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Research article/Araştırma makalesi

Morphological, anatomical and karyological investigations on the genus *Paris* in Turkey

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Abstract

This study presents morphological, anatomical and karyological characteristics of the genus *Paris* L. represented by 2 species in Turkey: *P. incomplete* M. Bieb. and *P. quadrifolia* L. Taxonomically detailed description of the species are based on collected and examined specimens; distributions and illustrations are presented along with anatomical cross sections of leaves, stems, rhizomes and roots and leaf surfaces. The differing anatomical structure of studied plant parts is suitable for use as an additional tool in their identification. Karyotypes of the species are determined, *P. incompleta*, as 2n = 10, diploid, chromosome formula is 2m+4sm+2st+2t and *P. quadrifolia* as 2n = 20, tetraploid, chromosome formula is 4m+8sm+2st+2t. Photographies of species in naturel habitat, distribution map of species in Turkey, detailed drawing of flowers and general view of species, drawings and microphotographies of the karyotypes are also presented.

Key words: *Paris*, morphology, anatomy, taxonomy, karyotype

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Türkiye'deki *Paris* cinsi üzerinde morfolojik, anatomik ve karyolojik araştırmalar

Özet

Bu çalışma Türkiye'de 2 tür (*P. incomplete* M. Bieb. ve *P. quadrifolia* L.) ile temsil edilen *Paris* L. cinsinin morfolojik, anatomik ve karyolojik özelliklerini içermektedir. Türlerin toplanan ve incelenen örneklere dayanarak yapılan detaylı tanımlamaları, dağılımları ve çizimleri ve yaprak, gövde, rizom ve köklerin enine kesitleri ve yaprakların yüzeysel kesitleri de yer almaktadır. Çalışılan bitki kısımlarının farklılık gösteren anatomik yapıları bitkileri ayırmak için kullanılabilmektedir. Türlerin karyotip özellikleri belirlenmiştir, *P. incomplete* kromozom sayısı 2n=10 diploid, kromozom formülü 2m+4sm+2st+2t, *P. quadrifolia*'nın kromozom sayısı 2n=20 tetraploid, kromozom formülü 4m+8sm+2st+2t olarak tespit edilmiştir. Türlerin doğal ortamda çekilmiş fotoğrafları, Türkiye'deki yayılışını gösteren harita, bitkilerin çiçekleri ve genel görünüşlerinin detaylı çizimleri, karyotiplerinin çizimleri ve mikrofotoğrafları da ayrıca yer almaktadır.

Anahtar kelimeler: *Paris*, morfoloji, anatomi, taksonomi, karyotip

1. Introduction

The genus *Paris* L. of the family *Melanthiaceae* (Ji et al., 2006; Reveal & Chase, 2011; Yue et al., 2011; Seberg et al., 2012) comprises approximately 24 rhizomatous perennial herb species that occur from Europe to eastern Asia (Li et al., 1998; Zomlefer et al., 2006; Jacquemyn et al., 2008). China is the center of the species diversity with 22 species, of which 12 are endemic (Liang & Soukup, 2000). According to analysis of DNA sequence, the genus *Paris* is monophyletic (Ji et al., 2006).

The classification of the genus is still unresolved. Hara (1969) divided it into 3 sections based on fruit and seed characteristics: Sect. *Paris*; Sect. *Kinugasa* Tatewaki et Suto; and Sect. *Euthyra* Salisb. According to Takhtajan's (1983) classification, the sections *Paris*, *Kinugasa* and *Daiswa* are different genera based on fruit types, ovary shapes,

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seed morphology and rhizome shapes. In the most recent taxonomic revision, Li (1998) following Hara's treatment, divided *Paris* s.l. into 2 subgenera; the subgenus *Daiswa* (Rafinesque) H. Li (13 species of 5 sections) and subgenus *Paris* (11 species of 4 sections) based on axile or incompletely axile placentation versus parietal placentation, respectively. Previous molecular data (Osaloo & Kawano, 1999; Farmer & Schilling, 2002) supported Takhtajan's treatment.

Different taxa of Liliaceae recently investigated according to their anatomical, cytological by different researchers (Dane, 2006; Gürsoy & Şık 2010; Kahraman et al. 2010; Ocak et al. 2004; Uysal 1999; Doğu et al. 2011) in Turkey. But this study is the first comprehensive survey of the genus *Paris* in Turkey. The current anatomical and karyological studies were initiated with a view to clarifying the taxonomic status of the species. Detailed descriptions and distributions with anatomical and karyological features are presented of 2 Turkish species collected from natural habitats.

The present study forms part of a doctoral thesis titled 'Pharmaceutical Botanical Studies on the Genera *Paris* L., *Polygonatum* Mill. and *Veratrum* L. in Turkey', carried out by Yeter Yeşil and supervised by Prof. Dr. Neriman Özhatay.

2. Materials and methods

Research specimens of *P. incompleta* were collected from natural habitats in the Artvin, Rize and Trabzon areas of Turkey during flowering and fruiting stages; specimens of *P. quadrifolia* were collected from Ardahan during July – August 2010 and 2011. Specimens were collected from as many different locations as possible in order to identify patterns of variation. In addition the collections of the following herbaria were examined: AEF, ANK, GAZI, Hb. Linn., HUB, ISTE, ISTF, ISTO, KATO, K, BATU, BM, E and P.

Anatomical research material was preserved in 70% ethanol. Cross-sections of leaves, stems, rhizomes and roots, and the surface sections of leaves were investigated. Sections were stained using Sartur (a compound reagent that consists of Lactic acid, Sudan III, Aniline, Iodine, Potassium iodide, alcohol and water) and chloral hydrate reagents. Drawings were made using a camera lucida drawing tube attached to a Leitz Wetzlar microscope. Photographs were taken with an Olympus BH– 2 microscope.

For karyological studies, rhizomes were collected from natural habitats and transplanted to the Alfred Heilbronn Botanic Garden, Istanbul University. Root tips were pretreated with 0.05% 1 –Bromonaphthalene solution at 4°C for 24 h. and then fixed in fresh Carnoy solution overnight. Root tips were hydrolyzed for 10 – 12 min. in 1NHCl at 60ºC and stained in Feulgen solution for 1 – 2 h. Stained meristems were squashed in a drop of 2% aceto orcein and permanent preparations were made by the liquid CO_2 method. Photographs were taken via a light microscope. Ideograms were prepared with measurements made on enlarged microphotographs of well-spread metaphase plates of different individuals. For all chromosome counts, a minimum of 10 plates (representing 10 different individuals of each locality) were examined for each taxon. The classification of chromosomes, length of the long and short arms, total length of arms, arm ratio, centromeric index, and relative chromosomal length were measured with an Image Analysis Systems KAMERAM©. Chromosomes were classified by the nomenclature of Levan et al. (1964); 1.00-1.7 median, 1.7-3.00 submedian, 3.00-7.00 subterminal, 7.00-∞ terminal.

3. Results

A detailed description of the Turkish species of *Paris*; illustrations, distribution and an identification key are given, based on the examined specimens. Anatomy of leaves, stems, rhizomes and roots are presented by drawings and microphotographs. Karyotypes of the species are also presented and compared with previous records..

3.1. Taxonomical and morphological characteristics:

The identification key of Turkish species:

Leaves 6 – 9 (– 13), perianth uniseriate, mucro of stamen 0.3 – 0.4 mm……..*P. incompleta*

Leaves 4 – 5, perianth biseriate, mucro of stamen 4 – 7 mm…… ……*P. quadrifolia*

***Paris incompleta* M. Bieb., Fl. Taur.–Cauc. 1: 306 (1808). (Figure 1).**

Syn: *P. apetala* Hoffm., Comm. Phys. Med. Mosc. I (1808) 5; *P. octophylla* Hoffm., Hort. Mosq. (1808) 27. **Ic:** M.B., Cent Pl. Rar. II – III (1843) tab. 74; Exs.: Pl. Or. Exs. No. 330; Fiori, Fl. Italy Illus. (1933) Pl. 718; Fl. Cauc. Exs. No. 30; Komarov, Fl. URSS 4 (1935) 360.

Perennial, rhizomatous herbs. Rhizomes creeping scaly, terete, 5 – 6 mm diam., odoratous. Stems 21 – 40 cm, 4 – 4.5 diam, dark green at the top, green–purple at the base. Leaves verticillate, 6 – 12 (– 13), usually obovate sometimes oblanceolate rarely eliptic, (6 –) 7.8 – 11 × (2 –) 3 – 5 cm, lower surface pale green, upper surface vivid green, prominently 3–veined, with faint secondary veins, apex usually acuminate, narrow acuminate, the base acute narrowed into short (2 – 3 mm) petiole. Pedicels 3 – 6.7 (–8) cm. The flowers terminal and solitary. Perianth segments,

uniseriate, usually 4, enlarged on bottom half, lanceolate or ovate–lanceolate, prominently 3–veined, green (25 –) 27 – 37 (– 40) × (2 –) 10 – 14 mm, acuminate at the apex, inner whorl absent. Stamens (6 –) 8 – 9. Filaments dark green, 6 – 10 × 1 mm, purple at the base in mature, some with basal oblong nectaries, length 2–3 mm. Anthers basifixed, longitudinalis, yellow, (5 –) 6–8 mm, apex mucronate with 0.3 – 0.4 mm mucro. Pollen grains yellow. Ovary black, subglobose with 4 loculus with 6 ovules in each loculus. If with 5 loculus 1 loculus in sterile. Styles black, 4 – 5 parted, (10 –) 14 – 18 mm, usually much longer than stamens. Berry black, globose, depressed on top, 5 – 7 (– 8) × 5 – 7 mm, glaucescent. Seeds brown, ovoid, about 3 × 3 mm diam. *F.l. 5 – 6, steep shady slopes, under Picea orientalis and Fagus orientalis forests and Rhododendron scrubs, 400 – 2200 m.*

Figure 1: *Paris incompleta* in natural habitat

Type: Described from the Caucasus (Tiflis) in Leningrad (LE).
Distribution: Caucasia and North Anatolia. Euxine element.
Distribution in Turkey: Central & East of Northern Turkey (App. I).

***Paris quadrifolia* L., Sp. pl. 1: 367 (1753). (Figure 2)**
Syn: *P. dahurica* Fisch., Bull. Soc. Nat. Mosc. II, 201. *P. verticillata* M. B., Fl. Taur-caus. III, 287; Ldb., Fl. Ross IV (1841) 121. *P. obovata* Ldb., Fl. Ross. IV, 120. **Ic:** Hegi, Illust. Fl. Mit. Eur. II (1908) p. 275; Bonnier, Fl. Comp. Fr. Suis. Belg. X (1911) Pl. 593; Komarov, Fl. URSS 4 (1935) 359; H. Hess, Landolt, Hırzel, Fl. Der Schweiz Band I (1967) p. 530; Ross–Craig, Draw. Br. Pl. XXIX (1972) Plate 33.
Perennial, rhizomatous herbs. Rhizomes creeping scaly, terete, 2 – 2.5 mm diam., odoratous. Stems (18 –) 23 – 40 cm, 2 – 2.5 mm diam., dark green at the top, green-purple at the base. Leaves verticillate, 4 – 5, usually broad obovate or obovate, (6 –) 8 – 10 (– 13) × 4 – 6 cm, lower surface pale green, upper surface vivid green, prominently 3 –veined, with faint secondary veins, apex usually acuminate with a mucro, the base cuneate, narrowed into short ((1.5 –) 2 – 3 mm) petiole. Pedicel 3 – 6 cm. The flower terminal and solitary. Perianth segments biseriate, usually 8. Outer perianth segments 4 – 5, enlarged in the middle, lanceolate, prominently 3-veined, green, 20 – 35 (– 40) × 3 – 4 mm, acute at the apex. Inner perianth segments 4-5, linear, prominently 1–veined, green, 4 – 5, (12 –) 18 – 24 × 1 – 2 mm, acuminate at the apex. Stamen 8–9. Filaments dark green, 3 – 5 × 1.5 mm, purple at the base in mature. Anthers basifixed, longitudinalis, yellow, (3 –) 4 – 6 mm, apex mucronate with 4 – 7 mm mucro. Pollen grains yellow. Ovary black, subglobose with 4 loculus with 6 ovules in each loculus. If with 5 loculus 1 loculus without ovules. Styles black, 4 – 5 parted, (3 –) 4 – 6 mm, much shorter than stamens. Berry black, depressed globose, on top, 6 – 8 × 7 – 9 × 5 – 7 mm. Seeds brown, ovoid, about 3 × 3 mm. *F.l. 5* – 6, *vet slopes, mixed coppice dominated by Betula litwinowii, 1800 – 2100 m.*

Type: Lectotype (designated by Mathew, in Regnum Veg. 127: 73, 1993): Right–hand specimen, Hb. Linn. 515.1.
Distribution: Europe & NE Anatolia Euxine element.
Distribution on Turkey: Ardahan and Artvin (App. II).
The distribution map of *Paris* species (As shown in Figure 3) and drawings are following (As shown in Figure 4).

Figure 2: *Paris quadrifolia* in natural habitat

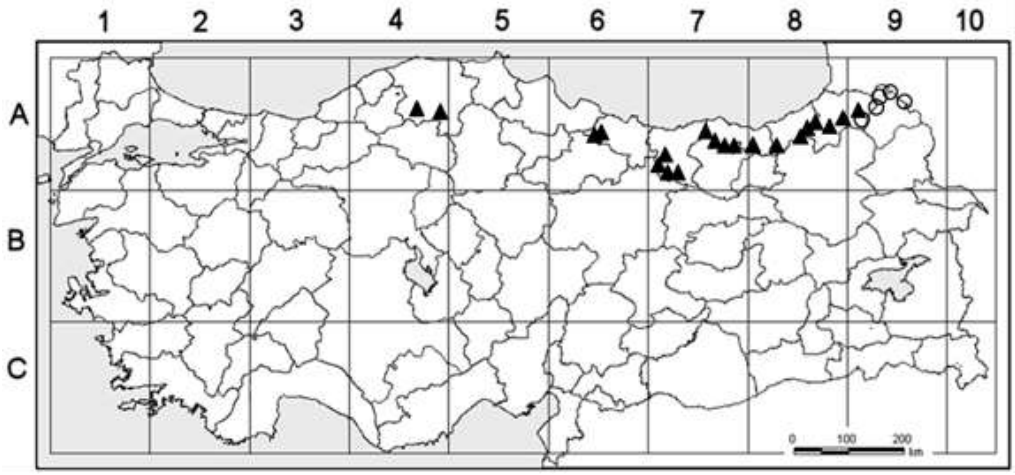

Figure 3: Distribution map of *P. incompleta* (▲) and *P. quadrifolia* (O) in Turkey.

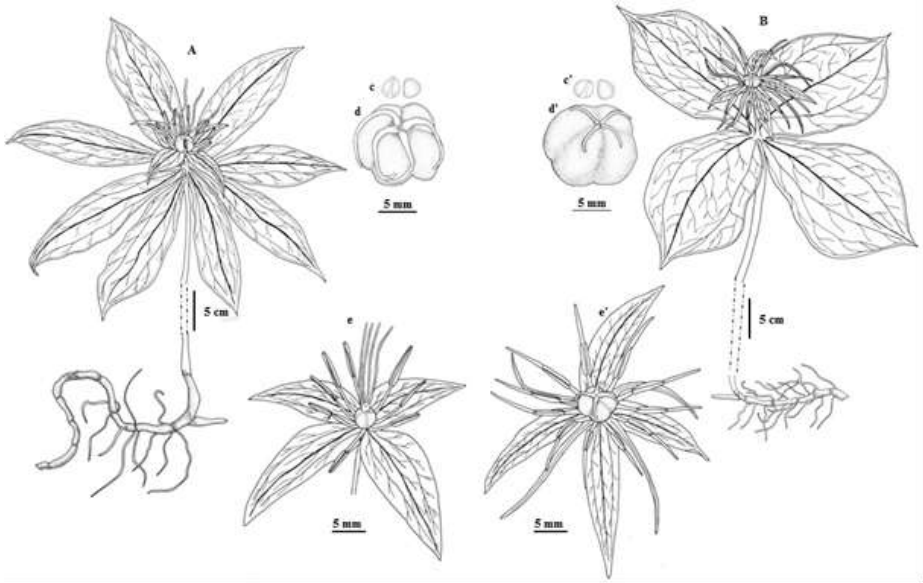

Figure 4: General view of A *P. incompleta* and B *P. quadrifolia*; c & c' seed, d & d' fruit, e & e' flowers.

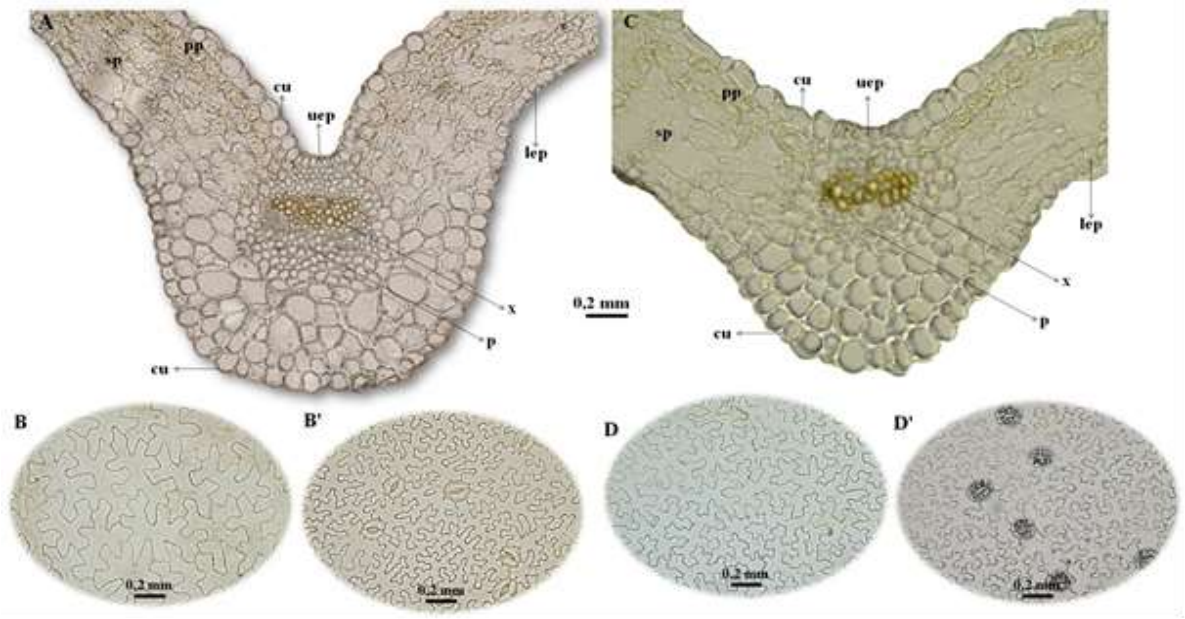

Figure 5: A Cross section of main vein and B upper surface section, B′ lower surface section of *Paris incompleta*, C Cross section of main vein and D upper surface section, D′ lower surface section of *Paris quadrifolia*.

3.2. Anatomical characteristics:

LEAF: Bifacial and hypostomatic

Mesophyll

Palisade parenchymatic tissues are 1 – 2 layered on adaxial surface; spongy parenchymatic tissues are 4 – 5 layered on abaxial surface. No crystals observed (As shown in Figure 5 A, B).

Vascular bundle

Leaves have 3 main parallel veins, interconnected by smaller veins. Closed collateral vascular bundles are larger in the main veins, and much smaller in the small veins without sclerenchymatous sheaths (As shown in Figure 5 A, B).

Epidermis

In the cross-section, the epidermis consists of a single layer with square or rectangular cells, upper epidermis cells are larger than lower ones. Stomata are at the same level with as the epidermis. The cuticle is thick on both epidermises. No hairs were observed (As shown in Figure 5 A, B).

In the surface section, epidermis cells are distinctly lobed. Stomata are anomocytic and only occur on the abaxial surface, neighboring cells are 4 – 5 (As shown in Figure 5 C, C', D, D').

STEM

The epidermis is composed of a single layered, with compactly arranged square cells, and is covered with a thick cuticula. *Paris incompleta* does not have angles, a layer of 1 – 3 layered sclerenchymatous cells is present beneath the epidermis (As shown in Figure 6 A–A'). *Paris quadrifolia* has angles, 2 layered collenchymatous cells occur on an angle beneath the epidermis, collenchymatous cells are single layered on inter-angle and are almost circular; this is followed by 4 – 5 layered sclerenchymatous cells in cortex (As shown in Figure 6 B–B'). Ground tissues are distributed throughout the space beneath the sclerenchyma, which is composed of circular cells. Vascular bundles are scattered throughout the cortex. However, vascular bundles are much smaller under sclerenchyma than those in the central area (As shown in Figure 6).

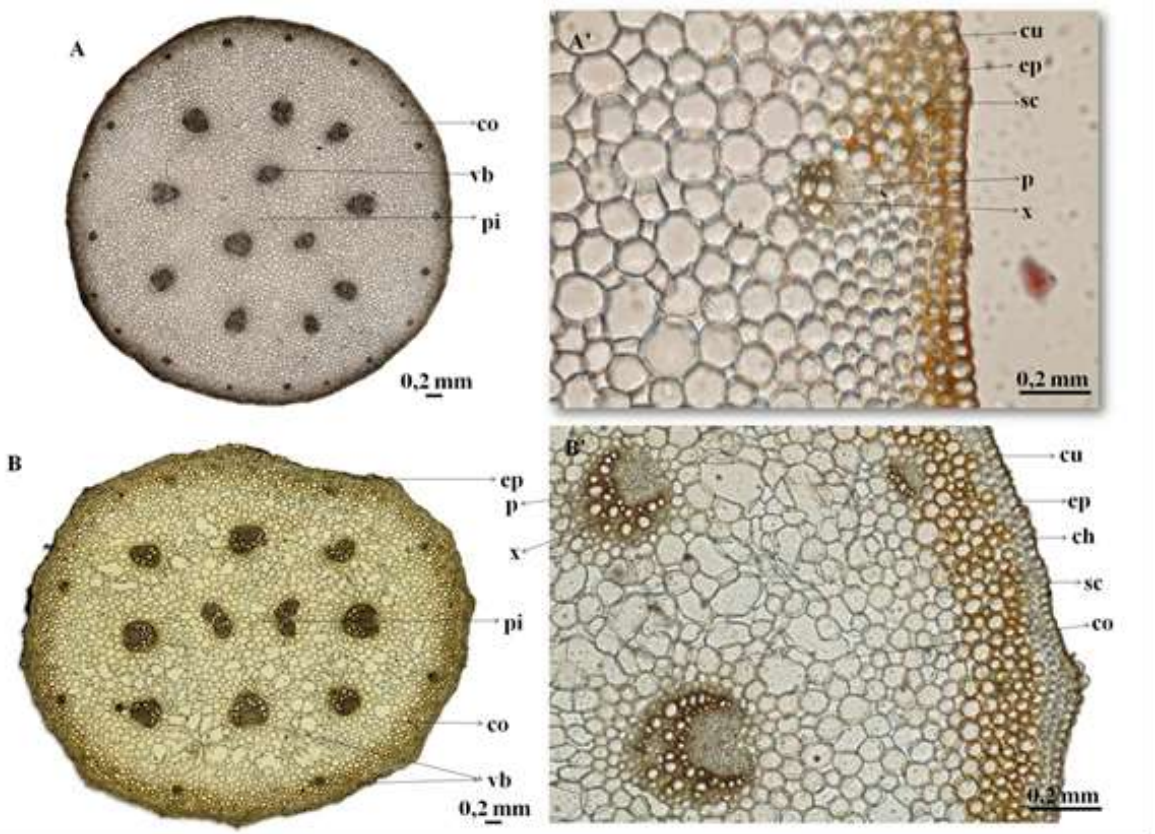

Figure 6: Cross section of stems.A – A' *Paris incompleta*, B – B' *Paris quadrifolia*; co cortex, col collenchyma, cu cuticula, ep epidermis, p phloem, pi pith, sc sclerenchyma, x xylem, vb vascular bundle.

RHIZOME

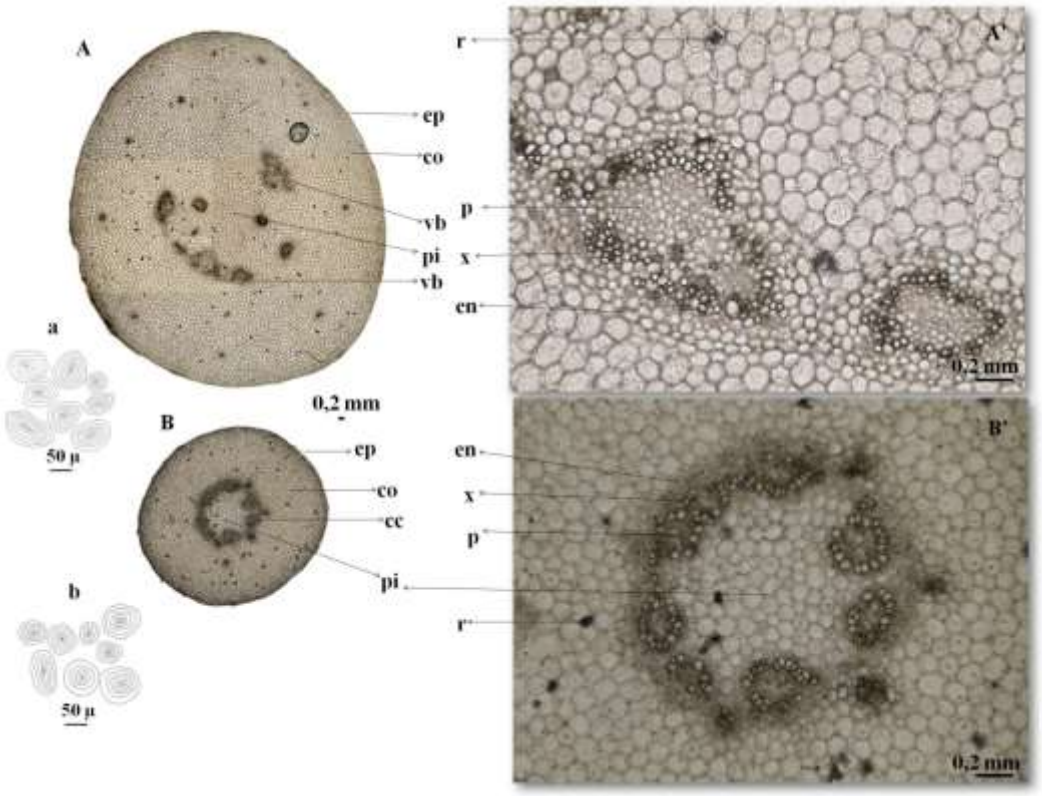

Figure 7.Cross section of rhizomes: A – A' *Paris incompleta*, B-B' *P. quadrifolia*, A' – B'Vascular bundle, a starch grain of *P. incompleta*, b starch grain of *P. quadrifolia*, cc central circle, co cortex, en endodermis, ep epidermis, p phloem, pi pith, x xylem, vb vascular bundle.

Epidermis is 2 layered, cells are square and rectangular, suberized. Cortex parenchymatic cells are filled with starch grain; raphides occur in the smaller size cells. The endodermis is either constant or inconstant. Vascular bundles are closed collateral and amphivasal type, 17 to 24. Small vascular bundles are spread throughout the cortex. Large vascular bundles are spread throughout the cortex or packed closely together. Some bundles can be seen on pith (As shown in Figure 7).

ROOT

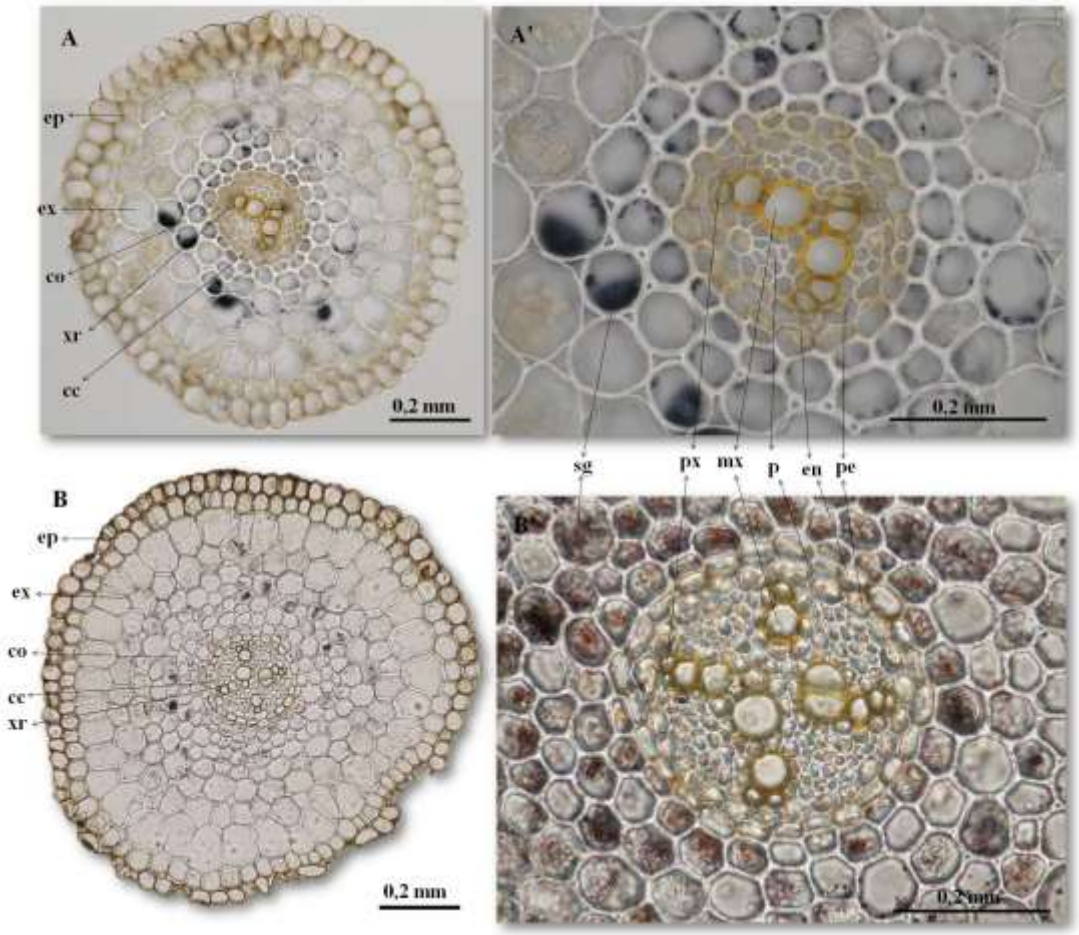

Figure 8.Cross section of roots: A – A' *Paris incompleta*, B – B' *Paris quadrifolia*; cc central circle, co cortex, cu cuticle, en endodermis, ep epidermis, ex exodermis, mx metaxylem, p phloem, pe pericycle, pi pith, px protoxylem, sg starch grain, x xylem, xr xylem ringe, vb vascular bundle.

Epidermis is single layered, cells are longitudinally elongated and isodiametric, often with persistent root hairs. The exodermis is single layered, cells are isodiametric and with thickened wall. Exodermal cells are smaller than epidermal cells. The cortex is 5 – 6 layered parenchymatic cells, bearing raphides and abundant starch grains. The endodermis is single layered, cell walls are of equal thickness. The pericycle is single layered and thin-walled. There are 4 or 5 xylem ridges located opposite the phloem. Metaxylem and protoxylem vessels have the same wall thickness and the central parenchymatous pith is present (As shown in Figure 8).
The starch grains observe in rhizomes and roots of species. Raphide crystals observes in stem, root and especially in rhizome.

3.4. Karyological characteristics:

The examined specimen measurement were presented in Table 1-2. Polymorphism in chromosome length and heterochromatin distribution was also observed between individuals within the same species, for almost all chromosome pairs, especially first pair and first set Intraindividual polymorphism in the relative length of homologous chromosome was also detected in the karyotypes. In this study, The karyotypes and the total and relative length of somatic chromosome pairs of Turkish species are reported here for the first time.

Table 1.Chromosome measurement of *Paris incompleta* collected from Artvin (Art.) Rize (Rize) and Trabzon (Trb.); AR Arm Ratio, As. K % The index of karyotypic asymetry, Chr. p. n. Chromosome pair number, CT chromosome type, TL Total Length of arms, LA Long Arm, RL Relative Length, SA Short Arm.

Chr	LA (µ)			SA (S) (µ)			TL (µm)			AR (L/S)			RL(%)			CT
p.n.	Art.	Rize	Trb.	Art.	Rize	Trb.	Art.	Rize	Trb.	Art.	Rize	Trb.	Art.	Rize	Trb.	
1	8.90	9.43	8,32	8.36	8.82	7.32	17.26	18.35	15.64	1.06	1.06	1.13	30.66	30.72	29.45	m
2	7.41	7.97	6.62	4.23	4.68	4.25	11.64	12.63	10.87	1.75	1.7	1.68	20.68	21.14	20,47	sm
3	5.55	5.60	5.10	3.18	3.33	3.02	8.73	8.93	8.12	1.74	1.68	1.68	15.51	14.95	15.29	sm
4	9.08	9.55	8.88	2.40	2.41	2.38	11.48	11.97	11.25	3.77	3.95	3.71	20.39	20.04	21.18	st
5	6.65	7.31	6.71	0.51	0.53	0.51	7.17	7.85	7.22	13.1	13.81	12.74	12.73	13.14	13.59	t

Table 2.Chromosome measurement of *P. quadrifolia*. AR Arm Ratio, Chr. p. n. Chromosome pair number, TL Total Length of arms, LA Long arm, RL Relative Length, SA Short Arm

Chr. p.n.	**LA (µ)**	**SA (µ)**	**TL (µ)**	**AR (L/S)**	**RL(%)**	**CT**
1	7.54	6.99	14.54	1.07	16.25	m
2	6.75	6.29	13.04	1.06	14.57	m
3	7.41	3.68	11.1	2	12.40	sm
4	4.65	2.72	7.37	1.71	8.23	sm
5	4.30	2.55	6.85	1.69	7.65	sm
6	4.41	2.63	7.04	1.68	7.86	sm
7	6.71	1.80	8.51	3.72	9.51	st
8	4.68	1.53	6.21	3.05	6.94	st
9	6.97	0.61	7.58	11.43	8,47	t
10	6.66	0.55	7.22	12.01	8.07	t

Karyotypes of *P. incompleta,*

Examined specimens: Artvin-Murgul (ISTE 97694, 97695), Rize-Çamlıhemşin (ISTE 97651, 97639), and Trabzon-Maçka (ISTE 97490, 97591, 97594, 97639). Detailed locality data are given App. I. Vouchers are hosted at ISTE.

Chromosomes numbers were counted as 2n = 10 (2x), diploid. The karyotype consists of 1 pair of median, 2 pairs of submedian, 1 pair of subterminal and 1 pair of terminal chromosomes. The chromosome formula can be summarized as 2n = 2x= 10 = 2m+4sm+2st+2t. Chromosomes of the fifth pair of Artvin and Rize populations have a satellite on the short arms, whereas the Trabzon population has a satellite on the short arm of only 1 chromosome (As shown in Figure 9).

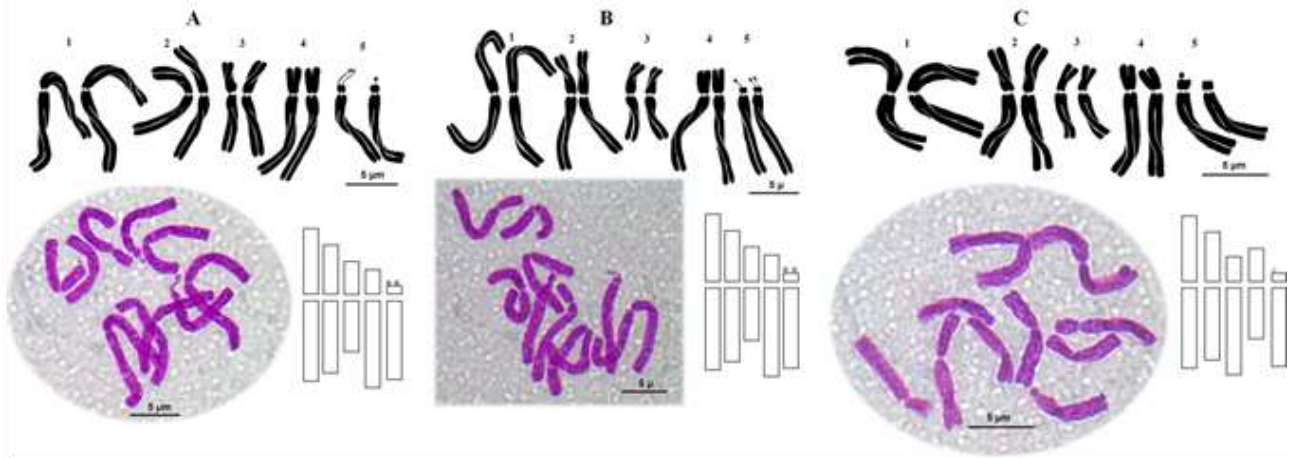

Figure 9. The somatic chromosomes of *Paris incompleta* in metaphase; karyogram and ideogram. A Artvin, B Rize, C Trabzon

Karyotype of *P. quadrifolia,*
Examined specimens: Ardahan (ISTE 97505). Detailed locality data are given App. I. Vouchers are hosted at ISTE.
Chromosome numbers were counted as 2n = 20 (4x) chromosomes tetraploid. The karyotype consists of 1 set of median, 2 sets of submedian, 1 set subterminal and 1 set terminal chromosomes. The chromosome formula can be summarized as 2n = 4x= 20 = 4m+8sm+4st+4t. A ninth pair has a satellite on the short arm (As shown in Figure 10).

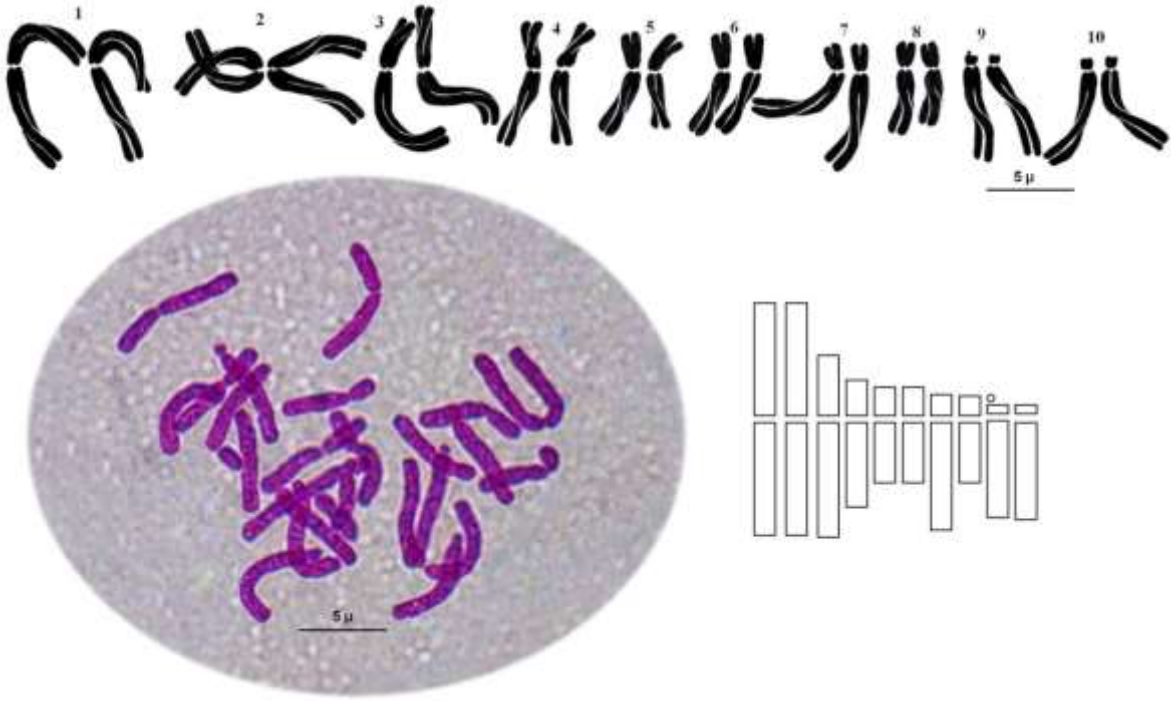

Figure 10: The somatic chromosomes of *Paris quadrifolia* in metaphase, karyogram and ideogram

4. Conclusions and Discussion

4.1. Morphology

The results (e.g., leaf, periant segment, filament, style) obtained from morphological investigations are mainly congruent with the description in *Flora of Turkey and the East Aegean Islands* (Miller, 1984), and recently recorded *Paris quadrifolia* (Terzioğlu, 2008). However morphological features show some differences from those given in *Flora of Turkey*. The dimensions of some characteristics such as size and shape of nectar, size of stamen mucro, diameter of rhizome, seed shape and size were not been reported in previous studies (Table 3).

Table 3. Morphological characteristics of *Paris* species

Species	**Morphological**					
	Leaf num.	**Length of style**	**Nectar**	**Stamen mucro size**	**Seed size**	**Rizom diameter**
P. incompleta	6-12 (-13)	(10-)14-18 mm	present	0.3-0.4 mm	3×3	5-7 mm
P. quadrifolia	4-5	(-3) 4-6	absent	4-7 mm	3×2.5	2-2.5 mm

4.2 Anatomy

Anatomical features of the roots of *Paris* species resemble the general characteristics of monocotyledones (Cutter, 1971). The comparative microscopic observations of the transverse section of leaf, stem, rhizome, and root and surface section of leaves of 2 species of *Paris* revealed that many of these anatomical characteristics are homologous. However, some differences were observed in the upper leaf surface section, the stomata cells size, the stomata index, cross-section of stem and rhizome, and characteristics of starch grains (Table 4).
Leaf, the upper epidermis cells of *Paris incompleta* have secondary lobes but the upper epidermis cell of *Paris quadrifolia* have primary lobes.
Rhizome, constant or inconstant endodermis, distribution and numbers of vascular bundles are the most useful characteristic in the general anatomy of the rhizome of *Paris* species (Xue et al., 2009). Endodermis of *Paris*

incompleta is inconstant. Vascular bundles are 20 – 24. Some bundles can be seen on pith. A few of the vascular bundles are closely packed, whereas the majorty is dispersed.
Endodermis of *Paris quadrifolia* is constant. Vascular bundles are 17 – 20. Most of the vascular bundles are closely packed whereas a minority dispersed.
The starch grains of *P. incompleta* are larger than those of *P. quadrifolia* and are of different shapes (Table 4).
Table 4. Anatomical characteristics of *Paris* species

Species	upper epidermis cells	stomata size	stoma index	Stem	Rizom		Starch g. shape & size
					endoderm is	vascular bundles	
P. incompleta	primary lobed	54.65×62 .31 µ	23.07	not angled, collenchyma absent in cortex	inconstant	interrupted circle	ellipsoidal 7,50×6,25
P. quadrifolia	secondary lobed	51.89× 2.45 µ	28.57	angled, collenchyma present in cortex	constant	a circle	spheroidal 5,64×5,44

4.3 Karyology
Paris incompleta, the index of karyotypic asymetry (As. K%) of Artvin population is 66.8%, Rize population is 67.6% and Trabzon population is 75.4% and for *P. quadrifolia* is 66,61%. Detailed measurements of the chromosomes are given on Table 1 and 2. This study confirms most of the earlier studies as tetraploid level with somatic chromosomes number of *P. incompleta* (Tifonovi, 1969; Gagnidze et al., 1985; Schneeweiss & Schneeweiss, 2003) and *P. quadrifolia* (2n = 20 Gotoh, 1933; Gotoh, 1937; Darlington, 1941; Love & Love, 1944; Kozlova, 1979; Bjerketvedt & Laane, 1982; Laane, 1990; Lavrenko & Serditov, 1991; Punina et al., 2000) but don't confirm some studies (2n = 10 Haga, 1934).

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Appendix I Examined specimens
P. incompleta
A4: Kastamonu**:** Ilgaz Mountain, N-NW. 1100 – 1300 m, 06.viii.1996, *Alper Yılmaz* (ISTO 28249!); Kastamonu–Pınarbaşı, Kurtgirmez Dağı, 1250 m, 24.viii.2002, forest, *Ufuk Özbek* (MUÖ 1353!); A6: Ordu: above Ünye, 1220 m, *Furse* & *Synge* 137! (ISTE)!; Ordu: Merkez, Çambaşı–Turnalık, 1820 m, 10.vi.1981, *H.Demir* & *Y. Karaağaçlı* (KATO 5569!); A7: Giresun**:** Merkez, Kulakkaya–Akçakbel yöresi, 1600 m, 22.v.1979, *Picea* forest, *R. Anşin* (KATO 3382!); Tirebolu Managment Directorate, Serial of Akılbaba, 1350 m, 05.ix.1989, *L. Görnaz* (ISTO 32450!); Bektaş-Bulancak, Bicik plateau, 1700 m, 22.viii.1995, *Abies nordmanniana* and *Picea orientalis* forest, *A.J.Byfield, S. Atay B1983* (ISTE 69335!); Bektaş–Bulancak, Bicik plateau, 1640 m, 02.08.2010, *Abies nordmanniana* and *Picea orientalis* forest, *Y.Yeşil* & *B.Sağlam* (ISTE 97754!); A7: Trabzon: Maçka, Hamsiköy, 1525 m, 1933, *Balls* 385! (E photo)!; Trabzon**:** Maçka, Hamsiköy future, Çifteköprü stream, vicinity of Yıkık Değirmen, 1450 m, 03.vi.1969, under forest, *A.Baytop* (ISTE 15310!); Maçka, Hamsiköy-village of Gürgenağaç, North West, 60%, 850 m, 25.v.1973, *Picea-Fagus* forest. *R.Anşin* (ISTO 16538!); Tonya, Valley of Fol Stream, 1100 m, 20.vii.1979, broadleaved forest, *O.Beşel* & *A.Demirci* (KATO 4137!); Maçka, Meryemana forest, Zalişki Mount., 1280 m, around Sümela and Altındere village, 19.v.1974, *Picea orientalis* forest, *R.Anşin* (KATO 1369!); Merkez, Yeşilova village, 350 m, 14.iv.1990, under forest, *A.Tüfekçioğlu* (KATO 8710!); Şalpazari, Kireçhane, 1450 m, 16.vii.2006, under forest, *S. P.Uzun* (KATO 16534!); Şalpazari, back of Kalpakkaya, Eskioba plataeu, 1790 m, 08.viii.2006, under forest, *S.P.Uzun* (KATO 16504!); Şalpazari, Geyikli near forest, outdoor, 1487 m, 11.viii.2007, *S.P.Uzun* (KATO 16501!); Maçka, Hamsiköy, stream edge, under forest, 147 m, 11.ix.2007, *M. Karaköse* (KATO 8687!); Maçka, stream edge 320 m, 01.vi.2008, broadleaved mixed forest, *S. Terzioğlu* (KATO 7856!); Maçka, Ormanüstü Köyü, 1280 m, 09.vii.2008, under forest, *A.Uzun* (KATO 18752!); Maçka, Ormanüstü Köyü, 1642 m, 15.vii.2008, under forest, *A.Uzun* (KATO 18753!); Maçka, Hamsiköy, around destroyed mill, upper side of Çifteköprü stream, north slope, 1534 m, 03.viii.2010, *Y.Yeşil, B.Sağlam* & *A.Alışkan* (ISTE 97488!); Maçka, between Hamsiköy-Zigana, Bekçiler side, 1640 m, 03.viii.2010, *Y.Yeşil, B.Sağlam* & *A.Alışkan* (ISTE 97490!); Maçka, Hamsiköy, the upper of Dikkaya site, 1031 m, 27.vi.2011, *Fagus orientalis* and *Carpinus orientalis* forest, *Y.Yeşil, A.Osançlıol* & *A.Alışkan* (ISTE 97591!); Maçka, lower of Zigana Mountain Rest Facilities (ZİTAŞ), Bekçiler side, 1600 m, 27.vi.2011, *Y.Yeşil, A.Osançlıol* & *A.Alışkan* (ISTE 97594!); Sürmene, Asot, Araklı-Bayburt road, Boğalı Stream, 1560 m, 03.viii.2010, broadleaved mixed forest, *Y.Yeşil* & *B.Sağlam* (ISTE 97639!); A7: Gümüşhane**:** Kürtün, Örümcek forest, environment of Elceyiz Plateau, 1970 m, 08.viii.1990, under forest, *M.Küçük*

(KATO 9152!); A8: Trabzon**:** Of, Karayer, 35 m, 01.iv.1993, wet and shadowy stream edge, *S.Terzioğlu* (KATO 10459!); Of, Çataldere Köyü, 120 m, 16.v.1996, under forest, wet area, *S.Terzioğlu* (KATO 13061!); A8: Rize: Uzunkaya, *A.Heil.* & *M.Baş.,* 06.vii.1947, (ISTF 7040!); Merkez, Çinçava 460 m; Çamlihemşin, upper of Ayder thermal water, 1800 m, 10.08.1979, *Picea orientalis* forest, *R.Anşin* (KATO 3925!); Rize**:** Çinçava, 460 m, *Guichard* TUR/12/59! (E photo)!; Çamlıhemşin, Aşağı Vice district, 400-900 m, 26.iv.1981, *A.Güner* 3462 (ISTE 50147!); within sight of Çamlıhemşin, Kaleköy, 1780 m, wet broadleaved mixed forest, *Y.Yeşil* & *A.Osançlıol* (ISTE 97651!); İkizdere, within sight of Cimil Yaylası, above Bulanıksu village, 1400 m, 28.vi.2010, wet broadleaved mixed forest, *Y.Yeşil* & *B.Sağlam* (ISTE 97639!); A8: Artvin**:** Çoruh, mt. Above Artvin, *Picea* forest, 1900 m, *Davis* & *Hedge* 29723a! (E photo)!; Çoruh Tiryal Dağı, above Damar ve Murgul, 1500 m, 03.vii.1994, volcanic rocky valleys, *A.J.Byfield* & *D.Pearman B.* 1006 (ISTE 67114!); Cerrahtepe, 1600 m, 28.vii.2004, *Pinus – Abies* and *Piceae* forest, *H. Altınözlü* 4129; Murgul – Damar, 1445 m, 04.vii.2011, broadleaved mixed forest opening area, border of stream, *Y.Yeşil* & *A.Osançlıol* (ISTE 97694!); between Hopa-Borçka, 700 m, 22.v.1980, *R.Anşin* (KATO 4902!); Hopa, Başköy, 250 m, 20.iv.1996, *Castanea, Carpinus* and *Buxus* forest, *M.Keskin* 532 (ISTE 77447!); A9: Artvin**:** Merkez, Soçundibi, 1450 m, 12.v.1978, forestation area, *R.Anşin* (KATO 1978!); Şavşat, Yavuzbey Köyü, Kışlalar, rigth side of Şavşat following hill, 2177 m, 02.vii.2011, broadleaved mixed forest, *Y.Yeşil* & *A.Osançlıol* (ISTE 97695!).

Appendix II, Examined specimens

P. quadrifolia

A9 Posof, Gönülaçan Köyü, 1930 – 2012 m, 17.viii.2007, wet area domination of *Betula litwinowii,* broadleaved mixed forest, *S.Terzioğlu* (KATO 10538!); Posof, Çamyazı, Asmakonak village, Posof, 1960 m, 07.viii.2010, *Betula pendula, B. litwinowii, B. recurvata* mixed forest, *Y.Yeşil* & *B.Sağlam* (ISTE 97502!); Posof Gönülaçan Village, 2015 m, 07.viii.2010, forest, *Y.Yeşil* & *B.Sağlam* (ISTE 97505!); Posof, Çamyazı village, 1978 m, 01.vii.2011, *Betula pedula, B. litwinowii, B. recurvata* mixed forest, *Y.Yeşil*, *A.Osançlıol, L.*& *S.Esen* (ISTE 97682!); Posof, Aşık Zülali village, Karakelle side, 2073 m, 01.vii.2011, *Y.Yeşil*, *A.Osançlıol, L.* & *S.Esen* (ISTE 97686!); Posof, above Baykent village, 2041 m, 01.vii.2011, *Betula pendula, B. litwinowii* ve *B. recurvata* and mixed forest, *Y.Yeşil*, *A.Osançlıol, L.* & *S.Esen* (ISTE 97737!); Posof, Gönülaçan village forest, 1860 m, 01.viii.2011, *Betula pendula, B. litwinowii* ve *B. recurvata* and mixed forest, *Y.Yeşil* & *Ş.Doğru* (ISTE 97747!); Posof, Baykent Köyü, Fado'nun köprüsü side, 1905 m, 01.vii.2011, *Betula pendula, B. litwinowii* ve *B. recurvata* and mixed forest, *Y.Yeşil*, *A.Osançlıol, L.* & *S.Esen* (ISTE 97757!); Artvin**:** Ardanuç, Kütül Mount., 15.vii.1947, forest, *A.Heil.* & *M.Baş,* (ISTF 7592!).

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