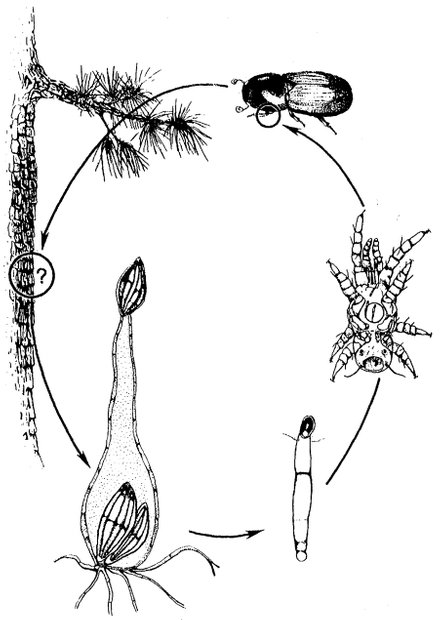

Fig. 2. Diagram of the life cycle of *Pyxidiophora* and its *Thaxteriola* anamorph. Ascospores are released to the perithecial ostiole where they may become attached to mites. Some mite hosts are phoretic on emerging southern pine beetles which disperse not only the mite, but also the endoconidium-forming fungus. Germination of endoconidia and establishment of the teleomorph have not been observed.

The morphological and behavioral specializations of the beetles and phoretic mites help to ensure fungal dispersal. Until now the role of the fungi was deemed passive and, except for the sticky spores of many fungal species associated with insects, no morphological specializations for dispersal had been reported. *Pyxidiophora* is part of the southern pine beetle assemblage. It is exceptional because it is the only fungus in this association that has been specialized for dispersal by arthropods in its anamorph form.

Although this species of *Pyxidiophora* currently is known only from loblolly pine (*Pinus taeda* L.) associated with *D. frontalis* galleries in Grant Parish, Louisiana, and Sabine County, Texas, we suspect that it is more widespread because of the broader anamorph distribution (Table 1). The *Thaxteriola* anamorph is known on 18 species of mites in seven families. The mites are associated with southern pine beetles or other beetles that often occupy the same trees. *Thaxteriola* has been found as a hyperphoront on phoretic stages of mites, but also on all active nonphoretic stages of at least one mite (Table 1). There is no strict host specificity.

Thaxteriola has not been found on adults of *D. frontalis* or *Ips* species, as it has been on other insects. We suspect that anamorphs may eventually be seen on *Ips* species, but not on *D. frontalis*. *Ips* species pupate in the inner bark, where the adults could presumably crawl over perithecia and acquire the anamorphs. *Dendroctonus frontalis*, however, pupates in the outer bark (*7*), where the adults never contact the perithecia and anamorphs.

The first observation of a close association between bark beetles and fungi was made in

Table 1. Host and geographical range of the *Thaxteriola* anamorph of *Pyxidiophora*.

Mite/infested stage	Scolytid association	Locality
Dendrolaelaps neocornutus (Hurlbutt)/larva, protonymph, deutonymph,* male, female	Galleries of *Dendroctonus frontalis* Zimmermann (SPB)	Louisiana
Dendrolaelaps neodisetus (Hurlbutt)/deutonymph,* female	*Ips grandicollis* Eichhoff, *Ips* spp., galleries SPB	Louisiana
Dendrolaelaps quadrisetus (Berlese)/deutonymph*	Reared adults of *Ips confusus* (LeConte)	California
Dendrolaelaps rotoni (Hurlbutt)/male, female	Galleries SPB	Louisiana
Longoseius brachypoda (Hurlbutt)/female	Galleries SPB	Louisiana
Mucroseius n. sp./female*	Reared adult of *Monochamus titallator* (Fabricius)	Louisiana
Ameroseius longitrichus Hirschmann/female*	Galleries SPB	Louisiana
Proctolaelaps fiseri Samsinak/male, female*	Galleries SPB	Louisiana
Proctolaelaps subcorticalis Lindquist/female*	Galleries SPB	Guatemala
Vulgarogamasus lyriformis (McGraw & Farrier)/female	Galleries SPB	Mississippi
Gamasolaelaps subcorticalis (McGraw & Farrier)/male	Galleries SPB	Louisiana
Uroobovella orri Hirschmann/female	Galleries SPB	Louisiana
Trichouropoda australis Hirschmann/protonymph	Galleries SPB	Louisiana
Cercoleipus coelonotus Kinn/male,* female*	Galleries SPB	Louisiana
Histiogaster rotundus Woodring/male, female	Galleries SPB	Louisiana
Tarsonemus krantzi Smiley & Moser/female*	Reared SPB	Louisiana, Texas
Tarsonemus ips Lindquist/female*	Reared SPB	Louisiana, Texas
Trichouropoda australis Hirschmann/protonymph	Galleries SPB	Louisiana

*Phoretic stages of mites.

the mid-19th century (*15*), but the complex interactions between the organisms are still not completely understood. This report emphasizes that point. Current outbreaks of southern pine beetle infestations in eastern Texas and western Louisiana and the inability to predict and control them provide a practical reason for continued study of these fascinating relationships.

REFERENCES AND NOTES

1. C. Spegazzini, *An. Soc. Cient. Argent.* **85**, 311 (1918); R. Thaxter, *Bot. Gaz. (Crawfordsville)* **58**, 235 (1914); *ibid.* **69**, 1 (1920); T. Majewski and J. Wiśzniewski, *Acta Mycol.* **14**, 3 (1978); *Mycotaxon* **7**, 508 (1978).
2. N. Lundqvist, *Bot. Not.* **133**, 121 (1980).
3. D. L. Hawksworth and J. Webster, *Trans. Br. Mycol. Soc.* **68**, 329 (1977).
4. M. Blackwell, T. J. Perry, J. R. Bridges, J. C. Moser, *Mycologia*, in press.
5. E. A. Gäumann and C. W. Dodge, *Comparative Morphology of Fungi* (McGraw-Hill, New York, 1928), p. 390.
6. R. K. Benjamin, *Bibl. Mycol.* **30**, 155 (1971).
7. T. L. Payne, *U.S. For. Serv. Tech. Bull. 1631* (1980), p. 7.
8. A. A. Berryman, in *Bark Beetles in North American Conifers*, J. B. Milton and K. B. Sturgeon, Eds. (Univ. of Texas, Austin, 1982), p. 264.
9. H. Francke-Grosmann, *Mater. Org.* **1**, 503 (1968); G. M. Happ, C. M. Happ, S. J. Barras, *Tissue Cell* **3**, 295 (1971).
10. S. J. Barras and T. Perry, *Z. Angew. Entomol.* **71**, 95 (1972); G. M. Happ, C. M. Happ, S. J. Barras, *Can. J. Bot.* **54**, 1049 (1975); *ibid.* **53**, 2702 (1975); J. R. Bridges, *Environ. Entomol.* **12**, 858 (1983).
11. J. C. Moser, *Trans. Br. Mycol. Soc.* **84**, 750 (1985).
12. L. M. Roton, *Can. Entomol.* **110**, 557 (1978).
13. J. R. Bridges and J. C. Moser, *Ecol. Entomol.* **8**, 9 (1983).
14. J. C. Moser and E. A. Cross, *Ann. Entomol. Soc. Am.* **68**, 820 (1975); R. L. Smiley and J. C. Moser, *Beitr. Entomol.* **26**, 307 (1976); G. W. Krantz, *A Manual of Acarology* (Oregon State Univ., Corvallis, ed. 2, 1978), p. 245.
15. T. Hartig, *Allg. Forst Jagdztg.* **13**, 73 (1844).

16. We thank R. L. Gilbertson and J. W. Kimbrough for making suggestions that improved the manuscript.

20 December 1985; accepted 25 February 1986

Propolypeptide of von Willebrand Factor Circulates in Blood and Is Identical to von Willebrand Antigen II

PHILIP J. FAY, YOHKO KAWAI, DENISA D. WAGNER, DAVID GINSBURG,* DAVID BONTHRON, BETSY M. OHLSSON-WILHELM,† STEPHEN I. CHAVIN, GEORGE N. ABRAHAM, ROBERT I. HANDIN, STUART H. ORKIN, ROBERT R. MONTGOMERY, VICTOR J. MARDER

The generally mild bleeding disorder of von Willebrand disease is associated with abnormalities of two distinct plasma proteins, the large multimeric von Willebrand factor (vWF), which mediates platelet adhesion, and von Willebrand antigen II (vW AgII), which is of unknown function. The two proteins were found to have a common biosynthetic origin in endothelial cells and megakaryocytes, which explains their simultaneous absence in the severe form of this hereditary disease. Shared amino acid sequences from a 100-kilodalton plasma glycoprotein and from vW AgII are identical to amino acid sequences predicted from a complementary DNA clone encoding the 5′ end of vWF. In addition, these proteins have identical molecular weights and immunologic cross reactivities. Monoclonal antibodies prepared against both proteins recognize epitopes on the pro-vWF subunit and on a 100-kilodalton protein that are not present on the mature vWF subunit in endothelial cell lysates. In contrast, polyclonal antibodies against vWF recognize both pro-vWF and vWF subunits. Thus, the 100-kilodalton plasma glycoprotein and vW AgII are identical proteins and represent an extremely large propolypeptide that is first cleaved from pro-vWF during intracellular processing and then released into plasma.

VON WILLEBRAND DISEASE (vWD) is characterized by a deficiency or structural defect in von Willebrand factor (vWF), a large glycoprotein that is involved in the binding of platelets to subendothelium after vascular injury and that is the carrier protein for procoagulant factor VIII (antihemophilic factor) (*1, 2*). Von Willebrand factor is present in plasma as a series of disulfide-bonded polymers of 220-kilodalton (kD) subunits (*3*). It is synthesized by megakaryocytes (*4, 5*) and by endothelial cells (*6*), in which it is stored in the endothelial cell-specific organelles, the Weibel-Palade bodies (*7*). In cultured endothelial cells, vWF is synthesized as a fully glycosylated large precursor (*8*) that is processed to the mature subunit (*9, 10*) and assembled into multimers before the protein is secreted (*9, 11*). A full-length complementary DNA (cDNA) for human vWF has been isolated (*12*), and analysis of the 8.15-kilobase (kb) sequence implies that the primary vWF translation product is 300 kD.

Von Willebrand antigen II (vW AgII) is a second protein that is deficient in the plasma and platelets of patients with severe vWD (*13*). It does not share antigenic determinants with vWF, but its deficiency in vWD could be explained by the hypothesis that both proteins are derived from a common precursor (*13, 14*). The antigen is also synthesized by endothelial cells (*14*), is found in the Weibel-Palade bodies (*14*), and is increased in plasma concomitant with vWF, after 1-desamino-8-D-arginine-vasopressin (DDAVP) stimulation (*15*). A complex between vW AgII and vWF is present in endothelial cells, although it is not completely understood (*14*).

A plasma glycoprotein of 100 kD, similar in size to vW AgII, was independently isolated from therapeutic factor VIII concentrates (*16*). Although it was initially believed to be human factor VIII (antihemophilic factor), comparison of partial sequences of this protein (*17*) with that of cloned factor VIII cDNA sequence (*18*) indicates that it is not factor VIII.

We now report that this 100-kD plasma glycoprotein and vW AgII are identical and represent circulating vWF propolypeptide, which is cleaved from pro-vWF subunits during intracellular processing and multimer assembly.

The full-length vWF cDNA is 8.3 kb and has a continuous open reading frame encoding 2813 amino acids (*12*). Amino terminal amino acid sequences of the 100-kD plasma glycoprotein (*17, 19*) and vW AgII (*20*) match amino acid sequences derived from vWF cDNA in clone pVWH33 (*12*), which represents the 5′ portion of vWF cDNA. The region of identity between the two proteins and the sequence predicted by the cDNA begins at nucleotide 67 (Fig. 1). Thrombin cleaves the 100-kD glycoprotein into two fragments of 75 and 26 kD (*16,*

P. J. Fay, D. D. Wagner, S. I. Chavin, G. N. Abraham, V. J. Marder, Department of Medicine, University of Rochester Medical Center, Rochester, NY 14642.
Y. Kawai and R. R. Montgomery, Departments of Pediatrics, Medicine, and Pathology, Medical College of Wisconsin, Milwaukee, WI 53233, and the Blood Center of Southeastern Wisconsin, Milwaukee, WI 53233.
D. Ginsburg, Hematology Divisions, Brigham and Women's Hospital, and Departments of Medicine and Pediatrics, Harvard Medical School, Boston, MA 02115.
D. Bonthron and S. H. Orkin, Hematology Division, Children's Hospital, and Departments of Medicine and Pediatrics, Harvard Medical School, Boston, MA 02115.
B. M. Ohlsson-Wilhelm, Department of Microbiology, University of Rochester Medical Center, Rochester, NY 14642.
R. I. Handin, Hematology Divisions, Brigham and Women's Hospital and Children's Hospital, and Departments of Medicine and Pediatrics, Harvard Medical School, Boston, MA 02115.

*Present address: Hematology and Oncology Division, University of Michigan Medical Center, Ann Arbor, MI 48109.
†Department of Medicine, Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.