

**TETRAPLOA ARISTATA BERKELEY & BROOME  
(FUNGI, PLEOSPORALES), A NEW TAXON TO POLAND**

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ABSTRACT

*Tetraploa aristata* Berkeley & Broome represents the anamorphic stage of a pleomorphic fungus with holomorph *Tetraplosphaeria tetraploa* (Scheuer) Kaz. Tanaka & K. Hiray (≡ *Lophiostoma tetraploa* (Scheuer) Aptroot & K.D. Hyde). Until now this taxon has not been reported in Poland, where it is probably on the fringe of its distribution. Conidia of *Tetraploa aristata* were found during palynological analyses of modern surface samples and later on the decaying leaves of *Phleum phleoides* from xeric grasslands in Kraków and its vicinity. Since *Tetraploa aristata* was discovered during palynological analysis, this method might be considered to be a useful tool for investigations of contemporary microfungual mycobiota.

KEY WORDS: *Tetraploa*, Hyphomycetes, Pleosporales, *Lophiostoma*, *Massarina*, *Tetraplosphaeria*, modern pollen spectra.

INTRODUCTION

*Tetraploa* Berkeley & Broome represents the anamorphic (asexual) stage of a pleomorphic fungus, a member of the dematiaceous staurospore hyphomycetes (Ellis 1971). A teleomorph of *Tetraploa aristata* was described by Scheuer (1991) as *Massarina tetraploa* Scheuer (Ascomycetes, Pleosporales). The holomorph of *Tetraploa aristata* and *Massarina tetraploa* was established by Hyde et al. (2002) as *Lophiostoma tetraploa* (Scheuer) Aptroot & K.D. Hyde (see also Index Fungorum 2009). Recently Tanaka et al. (2009) created a new pleosporan family Tetraplosphaeriaceae Kaz. Tanaka and K. Hiray to integrate *Tetraploa*-like fungal anamorphs and created a new combination *Tetraplosphaeria tetraploa* (Scheuer) Kaz. Tanaka & K. Hiray for holomorph *Lophiostoma tetraploa*.

Pleosporales are a scarcely recognized group in Poland; only 11 species of genus *Lophiostoma* Ces. & De Not. and 6 of *Massarina* Sacc. have been reported (Mulencko et al.

2009). Until now 14 members of the genus *Tetraploa* have been described worldwide (Index Fungorum 2009).

This taxon has not been hitherto reported in Poland. In 2008 and 2009, during palynological analysis of modern surface samples collected from xeric grasslands in Kraków and its vicinity, *Tetraploa aristata* was discovered at several localities within this area. The purpose of this paper is to present the morphology of Polish specimens of the fungus, give ecological notes and some information about its distribution in Poland.

*Palaeobotanical data*

Fossil remains of the genus *Tetraploa* (also known as *Frasnacritetrus* Taugourdeau, see Worobiec et al. 2009) are definitely known from the Late Cretaceous and regularly reported from the Miocene from pollen spectra mainly outside Europe (Samant and Mohabey 2009; Worobiec et al. 2009). Identical to *Tetraploa aristata* are microfossils Type 89: *Tetraploa aristata* Berkeley & Broome found in

Holocene peat deposits in the Netherlands and Germany (Van Geel 1978). *Tetraploa aristata* was also found in Poland in the Upper Miocene deposits of Józefina (Worobiec et al. 2009) and in sediments of the Gołębiewo I site (a peat bog in the Gdańsk Uplands) and occurrence of its conidia was dated back to about 4200-3700 yrs cal. BP (Pędziszewska 2008).

According to Worobiec et al. (2009) the presence of *Tetraploa aristata* in fossil pollen spectra indicates a warm temperate climate. Sharma (1976), Saxena and Sarkar (1986) and Worobiec et al. (2009) found fossil conidia of *Tetraploa* associated with graminaceous pollen grains, sometimes in quantity (Worobiec et al. 2009). Taking into consideration that contemporary *Tetraploa aristata* is usually found on grasses and other monocotyledons, the incidence of fossil conidia of *Tetraploa aristata* implies a considerable share or even dominance of monocotyledons (especially grasses) in local vegetation and possibly the presence of open vegetation e.g. grasslands (comp. Peglar 1993).

## MATERIAL AND METHODS

More than 10 subsamples as material for analysis were taken using a random pinch technique from an area ranging from 1 and 4 m<sup>2</sup> in Piekary up to an area of 100 m<sup>2</sup> within Kraków. The subsamples were usually parts of mosses, soil surface samples and detritus, which were homogenized together in plastic bags in order to obtain one amalgamated sample for palynological analyses (Adam and Mechringer 1975). A description of the surrounding vegetation was formulated according to the Braun-Blanquet (1965) method. The identification of plant communities was done using the Matuszkiewicz manual (2005).

For palynological analyses subsamples of 1 cm<sup>3</sup> from every sample were acetolyzed using a modified Erdtman method (Faegri and Iversen 1989). One *Lycopodium* tablet of a known number of spores was added to every sample in order to evaluate the concentration of counted sporomorphs (Stocmarr 1971). After this preparation, slides for microscopic analyses were made and were then analyzed under a ZEISS AMPLIVAL microscope using 400× and 1000× magnification. The conidia of *Tetraploa* found in the slides were assigned to the species *Tetraploa aristata* on the basis of a paper by Van Geel (1978). This identification was checked later with experts who confirmed it (C. Scheuer and K.D. Hyde person. comm.).

In order to find this taxon in situ on its possible host, supplementary samples of the dead leaves of grasses were collected in July 2009 in previously chosen areas in Piekary. Macroscopic analyses were done using a Nikon SMZ 800 stereoscopic microscope under 63× magnification. The investigated leaf remains were then acetolyzed and the slides subsequently prepared were scanned under a microscope to finally confirm the presence of conidia of *Tetraploa aristata*.

## RESULTS

### *Taxonomy and morphology*

Anamorph: *Tetraploa aristata* Berk. & Broome, Ann. Mag. nat. Hist., Ser. 2 5: 459 (1850).

Teleomorph: *Massarina tetraploa* Scheuer, Mycol. Res. 95(1): 126 (1991); Ascomycetes, Dothideomycetes, Pleosporales, Massarinaceae.

Holomorph: *Tetraplospheeria tetraploa* (Scheuer) Kaz. Tanaka & K. Hiray, in Tanaka et al., Stud. Mycol. 64: 182

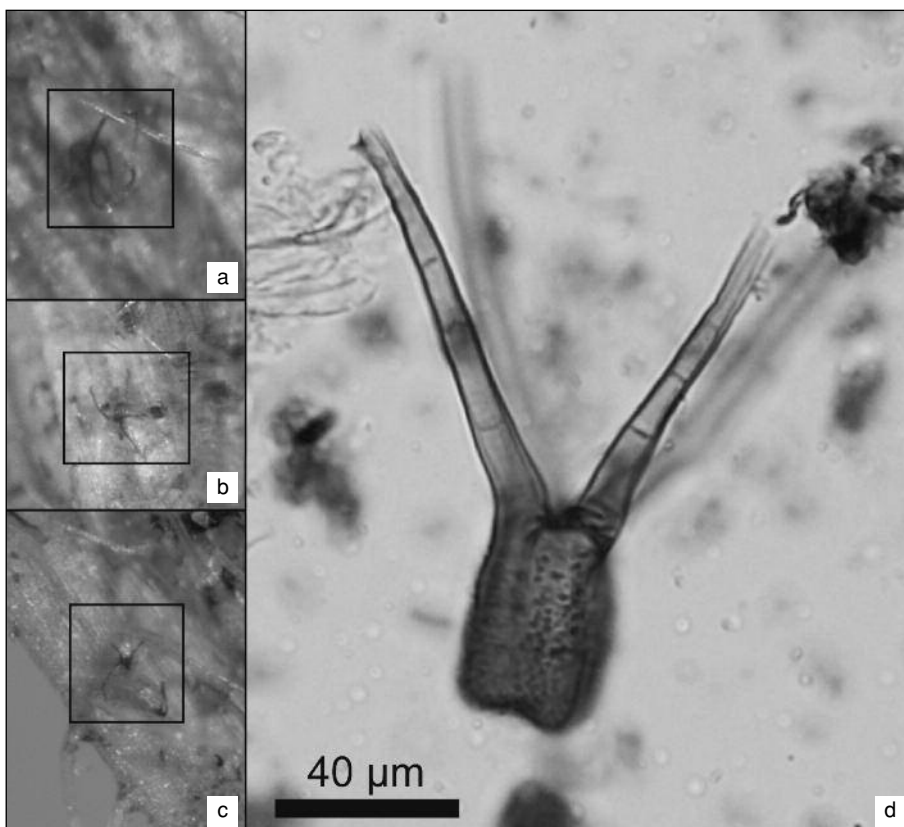


Fig. 1. Conidia of *Tetraploa aristata*: a-c – on dead leaves of *Phleum phleoides* (63× magnification); d – from modern surface sample (400× magnification). Photo: M. Karpńska-Kończak.

TABLE 1. A description of vegetation at the localities with the occurrence of *Tetraploa aristata* with a number of conidia found in the surface soil sample and their estimated concentration.

No.	Site	Association type; main species occur within analysed plot	Number of conidia found in sample	Estimated concentration of conidia (/cm <sup>3</sup> of surface sample)
1	Piekary I	<i>Koelerio-Festucetum rupicolae</i> ; <i>Phleum phleoides</i> (20%) and <i>Stachys recta</i> (10%)	33	2619
2	Piekary II	<i>Geranium sanguineum</i> aggregation; <i>Geranium sanguineum</i> (about 95%)	1	107
3	Mydlniki	<i>Koelerio-Festucetum rupicolae</i> (initial phase); <i>Festuca rupicola</i> (20%), <i>Phleum phleoides</i> (10%), <i>Vicia grandiflora</i> (10%) and <i>Artemisia campestris</i> (10%)	10	2255
4	Krzemionki	<i>Koelerio-Festucetum rupicolae</i> ; <i>Festuca rupicola</i> (25%), <i>Thymus austriacus</i> (25%) and <i>Brachypodium pinnatum</i> (10%)	16	2094
5	Wzgórze Benedykta	<i>Koelerio-Festucetum rupicolae</i> (degenerated); <i>Festuca rupicola</i> (40%), <i>Koeleria macrantha</i> (30%) and <i>Thymus</i> sp. (30%)	1	177
6	Bielany I	<i>Koelerio-Festucetum rupicolae</i> ; <i>Festuca rupicola</i> (30%) and <i>Koeleria macrantha</i> (20%)	1	166
7	Bielany II	<i>Koelerio-Festucetum rupicolae</i> ; <i>Festuca rupicola</i> (30%) and <i>Arrhenatherum elatius</i> (20%)	4	719
8	Bielany III	<i>Koelerio-Festucetum rupicolae</i> ; <i>Festuca rupicola</i> (30%), <i>Koeleria macrantha</i> (20%) and <i>Phleum phleoides</i> (5%)	5	926

(2009); Ascomycetes, Dothideomycetes, Pleosporales, Tetraplosteriaceae [= *Lophiostoma tetraploa* (Scheuer) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Diversity Res. Ser. (Hong Kong) 7: 108 (2002); Ascomycetes, Dothideomycetes, Pleosporales, Lophiostomataceae].

In the investigated material the conidia of typical *Tetraploa aristata* are verrucose and consist of 4 columns of two or four cells (every column is 38-42 µm long and 16-19 µm width), which terminates in septate appendages, which are verrucose pigmented and up to 80 µm long (Fig. 1).

This description was prepared on the basis of the selected well-preserved forms, due to the fact that most of the conidia found were severely damaged.

#### Ecological notes

*Tetraploa aristata* is usually found as a saprophyte on grasses and other monocotyledons (Ellis 1971) although it has rarely been recorded on dicotyledons (*Citrus*, *Eucalyptus*, *Heracleum*, *Impatiens*, *Liquidambar*, and *Quercus*) and occasionally also on *Pinus* (Farr et al. 2009). It is sometimes found on decaying wood and leaf litter (Vittal 1976; Révay 1993) as well. Some researchers (e.g. Schoenlein-Crusius and Piccolo Grandi 2003) have reported *Tetraploa aristata* together with aquatic hyphomycetes. *Tetraploa aristata* is reported on rare occasions as a human pathogen (Markham et al. 1990).

Within the area of Poland the presence of *Tetraploa aristata* conidia was confirmed on the dead leaves of *Phleum phleoides* from the Piekary I site near Kraków (Table 1, Fig. 2). In this and other sites the taxon was detected in modern surface samples. Specimens of *Phleum phleoides* occurred within most of examined plots and in the vicinity of every of them. The highest concentration of conidia was found in modern surface samples collected from the Piekary I, Mydlniki and Krzemionki sites and exceeded a value of 2000 specimens/cm<sup>3</sup> (Table 1). What is more in these plots *Phleum phleoides* occurred more numerous than in the others. Taking into consideration a broad spectrum of monocotyledonous hosts of *Tetraploa aristata*, the presence of this taxon is also possible on dead leaves of other

grass species which were detected in the analyzed plots e.g. *Agrostis capillaris*, *Arrhenatherum elatius*, *Briza media*, *Dactylis glomerata*, *Elymus repens*, *Festuca rubra*, *F. rupicola*, *Koeleria macrantha* and *Poa pratensis*.

#### Distribution

*Tetraploa aristata* is distributed mainly in subtropical and tropical regions (Ellis 1971; Farr et al. 2009). Until now neither *T. aristata* nor other species of *Tetraploa* have been reported in the contemporary mycobiota of Poland, and neither have *Massarina tetraploa* and *Lophiostoma tetraploa* (Mułenko et al. 2009). In other European countries conidia of *Tetraploa aristata* have been reported in Belgium (de Meulder 2002), Hungary (Révay 1993), Denmark, Germany, Italy, and United Kingdom (Farr et al. 2009), all countries with a mild climate. *Tetraploa* sp. (probably *T. aristata*) has been found in Spain (Diaz et al. 1998) and France (Proctor and Lambert 1961).

Conidia of the genus *Tetraploa* (mainly of *Tetraploa aristata*) are found as an accessory element among airborne fungal spores in warm regions of the world, e.g. Taiwan, Mexico, Spain, Australia, Canary Island, India, Martinique, USA, Italy (Tseng and Chen 1982; Calderón et al. 1997; Diaz et al. 1998; Mitakakis and Guest 2001; La-Serna et al. 2002; Jothish and Nayar 2004; Desbois et al. 2006; Codina 2008; Magyar et al. 2009). In Poland they have been observed in the course of aerobiological investigations only in Kraków (D. Myszkowska and D. Stępałska pers. comm.). Conidia of *Tetraploa* are sporadically transported by wind to such far-flung places as Antarctica (Marshall 1996).

Conidia of *Tetraploa aristata* were found in eight sites in Poland, six of which are located within the area of Kraków and two in Piekary (6 km to the south-west of Kraków) (Table 2, Fig. 2).

#### CONCLUSIONS

1. *Tetraploa aristata* is a new species for contemporary Polish microfungi mycobiota.

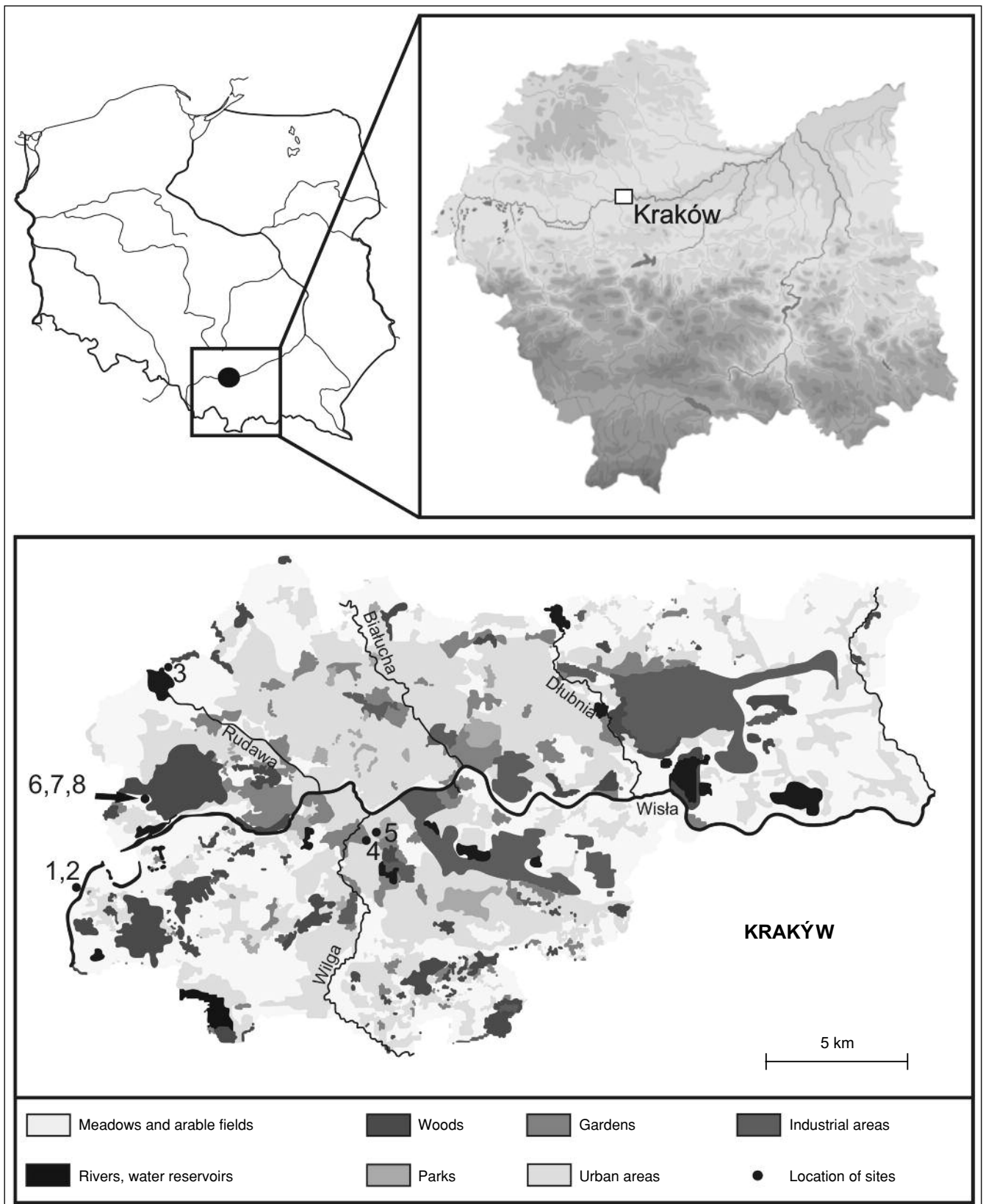


Fig. 2. Location in Poland and distribution of *Tetraploa aristata* in Kraków and its vicinity. Numbers of sites follows numbers in Table 1. Based on the map – Xerothermic grasslands in Kraków (2009).

2. This taxon occurs in Europe mainly in the Mediterranean area and Western Europe, and its closest sites are in Hungary and Germany. Polish sites of *Tetraploa aristata* located in Kraków and its vicinity are probably on the fringe of its distribution, hence its connection with xeric habitats with strong exposure to the sun and a warm microcli-

mate. This microfungus usually grows on decaying leaves of grasses, and its occurrence in situ was confirmed on *Phleum phleoides* specimens.

3. Palynological analysis of modern surface samples might be useful for analyses of microfungal mycobiota in different habitats and may contribute to the finding of new taxa.

TABLE 2. Description of localities of *Tetraploa aristata*. The numbers follow Table 1 and Figure 2.

No	Site	Site description	Latitude (°N)	Longitude (°E)	Altitude (m a.s.l.)	Exposure	Inclination	Date of sample collection	Leg.
1	Piekary I	Part of a rocky limestone slope	50°05'40.6''	19°47'39.1''	207	SW	65.00%	10.06.2008	M. Karpińska-Kołaczek
2	Piekary II	Part of a limestone hill	50°05'40.7''	19°47'39.3''	207	S	10.00%	10.06.2008	M. Karpińska-Kołaczek
3	Mydlniki	Part of the north-western slope of Krzemionki hill within a limestone quarry that was working until the beginning of the 20th century	50°05'15.6''	19°50'38.3''	230	SEE	10.00%	07.05.2009	M. Karpińska-Kołaczek
4	Krzemionki	Part of the north-western slope of Krzemionki hill within a limestone quarry that was working until the beginning of the 20th century.	50°02'09.6''	19°56'39.7''	220	NE	40.00%	28.04.2009	M. Karpińska-Kołaczek
5	Wzgórze Benedykta	An area on the western slope of Krzemionki hill that is being quarried for limestone (The "Nad Matecznym" quarry)	50°02'12.5''	19°56'45.0''	215	S	40.00%	03.06.2009	M. Karpińska-Kołaczek
6	Bielany I	Part of the southern slope of Srebrna Góra located within the main part of the Wolski Forest on an upper section of the hill in a part of the slope which is seasonally exploited as arable land.	50°02'41.2''	19°49'53.4''	250	SW	10.00%	14.06.2009	M. Karpińska-Kołaczek
7	Bielany II	Part of the southern slope of Srebrna Góra located within the main part of the Wolski Forest on the slope of a gully that has been cut into the hill from the south with numerous outcrops of limestone	50°02'41.7''	19°49'53.6''	254	SW	10.00%	14.06.2009	M. Karpińska-Kołaczek
8	Bielany III	Part of the southern slope of Srebrna Góra located within the main part of the Wolski Forest on the top part of the hill on the boundary between seasonally exploited arable land and a road	50°02'42.1''	19°49'53.5''	251	NW	5.00%	14.06.2009	M. Karpińska-Kołaczek

4. Palaeobotanical analysis combined with an analysis of modern pollen and spore influx might give an answer to the question as to whether *Tetraploa aristata* in Poland is a relic, an invasive taxon, or a southern (southwestern) element on the fringe of its distribution.

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