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Trochila ilicina (Helotiales, Ascomycota), a fungus newly found in Poland

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The morphological properties of *Trochila ilicina* (Helotiales, Ascomycota), a fungus previously not recorded in Poland, were described and illustrated. *Trochila ilicina* was associated with living, decaying and fallen leaves of *Ilex aquifolium*. However, this fungus formed apothecia only on leaves isolated from their plant host. Hence, *T. ilicina* was considered both a weak parasite and a saprotroph preferring decaying and necrotic leaves, and not only a saprotroph as all earlier published data suggested. This fungus has been found in different places of Szczecin, as well as in the Arboretum Glinna and the Arboretum Przelewice, north-western Poland. Additionally, the known distribution of *T. ilicina* in the world is presented.

Key words: distribution, north-western Poland, *Ilex aquifolium*, *Trochila ilicina*

INTRODUCTION

According to Bugała (2000), of the ca. 100 plant species of the genus *Ilex* L., only a few may be cultivated in the Western Pomerania and the Lower Silesia, i.e., in warmer regions of Poland. One of them is *I. aquifolia* L.

While collecting specimens of diseased plants of the Western Pomerania province, a common occurrence of small spots on leaves of *I. aquifolia* has been found. In the laboratory, a few days after the location of the leaves in wet chambers, apothecia of *Trochila ilicina* (Nees ex Fr.) Greenhalgh et Morgan-Jones (Helotiales, Ascomycota), a fungus previously not recorded in Poland, developed on their upper side.

The aim of this paper was to describe and illustrate *T. ilicina* found in Poland, to compare its characters with those earlier published, and to present the known distributions of this fungus in the world.

MATERIALS AND METHODS

Blotted leaves *I. aquifolia* and leaves of this plant with no disease symptoms were collected in Szczecin (53°26'N, 14°35'E), as well as in the Arboretum Glinna

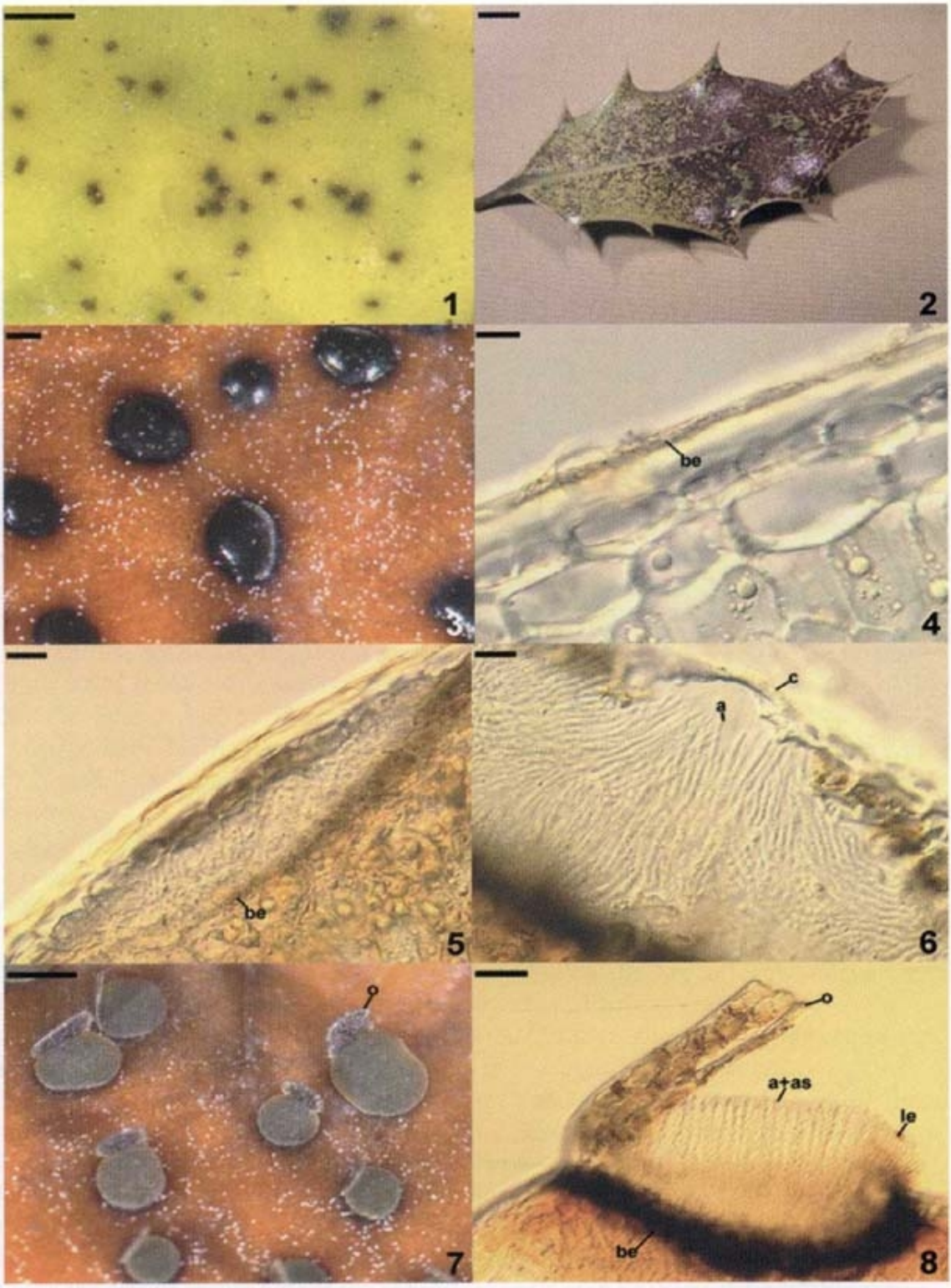
(53°18'N, 14°43'E) and the Arboretum Przelewice (53°06'N, 15°05'E), in April to July of 2004. In the laboratory, a part of the leaves were dried between sheets of a blotting-paper, and the others were incubated in wet chambers for up four weeks. Subsequently, the leaves were daily observed both with the naked eye and under an OLYMPUS SZx9 dissecting microscope. Using a safety razor, thin cuttings were taken from transverse sections of the fungal fruit bodies originating and the leaves of its host. The cuttings were placed in drops of lactic acid, polyvinyl alcohol/lactic acid/glycerol (PVLG; Koske and Tessier 1983), and a mixture of PVLG and Melzer's reagent (1:1 v/v). The cuttings were examined under an Olympus BX 50 compound microscope equipped with differential interference contrast optics. Measurements of the fungal structures were made using an ocular micrometer. Microphotographs were captured in a Sony 3CCD colour camera coupled to the microscopes mentioned above. Colour names are from Kornerup and Wanscher (1983). Nomenclature of plants is according to Mirek et al. (1985). Dried leaves of *I. aquifolia* hosting T. *ilicina* and microscopic specimens of this fungus mounted in PVLG on slides are deposited in the Department of Plant Pathology (DPP), University of Agriculture, Szczecin, Poland.

DESCRIPTIONS OF THE SPECIES AND DISCUSSION

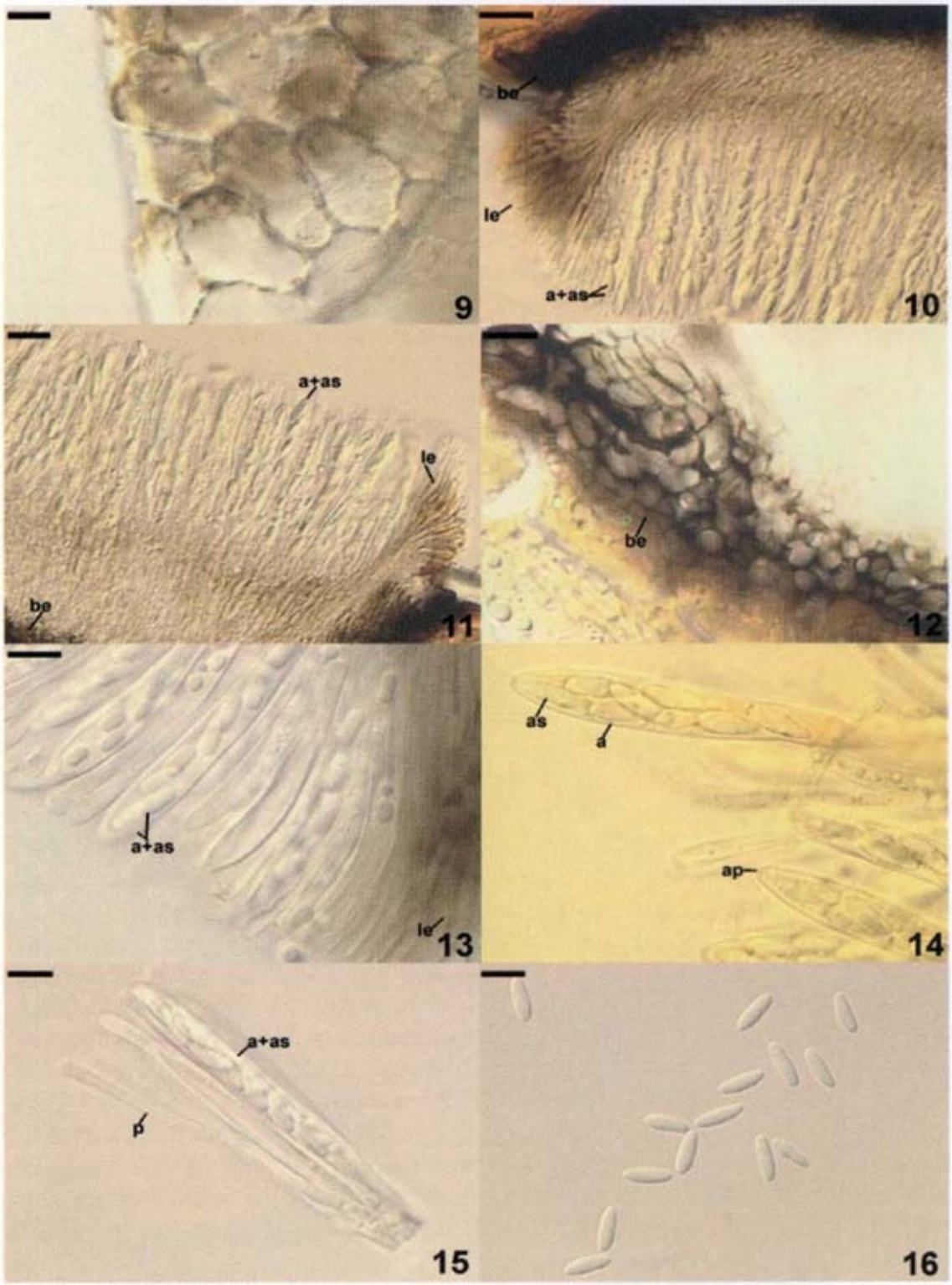
Trochila ilicina (Nees ex Fr.) Greenhalgh et Morgan-Jones

Leaf spots at first single, small, 1-2 mm diam (Fig. 1), later numerous, randomly distributed on the upper leaf surface, finally confluent and covering almost the whole upper leaf surface (Fig. 2). In the laboratory, in wet chambers, first glistening swells, 0.6-1.0 mm diam, and later apothecia appear on the spots (Fig. 3). *Apothecia* sessile, slightly immersed in the tissue of the upper side of infected leaves, 0.6-1.0 mm diam, usually with upraised operculum attached to one of the lateral excipulum when mature (Figs 7 and 8). Operculum consists of angular cells, 20-30 x 8-20 μm (Fig. 9). *Lateral excipulum* hyaline, maize yellow (4A6) to golden wheat (4B5), (16.5-)22.5-28.0(-27.9) μm thick, consisting of prolate, thin- to thick-walled cells, 2.0-6.6 μm wide, slightly broadened and rounded at their tops, usually forming feathery walls bended toward the outside of the apothecium (Figs 10 and 11). *Bottom excipulum* yellowish brown (5D8) to brown (5E8), (13.2-)20.8-36.5(-42.6) μm thick, consisting of subcircular, angular, (3.2-)5.8(-9.3) μm diam, ovoid to irregular, 3.2-4.2 x 6.1-12.5 μm, thick-walled cells, tightly adherent to each other (Figs 10-12). *Asci* clavate, straight or slightly curved, 6.6-9.6 x 57.6-93.4 μm, narrowed up to 2.5 μm at the base, with eight ascospores, and an apical pore, ca. 0.5 μm diam; asci separated by filiform, unbranched, hyaline paraphyses, 1.8-2.2 μm and up to 4.2 μm wide at the base and the top, respectively, usually slightly longer than the asci (Figs 13-15). *Ascospores* elliptical, hyaline, euseptate, 2.7-5.1 x 9.8-15.9 μm (Figs 13-16). In Melzer's reagent, the apical pore of asci sometimes stained violet white (15A2), whereas ascospores always remained non-reactive (Fig. 14).

The apothecium of *T. ilicina* started to form under the cuticule, directly below the spots of the upper leaf surface (Figs 4-6). The first structure being formed was the bottom excipulum (Fig. 4). Later, asci and paraphyses gradually rose from the bot-



Figs 1-8. Disease symptoms of leaves of *Ilex aquifolium* infected by, as well as macro- and microscopic characters of *Trochila ilicina*. 1 and 2. Leaf spots. 3. Swells on the upper leaf side. 4. Bottom excipulum developing under the cuticule. 5 and 6. Developing asci (a) inside leaves; bottom excipulum (be) and the cuticule (c) are indicated. 7 and 8. Burst apothecia with attached operculum (o); asci with ascospores (a+as), as well as lateral (le) and bottom (be) excipulum are visible. Figs 1-3, bright field; Figs 4-8, differential interference contrast. Figs 4-8, specimens in PVLG; Bars: Figs 1 and 7=0.5 mm; Fig. 2=1 cm; Figs 3=200 μm; Figs 5, 6=20 μm; Fig 4=10 μm.



Figs 9-16. Disease symptoms of leaves of *Ilex aquifolium* infected by, as well as macro- and microscopic characters of *Trochila ilicina*. 9. Operculum. 10-11. mature apothecia with ascospores (as) inside asci (a), as well as lateral (le) and bottom (be) excipulum. 13. Asci (a) with ascospores (as); prolate cells of lateral excipulum (le) are showed. 14. Ascospores (a) and apical pore (ap) of asci (a). 15. Asci with ascospores (a+as) and paraphyse (p). 16. Ascospores. Figs 9-16, differential interference contrast. Figs 9-13, 15 and 16, specimens in PVLG; Fig. 14, specimen in PVLG+Melzer's reagent. Bars: Figs 10 and 11=20 μm; Fig 9 and 12-16=10 μm.

tom excipulum (Figs 5 and 6). When the development of the fungus proceeded, the fungal stroma increased the extent of its occurrence to the next layers of epidermis and slightly below it. In the last stage of development, ascospores originated. The developing apothecium inside the leaves, pressing on the under surface of the cuticule, first resulted in the origin of glistening swells on the leaf surface (Fig. 3). Later, the swells burst and exposed the ascoma of the fungus (Fig. 7). Freshly protruded apothecia were partly covered with the operculum that later detached.

The genus *Trochila* has been erected by Fries (1849). The first species of the genus was *T. craterium* (DC.) Fr. Other fungi included also were *T. taxi* Fr., *R. ilicis* (Schleicher) Fr., and *T. laurocerasi* (Desm.) Fr. In the same paper, Fries also listed *Stegia ilicis* Fr. Earlier, Chevalier (1826) transferred *S. ilicis* to the genus *Eustegia* Fr., and Rabenhorst (1844) to the genus *Stegilla* Reichb. Later, both Crouan and Crouan (1867) and Rehm (1896) transferred this fungus from *Eustegia* to *Trochila* as *T. ilicis* (Rabenh.) Crouan and *T. ilicis* (Chev.) Rehm, respectively. However, Dennis (1968) and Greenhalgh and Morgan-Jones (1964) concluded that the name *T. ilicis* (Chev.) Crouan used was a later homonym of *T. ilicis* (Schleicher) Fr. and, hence, invalid. Greenhalgh and Morgan-Jones (1964) examined specimens of *Sphaeria ilicina* Nees, a fungus mentioned by Fries (1823), and found it to be conspecific with *T. ilicis* (Chev.) Crouan. Consequently, they recombined this epithet as *T. ilicina* using the basionym *S. ilicina* Nees ex Fr.

Collection examined. POLAND. Szczecin, April-July 2004; the Arobretum Glinna, 8 July 2004; the Arboretum Przelewice, 7 June 2004; associated with leaves of *I. aquifolia*; Błaszkowski J., 2573 and 2574 (DPP).

Distribution and habitat. Poland. The Western Pomerania province. On leaves of *I. aquifolium* growing in Szczecin, the Arboretum Glinna, and the Arboretum Przelewice.

No other report exists of the occurrence of *T. ilicina* in Poland.

General distribution. Literature data inform of records of *T. ilicina* in Germany (Rabenhorst 1896), Great Britain (Dennis 1968; Greenhalgh and Morgan-Jones 1964), temperate regions of USA (Farr et al. 1989), and Canada (Ginns 1986). According to G. Shannon (http://www.nifg.org.uk), this fungus was also found in many regions of Asia, Russia, southern Europe, and Africa.

Discussion. The genus *Trochila* comprises ca. 15 fungal species. Of them, the only member of this genus found to colonize leaves of *I. aquifolia* is *T. ilicina*.

The morphological properties of *T. ilicina* encountered by the authors of this paper generally fit those given by Rehm (1896), Greenhalgh and Morgan-Jones (1964), and Dennis (1968). Small differences found regard the size of asci and ascospores, as well as the reactivity of the apical pore of asci in Melzer's reagent. The asci examined in this study were slightly longer (up to 93.4 μm) than those earlier encountered (up to 80 μm; Rabenhorst 1896; Dennis 1968). Although the size of ascospores of the specimens examined by the authors of this paper more or less overlapped with that cited in the literature, the ascospores of specimens coming from Poland were both slightly longer (up to 15.9 μm) and wider (up to 5.1 μm) than the longest (up to 12.5 μm; Greenhalgh and Morgan-Jones 1964) and the widest (up to 4.5 μm; Rabenhorst 1896; Greenhalgh and Morgan-Jones 1964; Dennis 1968) ascospores of specimens collected in other regions of Europe. Finally, all the authors mentioned above informed of amyloid properties of

the apical pore of asci of *T. ilicina*, whereas the apical pore of asci of the Polish specimens generally remained non-reactive or infrequently stained violet white (17A2) in Melzer's reagent and a mixture of PVLG and Melzer's reagent.

Although *T. ilicina* has been so far considered a saprotroph (Greenhalgh and Morgan-Jones 1964; Dennis 1968; Rehm 1896), the presence of spots on and the development of apothecia from leaves with no disease symptoms suggest this fungus to be both a weak parasite and a saprotroph.

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Trochila ilicina (Helotiales, Ascomycota), grzyb po raz pierwszy znaleziony w Polsce

Streszczenie

Opisano i zilustrowano cechy morfologiczne *Trochila ilicina* (Helotiales, Ascomycota), gatunku grzyba wcześniej nie notowanego w Polsce. *Trochila ilicina* była związana z żywymi, zamierającymi i nekrotycznymi liśćmi *Ilex aquifolia*. Jednak grzyb ten formował apotecja tylko na liściach odizolowanych od ich rośliny gospodarza. Dlatego, *T. ilicina* została uznana za słabego pasożyta i saprotrofa preferującego zamierające i nekrotyczne liście, a nie tylko saprotrofa, jak sugerowały wszystkie dotychczas opublikowane dane. Grzyb ten znajdywano w różnych miejscach Szczecina, jak również w Arboretum Glinna i Arboretum Przelewice położonych w północno-zachodniej Polsce. Ponadto przedstawiono poznane rozmieszczenie *T. ilicina* w świecie.