

## The type species of the genus *Gnomonia*, *G. gnomon*, and the closely related *G. setacea*

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Species of the diaporthean genus *Gnomonia* are common yet inconspicuous and poorly known microfungi occurring mostly on overwintered leaves of trees and shrubs in the temperate zone of the northern hemisphere. Confusion exists about the type species of *Gnomonia* because a type was not designated. Three specific names have been mentioned in the literature as the type species: *G. gnomon*, *G. vulgaris* and *G. setacea*. *Gnomonia gnomon* was designated as the lectotype species; however, some authors have considered all three taxa to be synonymous with *G. setacea* having priority. *Gnomonia vulgaris* is a nomenclatural synonym of *G. gnomon*. Observations of type specimens and fresh material of *G. gnomon* and *G. setacea* reveal that these two species are distinct and can be distinguished by the position of perithecia in the leaf tissue and ascospore morphology. Descriptions and illustrations of *G. gnomon* and *G. setacea* are provided. A lectotype specimen and an epitype specimen with ex-type culture for *G. gnomon* are designated; an epitype for *G. setacea* is designated. The distinction between *G. gnomon* and *G. setacea* is supported by ribosomal DNA sequence analysis.

Keywords: Ascomycetes, Diaporthales, Gnomoniaceae, ITS, LSU, systematics, taxonomy

*Gnomonia* Ces. & De Not. is the type genus of the family Gnomoniaceae (Winter, 1887; Hawksworth & Eriksson, 1988) in the order Diaporthales. The taxonomic structure of the Diaporthales has undergone a number of changes over the past 30 years as summarized by Castlebury & al. (2002). Most authors have recognized the

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Gnomoniaceae although the concept of the family has varied (Wehmeyer, 1975; Barr, 1978, 1990; Monod, 1983; Vasilyeva, 1998). Cannon (1988) merged the Gnomoniaceae into the Valsaceae and this concept was followed by authors of 'Outlines of the Ascomycetes' and 'Outlines of the Ascomycota' from 1990 to 2003 (Eriksson & Hawksworth, 1990, 1991, 1993, 1998; Eriksson, 1999; Eriksson & al., 2001, 2003). Recent molecular work provided conclusive support for the Gnomoniaceae as a separate family (Castlebury & al., 2002) and this concept was accepted in 'Outlines of the Ascomycetes-2004' (Eriksson & al., 2004).

Some members of the Gnomoniaceae are known to cause plant diseases. One of the most important and well known is *Discula destructiva* Redlin, an agent of serious disease of dogwood trees (*Cornus florida* L. and *C. nuttallii* Audubon) in the northeastern and northwestern United States (Hibben & Daughtrey, 1988; Redlin, 1991; Windham & al., 1994; Daughtrey & al., 1996; Zhang & Blackwell, 2001). *Apiognomonium veneta* and *A. errabunda* and their associated anamorphs are known to be the causal agents of anthracnose diseases of oaks, beeches, plane trees and sycamores (Hepting, 1971; Sinclair & al., 1987; Fell, 1996; Tello & al., 2000). Members of the Gnomoniaceae, including the above-mentioned parasites, are also known to be symptomless endophytes inhabiting leaves and twigs (Sieber & al., 1991; Pehl & Butin, 1994; Wilson & Carroll, 1994; Fell, 1996; Ragazzi & al., 1999; Sahashi & al., 1999).

Although the Gnomoniaceae has been clearly defined by molecular criteria (Castlebury & al., 2002), the morphological criteria that distinguish this family from others in the Diaporthales have not been delineated. Furthermore, the taxonomy of the genera and species in the Gnomoniaceae has not been resolved. As defined in the most comprehensive monographs on the Gnomoniaceae (Barr, 1978; Monod, 1983), the main morphological characters that distinguish genera are the absence or presence of stroma, type of stroma, position of the beak on the perithecium, and septation of ascospores. According to these monographs, *Gnomonia* is characterized by the lack of a stroma, solitary perithecia that occur primarily on overwintered, rarely living, leaves, usually with a central beak that may or may not be long, and ascospores that tend to be oval to fusiform but never filiform, having one median septum.

Molecular evidence suggests that *Gnomonia* as defined by Barr (1978) and Monod (1983) may not be monophyletic (Castlebury & al., 2002). Knowledge of the type species of *Gnomonia* and any closely related species is essential for circumscribing a monophyletic genus that also typifies the family. Confusion exists in the literature about the type species of *Gnomonia* because as a type of the genus was not designated by the authors. Three specific names have been men-

tioned as the type: *G. gnomon*, *G. vulgaris* and *G. setacea*. *Gnomonia gnomon* was designated as the lectotype by Höhnelt (1917). However, some authors have considered all three taxa to be synonymous with *G. setacea* having priority. In this study, we examined type and herbarium specimens as well as fresh material of *G. gnomon* and *G. setacea* to determine morphological differences. In addition, we sequenced the internal transcribed spacer (ITS) regions 1 and 2 and the nuclear large subunit (nLSU) ribosomal DNA (rDNA) genes in order to determine the relationships of these taxa to each other and to other taxa in the Gnomoniaceae. Descriptions and illustrations of *G. gnomon* and *G. setacea* are provided.

## Materials and methods

### Preparation and morphological examination of specimens and cultures

Fresh material was collected in the mid-Atlantic USA (Maryland, Pennsylvania, and Virginia) in the spring of 2004. Additional fresh material was obtained from Austria, Finland, and Russia. Overwintered leaves were examined for the presence of perithecia. Those containing perithecia were air dried and stored in paper bags or envelopes.

Herbarium specimens were obtained from the U.S. National Fungus Collections (BPI), the Eidgenössische Technische Hochschule in Zürich (ZT), the Musée et Jardins Botanique Cantonaux in Lausanne (LAU), the Mycology Herbarium of Royal Ontario Museum (TRTC), the Farlow Reference Library and Herbarium of Cryptogamic Botany in Harvard University (FH).

For the microscopic examination of both fresh and herbarium material, perithecia were extracted from leaf tissue with insect pins under a dissecting microscope, placed into a drop of in 3 % aqueous KOH or 7 % aqueous sodium acetate solution on a microscope slide. After rehydration perithecia were observed under the Wild M5A dissecting microscope (Wild Heerbrugg Ltd., Heerbrugg, Switzerland) and photographed with DXM 1200 digital camera (Nikon Instruments Inc., Melville, NY, USA). Perithecia were crushed to release their contents, which were transferred with an attenuated glass capillary or a scalpel to a clean area of the slide. The perithecial contents were covered with a cover slip and observed under brightfield and differential interference contrast (DIC) with an Axioplan2 microscope (Carl Zeiss, New York, NY, USA) and photographed with DXM 1200 digital camera.

For the isolation of pure cultures, fresh material was rehydrated in 7 % sodium acetate solution and crushed. Ascospores and asci were removed with an attenuated glass capillary and plated on either cornmeal agar (CMA, Sigma<sup>®</sup>, Sigma Chemical Co., St. Louis, MO, USA) or potato dextrose agar (PDA, Bacto<sup>®</sup>, Becton, Dickinson & Co., Sparks, MD, USA) plates containing 1 % (v/v) of an antibiotics solution (0.2 % streptomycin sulfate and 0.2 % neomycin sulfate in sterile distilled water). Plates were incubated at room temperature for 24 hours. Germinated ascospores were transferred with a micromanipulator and glass needles at 100 fold magnification (Samuels, 1979) or by hand with a glass needle to fresh CMA or PDA and incubated at room temperature. For macroscopic descriptions of colonies, strains were grown on malt extract agar (MEA, Bacto<sup>®</sup>, Becton, Dickinson & Co., Sparks, MD, USA). Cultures were incubated at 25 °C with a 12 hour day/night cycle. Colors were determined according Kornerup & Wanscher (1978).

Measurements in descriptions are minimum/maximum value observed and range from first to third quartile. Arithmetic means, standard deviations (SD), and number of measurements (n) are given in parentheses. Thus measurements are provided as (min-)Q<sub>1</sub>-Q<sub>3</sub> (-max) × (min-)Q<sub>1</sub>-Q<sub>3</sub>(-max) μm (mean, SD, n). Images were processed with Adobe Photoshop 5.0 (Adobe Systems, Inc., San Jose, CA, USA)

### PCR amplification and sequencing

Genomic DNA was extracted directly from actively growing surface mycelium scraped from PDA plates with the Plant DNeasy Mini kit (Qiagen Inc., Chatsworth, CA, USA) or the PUREGENE Cell and Tissue kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions using approximately 50 mg fresh mycelium. The nLSU rDNA genes were amplified in 50 μL reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) under standard reactions conditions as in Castlebury et al (2002). The thermal cycler program was as follows: 2 min at 95 °C followed by 35 cycles of 30 sec at 94 °C, 30 sec at 55 °C, 1 min at 72 °C, with a final extension period of 10 min at 72 °C. Following amplification, the PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA) according to the manufacturer's instructions.

Amplified products were sequenced with the BigDye<sup>®</sup> dye terminator kit version 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI 3100 automated DNA sequencer using the following primers: LR0R, LR3R, LR5R, LR7, LR5, LR3 (Vilgalys & Hester, 1990; Rehner & Samuels, 1994). Internal transcribed spacer regions 1 and 2,

including the 5.8S rDNA, were amplified and sequenced using the primers ITS5 and ITS4 (White & al., 1990) and the same PCR conditions and sequencing protocols as for the nLSU rDNA. The following new sequences were generated in this study: *G. gnomon* AR 4062 (ITS & nLSU), AR 4071 (ITS & nLSU), CBS 829.79 (ITS & nLSU), CBS 199.53 (ITS); and *G. setacea* AR 3451 (ITS), AR 3814 (ITS & nLSU), CBS 859.79 (ITS & nLSU). GenBank accession numbers for the additional taxa included in the analyses are indicated on the tree. Cultures that were sequenced for this study were deposited at CBS if not already there.

### Sequence alignment and analyses

Raw sequences were edited using Sequencher version 4.2 for Windows (Gene Codes Corporation, Ann Arbor, MI, USA). Alignments were manually adjusted using GeneDoc 2.6.001 (<http://www.psc.edu/biomed/genedoc/>). Sequences are deposited in GenBank as AY818952–AY818964. The alignment was deposited in TreeBase C. Trees were inferred by the neighbor-joining (NJ) method (Kimura 2-parameter distance calculation) and by maximum parsimony (MP) using the heuristic search option with the random addition sequence (1000 replications), MULTREES off and the branch swapping (tree bisection-reconnection) option of PAUP\* 4.0b10 (Swofford, 2002). All aligned positions were included in the analysis. All characters were unordered and given equal weight during the analysis. Gaps were treated as missing data in the parsimony analysis and the neighbor joining analysis; missing or ambiguous sites were ignored for affected pairwise comparisons. Heuristic searches for most parsimonious trees (MPT) with the MULTREES option in effect resulted in large numbers of trees and did not search to completion. Relative support for branches was estimated with 1000 bootstrap replications (Felsenstein, 1985) with MULTREES and TBR off and 100 random sequence additions for the MP bootstraps.

## Results

### Taxonomy

***Gnomonia gnomon*** (Tode: Fr.) J. Schröt. in Cohn's Krypt. Fl. Schles. 3 (2): 390 (1897) – Figs. 1–11.

≡ *Sphaeria gnomon* Tode: Fr., Fungi mecklenb. 2: 50 (1791): Syst. mycol. 2: 517 (1823).

≡ *Cryptosphaeria gnomon* (Tode: Fr.) Grev., Fl. edin.: 360 (1824).

- ≡ *Gnomonia vulgaris* Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 232. (1863).
- ≡ *Gnomoniella vulgaris* (Ces. & De Not.) Sacc., Syll. Fung. 1: 416 (1882).
- ≡ *Gnomoniella gnomon* (Tode : Fr.) Magnus, Pilze von Tirol.: 490 (1906)

Anamorph: Unknown

*Perithecia* hypophyllous, scattered evenly over leaf blade, immersed at first, erumpent later, black, oblate, diam × height = (144–)204–263(–318) × (58–)167–210(–276) μm (mean = 230 × 187 μm, SD 40, 38, n<sub>1</sub> = 122, n<sub>2</sub> = 71), collapsing cupulate from the top while dry. Beaks central or occasionally eccentric, straight or slightly sinuous, length (159–)259–346(–654) μm (mean = 313 μm, SD 100, n = 93), basal diameter (18.2–)27.4–37.5(–53.0) μm (mean = 32.5 μm, SD 6.9, n = 98), distal diameter (14.5–)24.1–33.1(–42.6) μm (mean = 28.8 μm, SD 6.9, n = 93). Asci fusiform with narrow tapering stipe, (28.6–)35.8–43.1(–56.6) × (5.25–)6.9–9.6(–11.7) μm (mean = 39.7 × 8.1 μm, SD 5.9, 1.7, n = 73), with eight ascospores fasciculate, parallel or slightly shifted along their length, apical ring 1.37–2.6 μm diam. Ascospores (16.6–)19.0–21.1(–23.3) × (1.3–)1.6–1.8(–2.3) μm (mean = 20.0 × 1.7 μm, SD 1.5, 0.2, n = 340), fusiform, length/width ratio (l:w) (8.1–)9.7–10.8(–13.8) (mean = 10.3, SD 0.93, n = 340), straight to slightly curved, ends blunt, rounded, with one median septum, each cell with several (usually 4–8) lipid guttules; usually one bigger guttule disposed close to septum; appendages usually 2.5–4 μm long, cuneiform, sometimes absent, sometimes whip-shaped, up to 27 μm long (WJ2501).

Colonies on MEA after 14 d of growth 50–70 mm diam, whitish, semitransparent, edges even. Aerial mycelium scarce, loosely velutinous or with few loose cottony tufts. Reverse colorless. No sporulation or formation of perithecia observed. No distinct odor or pigment observed.

Habitat. – On overwintered leaves of *Corylus* spp., rarely *Populus* sp.

Geographic distribution. – Austria, Canada (ON), Czech Republic, Finland, Germany, Russia (Central European part), Slovakia, Sweden, Switzerland, Ukraine, United Kingdom, United States (CO).

Lectotype of *G. gnomon* designated herein. – GERMANY: Mecklenburg, *Corylus avellana*, 1791, H.I. Tode, illustration in 'Fungi mecklenb.', Tab. 16, figs. 125a–125f.

Epitype of *G. gnomon* designated herein. – Finland: Helsinki, Helsinki University Botanical Garden, *C. avellana*, 19 Apr.

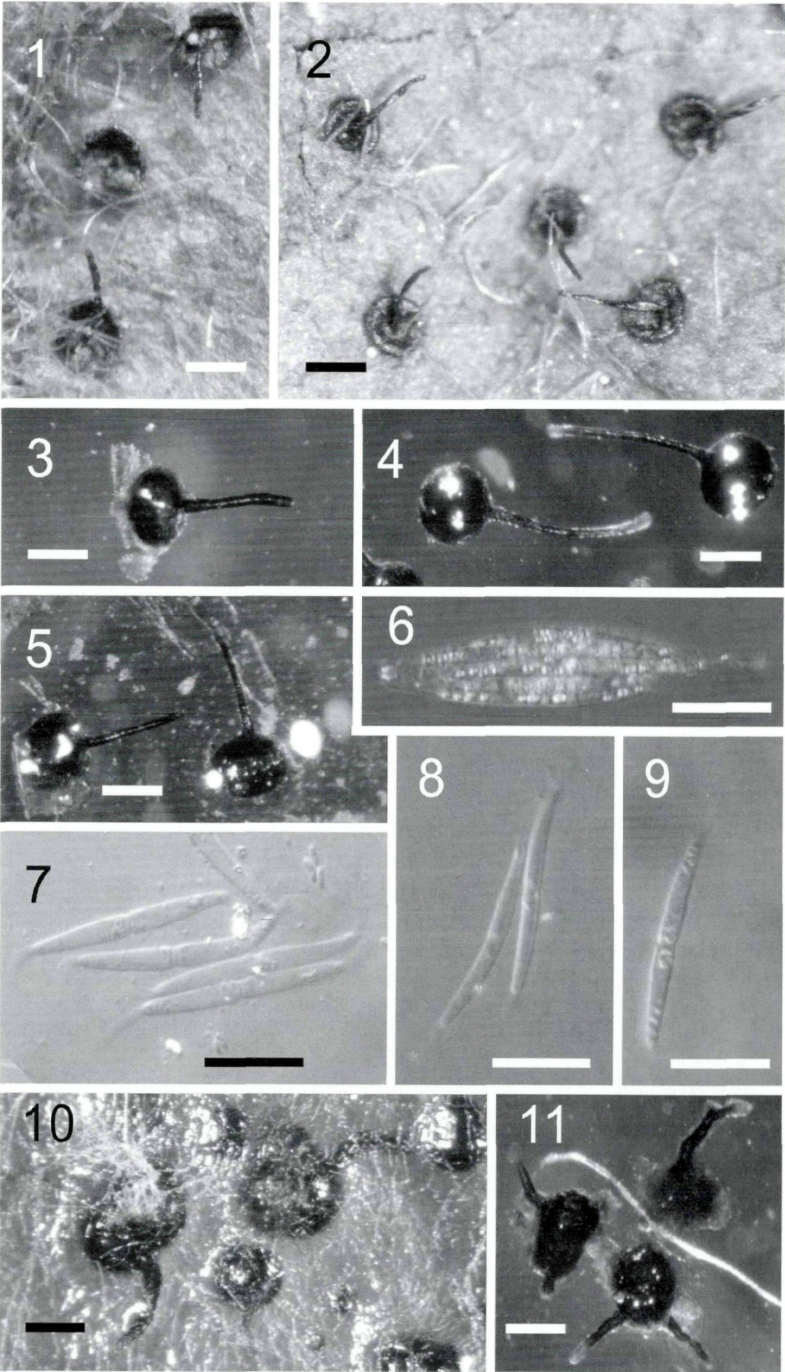
2004, leg. D. S. Shchigel MS0036 (BPI 844273, culture CBS 116383 = AR 4062).

Additional specimens examined. – Austria: Lower Austria, Krems, *C. avellana*, Apr. 1871, leg. Thümen (BPI 611815); Vienna, *C. avellana*, 1 May 2004, leg. W. Jaklitsch WJ2501 (BPI 844279, culture CBS 116384 = AR 4071). CANADA: ONTARIO: Lake Temagami, Sandy Inlet, *C. rostrata*, 12 June 1931, leg. R. F. Cain (TRTC 2589); T. F. R., Lake Temagami, Bear Isl., *C. rostrata*, 19 June 1932, leg. R. F. Cain (TRTC 10692); same location, 17 June 1932, leg. R. F. Cain (TRTC 10693). Czech Republic: Bohemia, Luditz, Krasch, *C. avellana* May 1913, leg. R. Steppan (BPI 611818); Bohemia, Turnov, *C. avellana*, 02 May 1907, leg. J. M. Kabat (BPI 611820); Hranice, *C. avellana*, 1913, leg. F. Petrak (BPI 611817). FRANCE: Haute-Savoie, Petit-Saleve, near Geneve, *C. avellana*, Feb. 1852, collector unknown (BPI 596635). Germany: *C. avellana*, date unknown, leg. J. C. Schmidt & G. Kunze, Deutschlands Schwämme 57, BPI (611814); Dillkreis, *C. avellana*, 02 Apr. 1934, collector unknown (BPI 596633); same details (BPI 596634); Dillkreis, Langenaubach, *C. avellana*, Apr. 1923, leg. unknown (BPI 611435); Hessen, Oestrich, near Dillkreis, Langenaubach, *C. avellana*, 30 Apr. 1933, leg. A. Ludwig (BPI 611808); Nossen, *C. avellana*, 24 Apr. 1889, leg. W. Krieger (BPI 611809); Leipzig, *C. avellana*, May 1871, leg. G. Winter (BPI 611810); same location, *C. avellana*, May 1874, leg. G. Winter (BPI 611811); Schleussig, near Leipzig, *C. avellana* May 1871, leg. G. Winter (BPI 611812); Thüringen, Steiger near Erfurt, *C. avellana*, 13 May 1905, collector unknown (BPI 596632); Brandenburg, Triglitz in Prignitz, *C. avellana*, 14 Apr. 1906, leg. O. Jaap, Fungi selecti exsiccati 220 (BPI 596638). RUSSIA: Nizhniy Novgorod prov., Pil'na, birch park close to the river P'yana, *C. avellana*, 20 May 2004, leg. G.M. Sogonova MS0103 (BPI 863598); SLOVAKIA: Preňčov, *C. maxima*, 28 Mar. 1887, leg. A. Kmet (BPI 611821). Sweden: *C. avellana*, leg. E. M. Fries, Scleromyceti Sueciae 285, BPI (bound). Switzerland: Bischofszell, date unknown, leg. H. Wegelin (ZT); Oberbuchsiten, *C. avellana*, 01 Mar. 1946, coll. J. A. von Ar x (ZT); Changins, *C. avellana*, 1 March 1976, leg. M. Monod, No. 2; Vaud, Bex, Le Bévieux, *C. avellana*, 13 May 1976, M. Monod, No. 47; Valais, *Populus nigra* var. *italica*, 13 May 1977, leg. A. Bolay, No. 267 (culture CBS 829.79); Misox, Grono, *C. avellana*, 17 May 1988 leg. E. Müller (ZT); Domleschg, Rodel, *C. avellana*, 04 May 1988, leg. E. Müller (ZT); Albulatal, Filisur, Solis, *C. avellana*, 20 May 1988, leg. E. Müller (ZT); Schanfigg, Lüen *C. avellana*, 24 May 1989, E. Müller (ZT); Vorderrheintal, Panix, *C. avellana*, 13 June 1989, leg. E. Müller (ZT). Ukraine: Ivano-Frankovsk, *C. avellana*, 12 May 1918, leg. F. Petrak (BPI 611816). UNITED KINGDOM: England, *C. avellana*, 1873?, leg. Plowright (BPI 611813). UNITED STATES: COLORADO: Boulder, 1600 m, on dead leaves of *C. rostrata*, 27 July 1907, leg. F.E. & E. S. Clements, Crypt. Format. Coloradensium 411 (FH). Country Unknown: *Corylus* sp., Mar 1880, leg. Thaxter? (BPI 611807).

At present, *Gnomonia gnomon* is primarily known from *Corylus avellana* (European filbert, European hazel) in Europe, although

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Figs. 1–11. *Gnomonia gnomon*. – 1, 2. Dried perithecia on natural substrata. Note dried perithecia collapsed cupulate from the top. – 3–5. Rehydrated perithecia from natural substrata. – 6. Ascus. – 7–9. Ascospores. – 10. Perithecia produced in pure culture on MEA. – 11. Perithecia produced in culture mounted in 7 % sodium acetate. – Figs. 1, 3, 7. Scleromyceti Sueciae 285. – 2, 5, 6, 10, 11. BPI 844273 (epitype specimen). – 4, 8. TRTC 2589. – 9. BPI 844279. – Figs. 1–5, 10, 11. Dissecting microscope. – 6–9. DIC. Scale bars: 1–5, 10, 11 = 200 µm; 6–9 = 10 µm.





there is one specimen from *Populus*, which the derived culture has proved to be *G. gnomon*. A few herbarium specimens of *G. gnomon* on *C. rostrata* exist from North America; however, neither living cultures nor DNA have been isolated from them. Therefore, their identification as *G. gnomon* is based only on morphological observations.

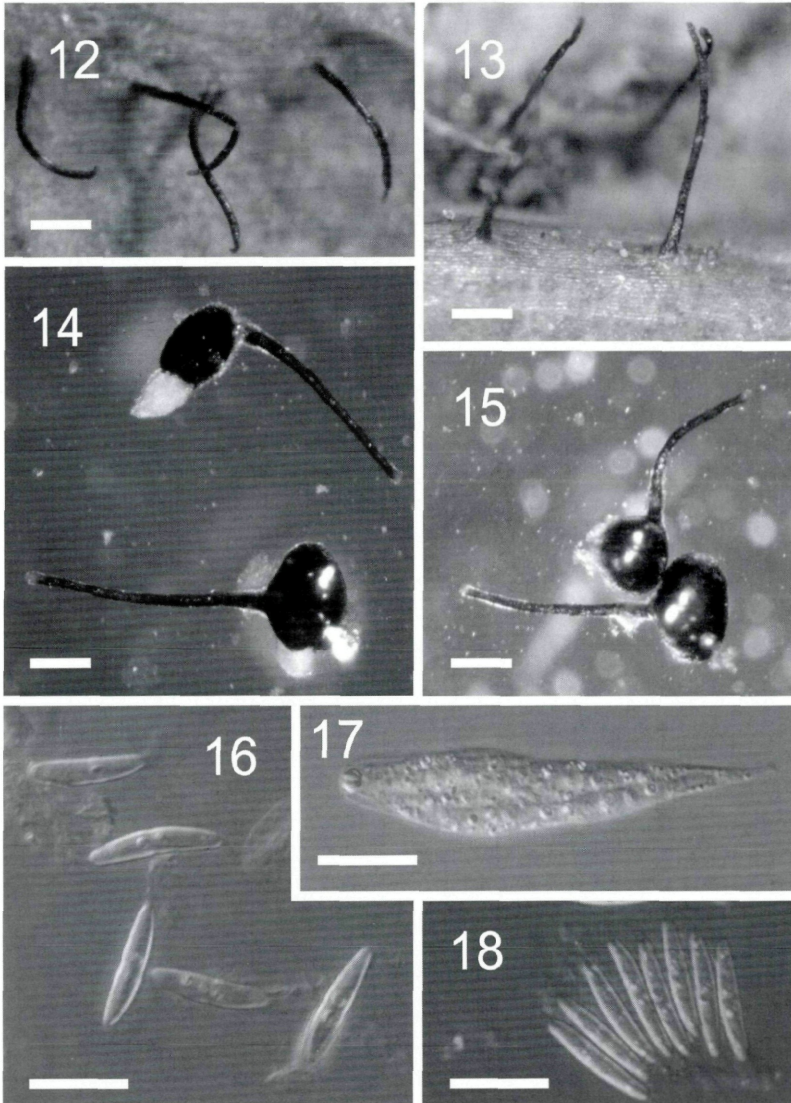
***Gnomonia setacea*** (Pers. : Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 232. (1863) – Figs. 12–18.

≡ *Sphaeria setacea* Pers. : Fr., Syn. Method. Fung. p. 62 (1801): Syst. mycol. 2: 517 (1823).

Perithecia hypophyllous, usually concentrated along edges of midribs, but also on midribs, side veins, and, occasionally, on leaf blades distant from veins, immersed, black, becoming dark brown or black when moist, oblate diam × height = (214–)266–350(–429) × (157–)198–246(–280) μm (mean = 308 × 217 μm, SD 55, 34.6, n<sub>1</sub> = 27, n<sub>2</sub> = 26) (usually circular in top view but sometimes oval), collapsing cupulate from the bottom while dry. Beaks central, eccentric, rarely lateral, straight or sinuous, always black, length (358–)571–723(–857) μm (mean = 634 μm, SD 130, n = 28), basal diameter (33.0–)41.5–49.8(–62.6) μm (mean = 46.0 μm, SD 6.9, n = 30), distal diameter (20.8–)27.0–34.0(–45.7) μm (mean = 31.3 μm, SD 6.2, n = 28). Asci ellipsoidal to fusiform with narrow tapering stipe, (25.3–)31.3–42.7(–0.5) × (6.1–)7.9–9.4(–12.6) μm (mean = 37.1 × 9.0 μm, SD 6.5, 1.4, n<sub>1</sub> = 39, n<sub>2</sub> = 40), with eight ascospores arranged irregularly fasciculate, apical ring 1.7–2.5 μm diam. Ascospores fusiform, inequilateral, (9.2–)11.5–12.9.0(–17.3) × (1.3–)2.0–2.3(–2.7) μm (mean = 12.3 × 2.1 μm, SD 1.2, 0.3, n = 387), l:w (4.6–)5.9–6.6(–8.8) (mean = 6.3, SD 0.7), with one median septum, ends blunt, rounded; every cell typically with two lipid guttules, sometimes some smaller guttules present as well; appendages frequently absent, if present, cuneiform, 2.5–4.0 × 0.9–1.2 μm, or setose, 5–6 × 0.3–0.6 μm.

Colonies on MEA after 14 d of growth 50–70 mm diam, whitish semitransparent, edges even. Aerial mycelium scarce, loosely velutinous to moderate in loosely cottony tufts. Reverse colorless. No sporulation or formation of perithecia observed. No distinct odor or pigment observed.

Habitats. – On overwintered leaves of *Quercus* spp. and *Castanea* spp.



Figs. 12–18. *Gnomonia setacea*. 12, 13. Dried perithecia on natural substrata. – 14, 15. Rehydrated perithecia from natural substrata. – 16, 18. Ascospores. – 17. Ascus. – Figs. 12, 14, 16. Deutshl. Schw. 132. – 13. BPI 844268. – 15, 17, 18. BPI 844261. – Figs. 12–15. Dissecting microscope. – 16–19. DIC. – Scale bars: 12–15 = 200  $\mu\text{m}$ ; 16–18 = 10  $\mu\text{m}$ .

Geographic distribution. – Austria, Canada (ON), Germany, Italy, Montenegro, Sweden, Switzerland, and USA (LA, MD, NJ, NY, OH, PA, TN, VA, WV).

Holotype. – Several specimens examined by Persoon were located at L (Nationaal Herbarium Nederland, Leiden, Netherlands) but were unavailable for examination. No evaluation of a type or potential type specimen is possible at this time.

Epitype of *G. setacea* designated herein. – SWITZERLAND: Vaud, Prilly, below the pathway La Fleur-de-Lys, *Quercus* sp., 08 May 1979, leg. M. Monod, No. 528 (LAU, derived culture CBS 859.79).

Additional specimens examined. – AUSTRIA: Wiener, *Quercus* sp. Apr. 1913, leg. Jos. Weese, Eumycetes selecti exsiccati 153 (BPI 611632, second specimen BPI 611636). CANADA: ONTARIO: York Co., Nashville, *Quercus alba*, 11 May 1955, leg. R. F. Cain (BPI 611641); York Co., Kleinburg, *Q. macrocarpa*, 02 June 1952, leg. R. F. Cain (BPI 611645). GERMANY: leg. Schmidt & Kunz, Deutschl. Schw. 132 (BPI bound). ITALY: Conegliano, *Quercus* sp., Aug. 1876, leg. Spegazzini (BPI 611637); Tirol, Arco, *Q. lanuginosa*, May 1915, leg. E. Diettrich-Kalkhoff, (BPI 611644); Vittorio, *Q. robur*, Sep. 1901, collector unknown (BPI 611651). SERBIA AND MONTENEGRO: Povzonica, *Q. cerris*, Apr. 1911, leg. L. Vlach (BPI 611643). SWITZERLAND: Vaud, Blonay, *Quercus* sp., 21 May 1979, leg. M. Monod, No. 539 (LAU); Valais, Vernayaz, *Castanea* sp., 05 June 1979, leg. M. Monod, No. 553 (LAU). UNITED STATES: LOUISIANA: *Quercus* sp., 07 Jan. 1890, leg. A. Langlois (BPI 611634); MARYLAND: Marlboro, *Q. alba*, 26 Apr. 1929, leg. C.L. Shear 6007, (BPI 611642); Montgomery Co., Chesapeake & Ohio Canal National Historical Park, *Q. alba*, 10 Apr.2004, leg. M.V. Sogonov MS0017 (BPI 844265); same location, *Q. prinus*, 10 Apr. 2004, leg. M.V. Sogonov MS0022 (BPI 844266, derived culture CBS 116408 = AR 4058); Prince George's Co., Beltsville, *Quercus* sp., May 1950, leg. F. Petrak (BPI 1111803); Beltsville, Cochran Rd., backyard, *Q. palustris*, 17 Mar. 2004, leg. M. V. Sogonov MS0002 (BPI 844255, derived culture CBS 116406 = AR 4056); same location, 23 Mar. 2004, leg. M. V. Sogonov MS0003 (BPI 844256); Prince George's Co., Beltsville Agricultural Research Center, near Indian Creek, swampy forest, *Q. bicolor*; 25 Mar. 2004, leg. M. V. Sogonov MS0004 (BPI 844257); same details MS0005 (BPI 844259); same location, *Q. palustris*, 25 Mar. 2004, leg. M. V. Sogonov MS0004a (BPI 844258, derived culture CBS 116407 = AR 4057); Prince George's Co., Beltsville Agricultural Research Center, Entomology Rd., hardwood forest, *Q. alba*, 30 Mar. 2004, leg. M. V. Sogonov MS0007 (BPI 844260); same location, *Q. macrocarpa* 30 Mar. 2004, leg. M. V. Sogonov MS0008 (BPI 844261); same location, *Q. phellos*, 01 Apr. 2004, leg. M. V. Sogonov MS0009 (BPI 844262). NEW JERSEY: Hunterdon Co., Califon, *Q. prinus*, 03 Mar 1998, leg. G. Bills (BPI 745881); same location, *Q. prinus*, Apr. 2000, leg. G. Bills 6418 (BPI 747274, derived cultures CBS 109781 = AR 3451, CBS 109523); Newfield, *Quercus* sp., Jul 1888, collector unknown (BPI 611638); same location, *Quercus* sp., 04 Jun 1877, collector unknown (BPI 611639). NEW YORK: Lott Wood, Flatbush, *Castanea dentata*, 02 Apr. 1890, leg. Zabriskie, Ellis 415 (BPI 611623). OHIO: Fairfield Co., Lancaster, *C. vesca*, Jun 1893, leg. W. A. Kellerman (BPI 611624). PENNSYLVANIA: State College, near North Frear building, *Quercus* sp., 02 May 2004, leg. M. V. Sogonov MS0029 (BPI 844268, derived culture AR 4078); Centre Co., Shingletown area, mixed forest, *Q. rubra*, 02 May 2004, leg. M. V. Sogonov MS0030 (BPI 844269, derived culture AR 4079).

same details MS0034 (BPI 844272); Centre Co., Shingletown area, deciduous forest, *Q. prinus*, 02 May 2004, leg. M. V. Sogonov MS0032 (BPI 844270, derived culture AR 4080); same location, *Q. rubra*, 02 May 2004, leg. M. V. Sogonov MS0032a (BPI 844271, derived culture AR 4081). TENNESSEE: Great Smoky Mountains National Park, on petioles and veins of *Quercus* sp., 25 Mar 2002, leg. Lar. N. Vasilyeva (BPI 843499, derived culture AR 3814). VIRGINIA: Fairfax x Co., Fairfax, Laurel St., *Q. prinus*, 18 Apr. 2004, leg. M.V. Sogonov MS0027 (BPI 844267, derived culture CBS 116409 = AR 4061); Madison Co., Nethers, *Q. prinus*, 16 May 2004, leg. M.V. Sogonov MS0040 (BPI 844275). WEST VIRGINIA: Fayette Co., *Q. prinus*, 08 Apr. 1898, leg. L. W. Nuttall 478 (BPI 611647); same details (BPI 611648); Fayette Co., *Q. prinus*, 15 Apr. 1898, leg. L. W. Nuttall (BPI 611649).

*Gnomonia setacea* is encountered on overwintered leaves of *Castanea* and *Quercus* (Fagaceae) in north temperate regions. The concept of *G. setacea* is here accepted in the narrow sense defined by Monod (1983) and one of Monod's specimens for which a culture exists is herein designated as epitype.

#### Sequence characteristics and phylogenetic reconstruction

The ITS regions, including the 5.8S rDNA and short regions at the 3' end of the nuclear small subunit (nSSU) and the 5' end of the nLSU rDNA, consisted of 538 base pairs (bp) for the four isolates of *G. gnomon*. For the same regions in *G. setacea*, 550 bp were sequenced for AR 3814 and CBS 859.79 and 551 bp were sequenced for AR 3451 (CBS 109523). Among the isolates of *G. gnomon*, the sequences differed by one C/T substitution in the ITS 1 region with AR 4071 (CBS 116384) and CBS 829.79 identical to one another and AR 4062 (CBS 116383) and CBS 199.53 identical to one another. Among the isolates of *G. setacea*, AR 3814 and CBS 859.79 were identical to one another and differed from AR 3451 at four positions, including one gapped position. The ITS sequences of the two taxa differed by 9.2% (51 of 553 aligned positions), not including positions that varied within taxa.

The nLSU sequences for *G. gnomon* were identical, as were the nLSU sequences for *G. setacea*. The nLSU sequences of the two taxa differed by 0.8% (10 of 1265 aligned positions). Figure 19 shows the placement of *G. gnomon* and *G. setacea* in the Gnomoniaceae as it was previously defined (Castlebury & al., 2002). Of the taxa identified as species of *Gnomonia* included in this study only *G. gnomon* and *G. setacea* group together indicating that, of the species sampled to date, only these two taxa represent a monophyletic group.

#### Discussion

The genus *Gnomonia* was described by Cesati & De Notaris (1863) who revised the enormously heterogeneous genus *Sphaeria*

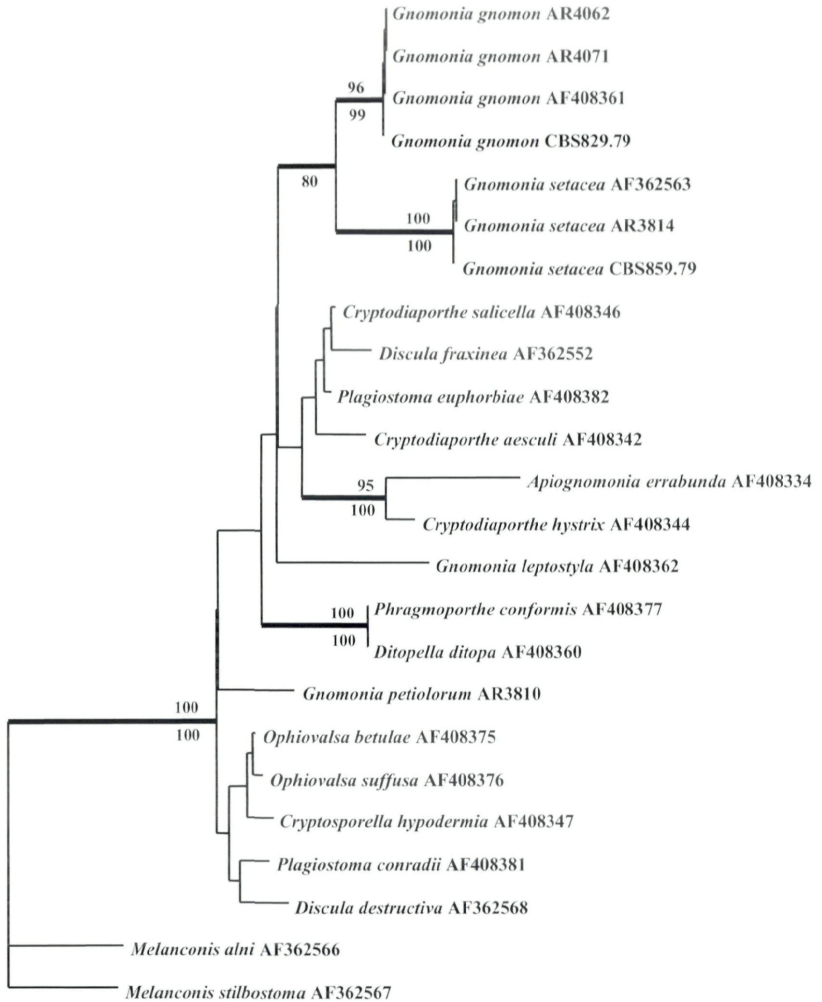


Fig. 19. – Phylogenetic tree resulting from Neighbor-Joining analysis of 1302 bp of the nLSU rDNA sequences of 22 strains representing the Gnomoniaceae. Bootstrap values greater than 70 % are shown above (based on maximum parsimony analysis) and below (NJ) each branch. Thickened lines indicate that branch appeared in the strict consensus of the 386 trees resulting from maximum parsimony analysis (length = 117, CI = 0.650, RI = 0.785, RC = 0.510).

and established several new segregate genera. They included eight species in *Gnomonia* with six additional species of *Sphaeria* as putative members of the genus. No type species for *Gnomonia* was designated at that time. Höhnelt (1917) selected *G. vulgaris* as the

lectotype species of the genus *Gnomonia* and that selection has not been challenged although this nomenclatural synonym is now recognized as *G. gnomon*.

The species *Gnomonia gnomon* was first described by Tode (1791) as *Sphaeria gnomon*, a name that was sanctioned by Fries (1823). Greville (1824) renamed *S. gnomon* as *Cryptosphaeria gnomon* when he established the new genus *Cryptosphaeria* Grev. based on *C. taxi* Grev., now considered a member of the Diatrypaceae (Glawe, 1984; Rappez, 1987). In placing *Sphaeria gnomon* in *Gnomonia*, Cesati & De Notaris (1863) considered the resulting name to be a tautonym and thus renamed the species *G. vulgaris* based on the same type specimen as *S. gnomon*. Saccardo (1881) established the genus *Gnomoniella* based on the type species, *G. tubaeformis* (Fr.) Sacc. for species like *Gnomonia* that have non-septate ascospores. Saccardo (1882) transferred *G. vulgaris* to *Gnomoniella* apparently not noticing the septate ascospores. Schröter (1897) placed the species back in *Gnomonia* using the original epithet, *G. gnomon*. Magnus (1905) recognized the species as *Gnomoniella gnomon*.

*Gnomonia setacea* has undergone fewer nomenclatural changes. The species *Sphaeria setacea* was first described by Persoon (1801) and sanctioned by Fries (1823). Cesati & De Notaris (1863) transferred it to their newly described genus *Gnomonia*. Confusion between *Gnomonia gnomon* and *G. setacea* began when Auerswald (1869) circumscribed *G. setacea* in a very broad sense to include *G. gnomon* and many other species of *Gnomonia*. Müller & von Arx (1962) and Kobayashi (1970) also considered *G. gnomon* (as *G. vulgaris*) to be a synonym of *G. setacea*, which they recognized to be the lectotype of the genus *Gnomonia*.

More recently, Barr (1978) and Monod (1983) both separated *G. gnomon* from *G. setacea*; however, their concepts of these species differ. Barr (1978) described *G. gnomon* as having 'ascospores (10–) 12–17.5 × 1.5–2.5 µm ... narrowly ellipsoid fusoid, ... septum usually suprmedian, occasionally nearly median,' which actually corresponds to *Apiognomonium ostryae sensu* Monod. The concept of *G. gnomon* presented by Monod (1983) agrees with our examination of the Friesian, epitype and additional specimens listed in this paper. *Gnomonia gnomon* and *A. ostryae* have the same major host plant species and regularly occur on the same leaf blades. The concept of *G. setacea* as applied by Monod (1983) and Barr (1978) is generally similar but that of Monod is limited to collections occurring on species of *Quercus* and *Castanea*, while Barr reported that *G. setacea* also occurs on *Alnus*, *Betula*, and *Lycopodium*. Monod's concepts of both species were accepted later by Barr (1991).

Morphological features of the perithecia and ascospores distinguish *Gnomonia gnomon* from *G. setacea*. These taxa both have

solitary perithecia with long beaks that occur on overwintered leaves of deciduous trees. The fusoid, unitunicate asci have a conspicuous ring in the apex and float free in the centrum as is characteristic of the Diaporthales. *Gnomonia gnomon* has erumpent, superficial perithecia becoming cupulate from the top when dry and occur throughout the leaf blade. In contrast, perithecia of *G. setacea* remain immersed in the substrate becoming cupulate from the bottom when dry and tend to be concentrated near the leaf midrib. This difference in perithecial shape when collapsed is apparently due to differences in thickness of the basal wall (Klebahn, 1918). The perithecial beaks of *G. gnomon* are shorter than those of *G. setacea* and are almost always central, very rarely eccentric, while the beaks on perithecia of *G. setacea* may be central, eccentric or even lateral. Arrangement of the ascospores within the ascus varies as well. In *G. gnomon* the ascospores are parallel to the main axis of the ascus while those of *G. setacea* are arranged obliquely within the ascus. Ascospores of *G. gnomon* are longer and narrower with a greater length-width ratio than those of *G. setacea*. In addition, cultures of *G. gnomon* on MEA grow more slowly than those of *G. setacea*. Finally, *G. gnomon* occurs on overwintered leaves of *Corylus* with one report on *Populus*, primarily in Europe, while *G. setacea* is known on overwintered leaves of *Castanea* and *Quercus* throughout north temperate regions.

*Gnomonia gnomon* typifies the genus *Gnomonia* and is closely related to *G. setacea*. Both ITS and nLSU DNA sequences support this close relationship, with the more variable ITS regions differing by less than 10 % and the more conservative nLSU sequences differing by less than 1 %. Although the nLSU sequences are too conservative to resolve relationships among genera in the Gnomonaceae (Fig. 19), they indicate that the genus *Gnomonia*, as it is currently conceived, is likely to be polyphyletic. Gene with regions more variable than the nLSU regions, but less variable than the ITS regions sequenced in this study will be required to determine generic relationships among these fungi. Among the species of *Gnomonia* that have been sequenced to date, only *G. gnomon* and *G. setacea* may actually represent the monophyletic genus.

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Artikel/Article: [The type species of the genus \*Gnomonia\*, \*G. gnomon\*, and the closely related \*G. setacea\*. 102-119](#)