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Molecular phylogeny of *Polygonum* L. s.l. (Polygonaceae, Polygonales), focusing on European taxa: preliminary results and systematic considerations based on *rbcL* plastidial sequence data

Abstract - The Polygonaceae (40-50 genera and approximately 1,100 species) are characterized by the presence of an ocrea, an hyaline or membranous tubular sheath, derived from the fusion of the stipules. Their classification is based principally on macromorphological characters. The broad *Polygonum* s.l. is a much debated genus which has been variously interpreted in the literature: its relation to associated genera is not clear and moreover, it has been subdivided into numerous more natural taxa considered at the rank of genus, subgenus or section.

In the present study, using analysis of the *rbcL* sequence the monophyly of the various taxa that *Polygonum* s.l. has been subdivided are investigated by focusing on the species present in Europe in order to reach a classification more in line with the molecular data.

The phylogenetic tree obtained with the maximum parsimony method confirms that Polygonaceae is monophyletic, however, the subfamily Polygonaceae seems to be paraphyletic as it includes the Eriogonoideae. On the basis of the principal clades identified the restriction of the Polygonaceae to only those taxa with an herbaceous habit is confirmed and a new classification articulated in 4 tribes is proposed: 1) Polygoneae, subdivided into Polygoninae (*Atraphaxis*, *Polygonum* -incl. *Polygonella* and, probably, *Calligonum*, *Oxygonum*, *Parapteropyrum* and *Pteropyrum*) and Reynoutriinae (*Fallopia*, *Homalocladium*, *Muehlenbeckia*, *Reyloppia*, *Reynoutria*) as well as an *incertae sedis* genus (*Knorringia*); 2) Persicarieae, subdivided into Persicariinae **subtrib. nov.** (*Persicaria*) and Koenigiinae (*Aconogonum*, *Bistorta*, *Koenigia*, *Rubrivena*); 3) Fagopyreae (*Fagopyrum*, *Harpagocarpus* and, probably, *Eskemukerjea* and *Pteroxygonum*); 4) Rumiceae (*Emex*, *Oxyria*, *Rheum*, *Rumex*).

Lastly new combinations and/or new names are proposed in the genus *Reyloppia*, *Persicaria* and *Rubrivena*.

Key words: Polygonaceae, *Polygonum* s.l., phylogenesis, *rbcL*, classification.

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Riassunto - Filogenesi molecolare di *Polygonum* L. s.l. (Polygonoideae, Polygonaceae), con particolare riferimento alle entità europee: risultati preliminari e considerazioni sistematiche basate sulle sequenze plastidiali *rbcL*.

Le Polygonaceae (40-50 generi e circa 1.100 specie) sono caratterizzate dalla presenza dell'ocrea, una guaina tubolare, membranacea o ialina, derivata dalla fusione delle stipole. La loro classificazione è basata principalmente su caratteri macromorfologici. Un genere molto dibattuto e variamente circoscritto dagli autori è l'ampio *Polygonum* s.l.: i suoi limiti verso i generi vicini non sono molto chiari ed inoltre è stato suddiviso in numerose entità più naturali, variamente considerate a rango di genere, sottogenere o sezione.

In questo lavoro, attraverso l'analisi delle sequenze *rbcL* è stato indagato il monofiletismo dei vari taxa nei quali è suddiviso *Polygonum* s.l., ponendo maggiore attenzione alle specie presenti in Europa, al fine di giungere ad una classificazione maggiormente aderente ai dati molecolari.

L'albero filogenetico ottenuto con il metodo della massima parsimonia conferma il monofiletismo delle Polygonaceae, ma la sottofamiglia delle Polygonoideae appare parafiletica poiché le Eriogonoideae sono annidate al suo interno. In base ai principali cladi individuati, viene confermato il restringimento delle Polygonoideae ai soli taxa ad habitus erbaceo e viene proposta una sua nuova classificazione, articolandola in 4 tribù: 1) Polygoneae, suddivise in Polygoninae (*Atraphaxis*, *Polygonum* -incl. *Polygonella*- e, probabilmente, *Calligonum*, *Oxygonum*, *Parapteropyrum* e *Pteropyrum*) e Reynoutriinae (*Fallopia*, *Homalocladium*, *Muehlenbeckia*, *×Reyllophia*, *Reynoutria*) più un genere *incertae sedis* (*Knorringia*); 2) Persicarieae, suddivise in Persicariinae **subtrib. nov.** (*Persicaria*) e Koenigiinae (*Aconogonum*, *Bistorta*, *Koenigia*, *Rubrivena*); 3) Fagopyreae (*Fagopyrum*, *Harpagocarpus* e, probabilmente, *Eskemukerjea* e *Pteroxygonum*); 4) Rumiceae (*Emex*, *Oxyria*, *Rheum*, *Rumex*).

Infine vengono proposte nuove combinazioni e/o nuovi nomi nei generi *×Reyllophia*, *Persicaria* e *Rubrivena*.

Parole chiave: Polygonaceae, *Polygonum* s.l., filogenesi, *rbcL*, classificazione.

Introduction

The Polygonaceae include approximately 40 to 50 genera and approximately 1,100 species (Dammer, 1892, 1893; Cronquist, 1981; Brandbyge, 1993; Stevens, 2001). They are either annual herbs (eg. *Koenigia islandica* of the Arctic regions), perennial herbs, shrubs, trees (eg. *Triplaris americana* of the equatorial rain forests) or lianas. The leaves develop from the often swollen nodes and are alternate, seldom opposite or whorled, simple, petiolate to sessile, with generally entire margins. The stipules are nearly always well developed and connate within a tubular sheath which may be either persistent or deciduous, hyaline to membranous, bilobed or fringed at the apex and is referred to as the ocrea. The presence of the ocrea is exclusive to the Polygonaceae, and is the most distinguishing morphological characteristic, even if at times it is reduced in size or absent in the subfamily Eriogonoideae. Extrafloral nectary pits are present at the base of the petioles and in the nodal area of the genera *Fallopia* s.l. (incl. *Reynoutria*) and *Muehlenbeckia* s.l. (incl. *Homalocladium*). The axillary or terminal inflorescence is composed of simple or branched thyrsi panicle-, raceme- or spike-like in appearance which, however, are formed of dichasia or helicoid cymes. The partial inflorescences are subtended by bracts while each flower or group of flowers is subtended by a persistent membranous ocreola which corresponds to the fusion of the bracteoles. The flowers are small, trimerous, hermaphrodite or unisexual, and in the latter case being mostly dioecious. Tepals 2-6, prevalently persistent and often accrescent in fruit, fused at the base in a \pm developed hypanthium, forming two whorls of 3 elements or one whorl of 5 elements; in the latter case a tepal is derived from the fusion of

a segment of the outer whorl with one from the inner whorl, determining the characteristic quincuncial aestivation. Stamens being equal, double or triple the number of tepals, ranging from 2 to 9, rarely more, free or basally connate, alternating with the tepals. Tricolporate to pantoporate pollen. Nectaries often present, located between the bases of the filaments or fused into an annular disc at the base of the ovary. Superior ovary, 2-4-carpellate (generally 3-carpellate), unilocular; styles 1-3, free or proximally connate; filiform, peltate or capitate stigmas, entire or variously fringed; unique ovule. Fruit an achene, trigonous or lenticular, mainly subtended by the often accrescent perianth; in more specific terms a diclesium (Spjut, 1994). *Fagopyrum* (buckwheat) and *Coccoloba* (sea grape) produce edible fruit; the petioles of *Rheum* (rhubarb) are edible, as are the leaves of some species of *Rumex* (sorrel). The rhizomes of *Rheum* are also renowned for their medicinal properties. Some genera include ornamental species and common weeds.

The classification of the Polygonaceae has to date been mainly defined on macromorphological characters (presence or absence of an ocrea, woodiness, tepal arrangement) and has been widely debated, so much so that numerous taxonomic schemes have been proposed (see Ronse Decraene & Akeroyd, 1988); two subfamilies Polygonoideae and Eriogonoideae are presently accepted almost universally, based on the presence or absence of the ocrea.

Polygonum s.l. is a widely debated genus which has been interpreted differently by various authors. Its relationships with related genera, such as *Atraphaxis*, *Koenigia*, *Muehlenbeckia* and *Polygonella*, are not very clear; moreover, it has been subdivided into numerous more natural taxa, considered variously at the rank of genus, subgenus or section (see Meisner, 1826, 1856, 1857; Bentham & Hooker, 1880; Dammer, 1892; Gross, 1913a, 1913b; Jaretzky, 1925; Hedberg, 1946; Roberty & Vautier, 1964; Graham & Wood, 1965; Holub, 1971; Soják, 1974; Haraldson, 1978; Tzvelev, 1987; Ronse Decraene & Akeroyd, 1988; Hassan, 1991, 1997; Hassan & Khan, 1992; Hong *et al.*, 1998; Ronse Decraene *et al.*, 2000). The recent biomolecular studies by Cuénoud *et al.* (2002), even if they were on only a few species, and above all those by Lamb Frye & Kron (2003), Kim *et al.* (2005), Kim & Donoghue (2008) and Sanchez & Kron (2008) have revealed that *Polygonum* s.l. is polyphyletic, and should be divided into several genera. The phylogenetic-molecular studies available include only a few species and scarce representation of the types of the genera segregated from *Polygonum*; consequently it is still not clear to what lengths this subdivision may be taken and what are their phylogenetic relationships. The systematic scheme mostly accepted in the literature (Tab. 1) includes the identification of at least 7 genera (some of which are articulated in sections) assigned, together with other genera, to two tribes: the Persicarieae and the Polygoneae. Lamb Frye & Kron (2003), Kim *et al.* (2005) and Sanchez & Kron (2008) have demonstrated that also the genus *Muehlenbeckia*, usually placed within the *Coccolobeae*, is related to *Polygonum* s.l.; this is also often united with the monotypic genus *Homalocladium*, characterised by flattened stems. *Knorringia*, segregated from *Polygonum* s.l. (or from *Aconogonum*) and placed in the *Coccolobeae* (Hong, 1989), may also belong to the *Polygoneae*.

Tab. 1 - Accepted classification of *Polygonum* s.l. species.Tab. 1 - Classificazione accettata delle specie appartenenti a *Polygonum* s.l.

Persicarieae	Tepals with three main nervatures which depart from the base (with some exceptions in <i>Fagopyrum</i>); epidermic cells rectangular to elongate with straight or undulating anticlinal walls, cuticles smooth or striate in longitudinal direction and often continuous.		
	<i>Aconogonum</i>	Inflorescences paniculate, ocreas oblique.	
	<i>Bistorta</i>	Inflorescences spicate, only terminal, rhizome robust and presence of basal leaves, ocreas oblique.	
	<i>Fagopyrum</i> s.l. (= incl. <i>Eskemukerjea</i>) (= incl. <i>Harpagocarpus</i>) (= incl. <i>Pteroxygonum</i>)	Tepals almost free, filaments winged. Asian genus from which the genera <i>Eskemukerjea</i> from Nepal, <i>Harpagocarpus</i> from eastern Africa and <i>Pteroxygonum</i> from China are often segregated, even if, according to Ohsako <i>et al.</i> (2001), <i>Eskemukerjea</i> would have an isolated position. Furthermore, the position of <i>Fagopyrum</i> s.l. within the Polygonoi-deae is still not clear (see Marek, 1958; Hong <i>et al.</i> , 1998; Ronse Decraene <i>et al.</i> , 2000) and its collocation within the Persicarieae is controversial and not accepted by many authors.	
	<i>Koenigia</i>	Inflorescences cymose, pollen spinulose. Some species of <i>Polygonum</i> (those of sect. <i>Eleutherospermum</i> Hook.f.) have been moved to this genus on the basis of the pollen morphology (Měsíček & Soják, 1973; Hedberg, 1997), as it was at first considered monotypic.	
	<i>Persicaria</i>	Inflorescences spicate or capitate (rarely paniculate in sect. <i>Rubrivena</i>). The following 5 sections are generally accepted (sometimes treated as autonomous genera), even if others have been described.	
		sect. <i>Persicaria</i> (incl. sect. <i>Amblygonon</i>)	Inflorescences spicate, ocreas truncate.
		sect. <i>Tovara</i> (= genus <i>Antenoron</i>)	Inflorescences spicate, tepals 4, styles 2, hooked and hardened at maturity.
		sect. <i>Cephalophilon</i> (= genus <i>Ampelygonum</i>)	Inflorescences capitate, ocreas truncate.
		sect. <i>Echinocaulon</i> (= genus <i>Truellum</i>)	Inflorescences capitate, stem angles and leaves with recurved prickles, ocreas oblique.
		sect. <i>Rubrivena</i> (= genus <i>Rubrivena</i>)	Inflorescences paniculate, ocreas oblique. Shows morphological affinities with <i>Aconogonum</i> and its species are often attributed to the latter genus.

Polygoneae	Tepals with one principal nervature, more or less branched; epidermic cells irregular to elongate, rarely rectangular with mostly sinuate anticlinal walls, cuticles rarely with longitudinal striation but with strong orthogonal to reticulate ridges or striae, often without correlation between cells.	
	Fallopia s.l.	Outer tepals expanded in fruit or carinate or winged. The following 4 sections are generally accepted.
		sect. Reynoutria (= genus <i>Reynoutria</i>)
		Stems erect, flowers functionally unisexual, stigmas fimbriate. Sometimes classed at genus rank.
		sect. Fallopia and sect. Paragonum
		Stems scandent, flowers hermaphrodite, stigmas capitate, inflorescences racemose; they are distinguished on the basis of the trichome features.
		sect. Pleuropterus (= sect. <i>Sarmentosae</i>)
		Stems scandent, flowers hermaphrodite, stigmas peltate, inflorescences paniculate.
	Polygonum	Outer tepals not expanded in fruit and not carinate or winged. The recent morphological revision by Ronse Decraene <i>et al.</i> (2004) includes <i>Polygonella</i> in <i>Polygonum</i> and recognizes the following 3 sections.
		sect. Polygonum
		Pollen <i>Avicularia</i> -type (Hedberg, 1946), achenes not winged.
		sect. Pseudomollia
		Pollen dimorphic (Ronse De Craene <i>et al.</i> , 2004), achenes winged along the beak.
		sect. Duravia (incl. genus <i>Polygonella</i>)
		Pollen <i>Duravia</i> -type (Hedberg, 1946), achenes not winged.
The phylogenetic molecular analyses by Lamb Frye & Kron (2003), Kim <i>et al.</i> (2005) and Sanchez & Kron (2008) show that also the genus <i>Muehlenbeckia</i> , usually placed within the <i>Coccolobeae</i> , has affinities with <i>Polygonum</i> s.l.; the monotypic genus <i>Homalocladium</i> , characterized by flattened stems, is often united with this genus.		
Also the genus <i>Knorringia</i> , segregated from <i>Polygonum</i> s.l. (or from <i>Aconogonum</i>) and placed within the <i>Coccolobeae</i> (Hong, 1989), may belong to the <i>Polygoneae</i> .		

The third tribe of Polygonoideae with an herbaceous habit is the Rumiceae (genera *Emex*, *Oxyria*, *Rheum* and *Rumex*), retained as being homogenous from a morphological viewpoint. The latter is not taken into consideration here, if only as an outgroup, however it may be the focus of later research. The main aim of this study is to verify the monophyly of the various taxa into which *Polygonum* s.l. (Tab. 1) has been subdivided and therefore the validity of the whole systematic model by mainly focusing on native and alien species present in Europe in order to obtain a classification more in line with the evolutionary history of the group.

Materials and methods

Sampling of taxa - In sampling for the phylogenetic analyses it was attempted to cover the majority of the natural groups belonging or correlated to the complex of *Polygonum* s.l. (tribes Persicarieae and Polygoneae), where possible also including the types of the genera. In particular, the species of the genera *Aconogonum*, *Atraphaxis*, *Bistorta*, *Eskemukerjea*, *Fagopyrum* s.s., *Fallopia* s.l. (3 sections out of 4), *Homalocladium*, *Koenigia*, *Muehlenbeckia* s.s., *Persicaria* (all 5 sections) and *Polygonum* s.s. (incl. *Polygonella*; 2 sections out of 3) have been utilized. Regarding the genus *Fallopia* it was not possible to find the type species (*F. scandens*), however, a closely related species was used (*F. dumetorum*); similarly the species of the sect. *Paragonum* was not found. In relation to *Fagopyrum* and its satellite genera the species and the types of *Fagopyrum* s.s. and *Eskemukerjea* have been recovered from the genetic data banks while the material of *Harpagocarpus* and *Pteroxygonum* is missing. The types of all the *Persicaria* sections have been included; from the sect. *Echinocaulon* also *P. bungeana* has been included, the systematic position of which is still controversial. Finally, for *Polygonum* the sect. *Pseudomollia* has not been included and for the sect. *Duravia* the type was not found. Concerning the genera related to *Polygonum* s.l. the material of *Calligonum*, *Knorringia*, *Oxygonum*, *Parapteropyrum* and *Pteropyrum* has not been found. Some taxa have been included from the Rumiceae (all the genera: *Emex*, *Oxyria*, *Rheum*, *Rumex*), Cocolobeae (*Antigonon*, *Brunnichia*, *Coccoloba*) and Triplareae (*Triplaris*) tribes and from the subfamily Eriogonoideae (*Eriogonum*), in order to clarify the relationships between the genera belonging to *Polygonum* s.l. and the other genera of Polygonaceae.

The outgroups have been selected from the Plumbaginaceae (*Armeria bottendorfensis* and *Plumbago auriculata*), the sister family of the Polygonaceae (Cuénoud *et al.*, 2002).

In Tab. 2 are listed the 96 sequences related to the 92 taxa included in the phylogenetic analyses of the *rbcL* data, with information on the samples utilized and the access number of the relative sequence in the EMBL/GenBank. 41 of the latter have been sequenced for the first time while the remaining 55 have already been published by Fay *et al.* (1997), Forest *et al.* (2007), Kim & Donoghue (2008), Lamb Frye & Kron (2003), Lledó *et al.* (1998), Ohsako *et al.* (2001), Silvertown *et al.* (2006) and Yasui & Ohnishi (1996, 1998b). The species utilized cover almost all the variability of *Polygonum* s.l. within Europe: except for *Polygonum* s.s. which is poorly represented in our samples (2 species out of circa 23), however, these species are very similar, the other genera are present in abundance and only 6 species are missing from among the spontaneous or naturalized species in Europe.

DNA isolation - Total DNAs were isolated from 50-100 mg of fresh, or 20-50 mg of silica-dried or herbarium leaves of individual plants using a commercial kit (DNeasy Plant Mini Kit; QIAGEN). For PCR, DNA samples were adjusted to a concentration of 2 ng/μl in 10 mM Tris, 1 mM EDTA, pH 8.0.

***rbcL* amplification** - Approximately 1.4 kb of the *rbcL* gene was amplified using primers *rbcL*-up (5'-GGACATTAATTGAATGCTACTG-3') and *rbcL*-down (5'-GCATGTACCTGCAGTAGCATT-3') by polymerase chain reaction (PCR, 30 cycles, 1-min denaturation at 94 °C, 30-s annealing at 51 °C, 1-min extension at 72 °C, 7-min final extension). For the amplification of *Persicaria* spp., primer *rbcL*-up was replaced with 1FS (5'-ATCTCAC-CACAAACAGAAAC-3'), as described by Lamb Frye & Kron (2003).

Tab. 2 - Taxa used in the phylogenetic analysis; the species present in Europe are shown in bold. / Elenco dei taxa utilizzati nell'analisi filogenetica; in grassetto le specie presenti in Europa.

Taxon	Section	Voucher/Source	Locality	Gene-Bank n
<i>Aconogonum alpinum</i> (All.) Schur		MSNM 32.861	Italy; Piemonte; VB; Formazza	FM883602
<i>Aconogonum angustifolium</i> (Pall.) H.Hara		Lamb Frye & Kron (2003)		AF297139
<i>Aconogonum divaricatum</i> (L.) Nakai		Only fresh material	Sweden	FM883603
<i>Aconogonum</i> × <i>fennicum</i> Reiersen (= <i>A. alpinum</i> × <i>weyrichii</i>)		Only fresh material	Sweden	FM883604
<i>Aconogonum molle</i> (D.Don) H.Hara (= <i>A. paniculatum</i> (Blume) Haraldson) ¹		Lamb Frye & Kron (2003)		AF297129
<i>Aconogonum weyrichii</i> (F.Schmidt) H.Hara		Lamb Frye & Kron (2003)		AF297145
<i>Antigonon leptopus</i> Hook. & Arn.		Lamb Frye & Kron (2003)		AF297146
<i>Atraphaxis spinosa</i> L.		Lamb Frye & Kron (2003)		AF297123
<i>Bistorta affinis</i> (D.Don) Greene		Only fresh material	Italy; Lombardia; MI; Milano; MSNM Botanic Garden	FM883605
<i>Bistorta amplexicaulis</i> (D.Don) Greene		Only fresh material	Italy; Lombard- dia; MI; Milano; MSNM Botanic Garden	FM883606
<i>Bistorta officinalis</i> Delarbre (= <i>Polygonum bistorta</i> L.)		MSNM 24.720	Italy; Lombardia; BS; Breno	FM883607
<i>Bistorta vivipara</i> (L.) Delarbre		MSNM 24.721	Italy; Lombard- dia; BS; Breno	FM883608
<i>Brunnichia ovata</i> (Walter) Shinners (= <i>B. cirrhosa</i> Gaertn.)		Lamb Frye & Kron (2003)		AF297136
<i>Coccoloba densifrons</i> Mart.		Lamb Frye & Kron (2003)		AF297138
<i>Coccoloba swartzii</i> Meisn.		Lamb Frye & Kron (2003)		AF297150
<i>Emex spinosa</i> (L.) Campd.		Lamb Frye & Kron (2003)		AF297142
<i>Eriogonum flavum</i> Nutt.		Fay <i>et al.</i> (1997)		Z97648
<i>Eriogonum tomentosum</i> Michx.		Lamb Frye & Kron (2003)		AF297134
<i>Eskemukerjea megacarpa</i> (H.Hara) H.Hara (= <i>E. nepalensis</i> Malick & Sengupta)		Ohsako <i>et al.</i> (2001)		AB056690
<i>Fagopyrum callianthum</i> Ohnishi		Yasui & Ohnishi (1998b)		AB000302

¹ The sequenced taxon corresponds to var. *frondosum* (Meisn.) H.Hara (= *A. paniculatum*). / L'entità sequenziata corrisponde alla var. *frondosum* (Meisn.) H.Hara (= *A. paniculatum*).

Taxon	Section	Voucher/Source	Locality	Gene-Bank n
<i>Fagopyrum capillatum</i> Ohnishi		Yasui & Ohnishi (1998b)		AB000303
<i>Fagopyrum dibotrys</i> (D.Don) H.Hara (= <i>F. cymosum</i> (Trev.) Meisn.)		Yasui & Ohnishi (1998b)		AB000304
<i>Fagopyrum esculentum</i> Moench subsp. <i>esculentum</i>		Yasui & Ohnishi (1998b)		AB000309
<i>Fagopyrum gilesii</i> (Hemsl.) Hedberg		Ohsako <i>et al.</i> (2001)		AB056689
<i>Fagopyrum gracilipes</i> (Hemsl.) Dammer ex Diels		Yasui & Ohnishi (1998b)		AB000311
<i>Fagopyrum homotropicum</i> Ohnishi		Yasui & Ohnishi (1998b)		AB000312
<i>Fagopyrum leptopodum</i> (Diels) Hedberg var. <i>leptopodum</i>		Yasui & Ohnishi (1998b)		AB000313
<i>Fagopyrum lineare</i> (Sam.) Haraldson		Yasui & Ohnishi (1998b)		AB000314
<i>Fagopyrum macrocarpum</i> Ohsako & Ohnishi		Ohsako <i>et al.</i> (2001)		AB056687
<i>Fagopyrum pleioramosum</i> Ohnishi		Yasui & Ohnishi (1998b)		AB000315
<i>Fagopyrum rubifolium</i> Ohsako & Ohnishi		Ohsako <i>et al.</i> (2001)		AB056686
<i>Fagopyrum statice</i> (H.L.Év.) H.Gross		Yasui & Ohnishi (1998b)		AB000317
<i>Fagopyrum tataricum</i> (L.) Gaertn. subsp. <i>potanini</i> Batalin		Yasui & Ohnishi (1998b)		AB000318
<i>Fagopyrum tataricum</i> (L.) Gaertn. subsp. <i>tataricum</i>		Yasui & Ohnishi (1998b)		AB000319
<i>Fagopyrum urophyllum</i> (Bureau & Franch.) H.Gross		Yasui & Ohnishi (1998b)		AB000321
<i>Fallopia baldschuanica</i> (Regel) Holub (= <i>F. aubertii</i> (L.Henry) Holub)	<i>Fallopia</i> sect. <i>Pleuropterus</i>	Only fresh material	Italy; Lombardia; LC; Rovagnate	FM883609
<i>Fallopia</i> × <i>bohemica</i> (Chrték & Chrtková) J.P.Bailey (= <i>F. japonica</i> var. <i>japonica</i> ♀ × <i>sachalinensis</i> ♂)	<i>Fallopia</i> sect. <i>Reynoutria</i>	MSNM 40.867	Italy; Lombardia; MI; Milano	FM883610
<i>Fallopia</i> × <i>bohemica</i> (Chrték & Chrtková) J.P.Bailey (= <i>F. japonica</i> var. <i>japonica</i> ♀ × <i>sachalinensis</i> ♂)	<i>Fallopia</i> sect. <i>Reynoutria</i>	MSNM 40.967	Italy; Lombardia; VA; Somma Lom- bardo	FM883611
<i>Fallopia convolvulus</i> (L.) Å.Löve	<i>Fallopia</i> sect. <i>Fallopia</i>	MSNM 40.162	Italy; Lombardia; MI; Milano	FM883612
<i>Fallopia dumetorum</i> (L.) Holub	<i>Fallopia</i> sect. <i>Fallopia</i>	MSNM 39.131	Italy; Liguria; SV; Pietra Ligure	FM883613
<i>Fallopia japonica</i> (Houtt.) Ronse Decr. var. <i>compacta</i> (Hook.f.) J.P.Bailey	<i>Fallopia</i> sect. <i>Reynoutria</i>	Only fresh material	Italy; Lombardia; MI; Milano; MSNM Botanic Garden	FM883614

Taxon	Section	Voucher/Source	Locality	Gene-Bank n
<i>Fallopia japonica</i> (Houtt.) Ronse Decr. var. <i>japonica</i>	<i>Fallopia</i> sect. <i>Reynoutria</i>	MSNM 40.965	Italy; Lombardia; MI; Milano	FM883615
<i>Fallopia multiflora</i> (Thunb.) Haraldson	<i>Fallopia</i> sect. <i>Pleuropterus</i>	MSNM 39.993	Italy; Lombardia; BG; Ponte San Pietro	FM883616
<i>Fallopia sachalinensis</i> (F.Schmidt) Ronse Decr.	<i>Fallopia</i> sect. <i>Reynoutria</i>	Lamb Frye & Kron (2003)		AF297125
<i>Homalocladium</i> <i>platycladum</i> (F.Muell.) L.H.Bailey		MSNM 34.030	Italy; Liguria; IM; Ventimiglia; Villa Hanbury Botanic Garden	FM883617
<i>Koenigia forrestii</i> (Diels) Mesicek & Soják		Lamb Frye & Kron (2003)		AF297144
<i>Koenigia islandica</i> L.		Kim & Donoghue (2008)		EF653763
<i>Muehlenbeckia australis</i> (G.Forst.) Meisn.		Only fresh material	Australia	FM883618
<i>Muehlenbeckia complexa</i> (A.Cunn.) Meisn.		MSNM 3.296	Italy; Lombardia; MI; Cuggiono	FM883619
<i>Oxyria sinensis</i> Hemsl.		Lamb Frye & Kron (2003)		AF297148
<i>Persicaria acuminata</i> (Kunth) M.Gómez	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 36.839	Brasil; RS; Caxias do Sul	FM883620
<i>Persicaria amphibia</i> (L.) Delarbre	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 39.400	Italy; Lombardia; MI; Milano	FM883621
<i>Persicaria amphibia</i> (L.) Delarbre	<i>Persicaria</i> sect. <i>Persicaria</i>	Silvertown <i>et al.</i> (2006)		AY395553
<i>Persicaria arifolia</i> (L.) Haraldson	<i>Persicaria</i> sect. <i>Echinocaulon</i>	Kim & Donoghue (2008)		EF653770
<i>Persicaria bungeana</i> (Turcz.) Nakai	<i>Persicaria</i> sect. <i>Echinocaulon</i>	MSNM 41.053	Italy; Veneto; VI; Grancona	FM883622
<i>Persicaria capitata</i> (Buch.- Ham. ex D.Don) H.Gross	<i>Persicaria</i> sect. <i>Cephalophilon</i>	Only fresh material	Italy; Liguria; IM; Ventimiglia; Villa Hanbury Botanic Garden	FM883623
<i>Persicaria decipiens</i> (R.Br.) K.L.Wilson (= <i>P. salicifolia</i> (Brouss. ex Willd.) Assenov, non Gray)	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 40.945	Italy; Sicilia; SR; Siracusa	FM883624
<i>Persicaria dubia</i> (Stein) Fourr. (= <i>P. mitis</i> (Schrank) Assen- nov)	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 26.625	Italy; Lombardia; MI; Milano	FM883625
<i>Persicaria filiformis</i> (Thunb.) Nakai	<i>Persicaria</i> sect. <i>Tovara</i>	MSNM 40.839	Italy; Lombardia; CO; Figino Serenza	FM883628
<i>Persicaria filiformis</i> (Thunb.) Nakai 'Painter's Palette'	<i>Persicaria</i> sect. <i>Tovara</i>	Only fresh material	Italy; Lombardia; MI; Milano; MSNM Botanic Garden	FM883626
<i>Persicaria filiformis</i> (Thunb.) Nakai 'Variegata'	<i>Persicaria</i> sect. <i>Tovara</i>	Only fresh material	Italy; Lombardia; MI; Milano; MSNM Botanic Garden	FM883627
<i>Persicaria hydroziper</i> (L.) Delarbre	<i>Persicaria</i> sect. <i>Persicaria</i>	Only fresh material	Italy; Lombardia; MI; Milano	FM883629

Taxon	Section	Voucher/Source	Locality	Gene-Bank
<i>Persicaria lapathifolia</i> (L.) Delarbre subsp. <i>lapathifolia</i>	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM	Italy; Lombardia; MI; Milano	FM883630
<i>Persicaria longiseta</i> (Bruijn) Kitag.	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 40.958	Italy; Lombardia; MB; Lesmo	FM883631
<i>Persicaria meisneriana</i> (Cham. & Schltdl.) M.Gómez	<i>Persicaria</i> sect. <i>Echinocaulon</i>	Kim & Donoghue (2008)		EF653772
<i>P. microcephala</i> (D.Don) H.Gross 'Red Dragon'	<i>Persicaria</i> sect. <i>Cephalophilon</i>	MSNM 41.049	Italy; Lombardia; MI; Milano; MSNM Botanic Garden	FM883632
<i>Persicaria minor</i> (Huds.) Opiz	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 41.046	Italy; Lombardia; MI; Trezzo sull'Adda	FM883633
<i>Persicaria nepalensis</i> (Meisn.) H.Gross	<i>Persicaria</i> sect. <i>Cephalophilon</i>	MSNM 39.369	Italy; Lombardia; VA; Casalzuigno	FM883634
<i>Persicaria odorata</i> (Lour.) Soják	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM	Italy; Lombardia; MI; Milano; Cascina Rosa Botanic Garden	FM883635
<i>Persicaria orientalis</i> (L.) Spach	<i>Persicaria</i> sect. <i>Persicaria</i>	Only fresh material	Italy; Lombardia; VA; Vergiate	FM883636
<i>Persicaria pensylvanica</i> (L.) M.Gómez	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 39.267	Italy; Lombardia; MI; Settimo Milanese	FM883637
<i>Persicaria punctata</i> (Elliott) Small	<i>Persicaria</i> sect. <i>Persicaria</i>	MSNM 24.827	Brasil; RS; Capão de Canoa	FM883638
<i>Persicaria runcinata</i> (Buch.-Ham. ex D.Don) Meisn.	<i>Persicaria</i> sect. <i>Cephalophilon</i>	Lamb Frye & Kron (2003)		AF297124
<i>Persicaria sagittata</i> (L.) H.Gross ex Nakai	<i>Persicaria</i> sect. <i>Echinocaulon</i>	Kim & Donoghue (2008)		EF653773
<i>Persicaria senticosa</i> (Meisn.) Nakai	<i>Persicaria</i> sect. <i>Echinocaulon</i>	Yasui & Ohnishi (1996)		D86289
<i>Persicaria thunbergii</i> (Siebold & Zucc.) H.Gross (≡ <i>Polygonum thunbergii</i> Siebold & Zucc.; = <i>Persicaria maackiana</i> (Regel) Nakai ≡ <i>Polygonum maackianum</i> Regel) ²	<i>Persicaria</i> sect. <i>Persicaria</i>	Kim & Donoghue (2008)		EF653771
<i>Persicaria tinctoria</i> (Aiton) Spach	<i>Persicaria</i> sect. <i>Persicaria</i>	Only fresh material	Italy; Lombardia; MI; Milano; Cascina Rosa Botanic Garden	FM883639
<i>Persicaria virginiana</i> (L.) Gaertn.	<i>Persicaria</i> sect. <i>Tovara</i>	MSNM 39.309	Italy; Lombardia; LC; Cernusco Lombardone	FM883641

² The sequenced taxon corresponds to *P. maackiana*, which Park (1988) nevertheless reduced to a simple variety of *P. thunbergii*; due to the lack of a specific study and a combination at varietal rank in *Persicaria*, it is preferred to use the binomial used in the present study. / L'entità sequenziata corrisponde a *P. maackiana*, che tuttavia Park (1988) riduce a semplice varietà di *P. thunbergii*; in mancanza di uno studio specifico e di una combinazione a rango varietale in *Persicaria*, preferiamo utilizzare il binomio qui riportato.

Taxon	Section	Voucher/Source	Locality	Gene-Bank n
<i>Persicaria wallichii</i> Greuter & Burdet	<i>Persicaria</i> sect. <i>Rubrivena</i>	MSNM 39.191	Italy; Piemonte; VB; Premeno	FM883640
<i>Polygonella robusta</i> (Small) G.L.Nesom & V.M.Bates ³	<i>Polygonum</i> sect. <i>Duravia</i>	Lamb Frye & Kron (2003)		AF297132
<i>Polygonum aviculare</i> L.	<i>Polygonum</i> sect. <i>Polygonum</i>	Lamb Frye & Kron (2003)		AF297127
<i>Polygonum erectum</i> L.	<i>Polygonum</i> sect. <i>Polygonum</i>	Lamb Frye & Kron (2003)		AF297128
<i>Polygonum rurivagum</i> Jord. ex Boreau	<i>Polygonum</i> sect. <i>Polygonum</i>	MSNM 40.903	Italy; Lombardia; BS; Manerba del Garda	FM883642
<i>Polygonum undulatum</i> (L.) P.J.Bergius	<i>Polygonum</i> sect. <i>Polygonum</i>	Forest <i>et al.</i> (2007)		AM235078
<i>Rheum kialense</i> Franch.				AB232461
<i>Rheum palmatum</i> L.				AB232452
<i>Rheum rhabarbarum</i> L. (= <i>R. undulatum</i> L.)				AB232458
<i>Rheum rhaponticum</i> L.				AB232459
<i>Rumex acetosa</i> L.		Silvertown <i>et al.</i> (2006)		AY395559
<i>Rumex acetosella</i> L.		Yasui & Ohnishi (1996)		D86290
<i>Rumex induratus</i> Boiss. & Reut.		Lamb Frye & Kron (2003)		AF297122
<i>Rumex obtusifolius</i> L.		Lamb Frye & Kron (2003)		AF297126
<i>Triplaris americana</i> L.		Lledó <i>et al.</i> (1998)		Y16910
<i>Triplaris poeppigiana</i> Wedd.		Lamb Frye & Kron (2003)		AF297137
Outgroup				
<i>Armeria bottendorfensis</i> A.Schulz (Plumbaginaceae)		Fay <i>et al.</i> (1997)		Z97640
<i>Plumbago auriculata</i> Lam. (Plumbaginaceae)		Lledó <i>et al.</i> (1998)		Y16906

The PCR amplification was conducted under the same conditions with the annealing temperature at 48 °C. All PCRs contained 10 µmol/L primers in 25-µL reactions by using Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) and following the manufacturer's standard protocol. The amplified product was empirically estimated by sight after electrophoresis of the fragments marked with 1-µL ethidium-bromide in a 1% agarose gel. The PCR products were then purified using QIAquick PCR purification kit (Qiagen). Cycle sequencing (25 cycles, 10-s denaturation at 96 °C, 5-s annealing at 56 °C, 4-min extension at 60 °C) with dye

³ As far as is known there is no specific binomial available for this species within *Polygonum*, also due to the presence of the earlier homonym *Polygonum robustum* Meisn.; in the present study the combination of the genus *Polygonella* has been retained. / Per questa specie non vi è, per quanto ne sappiamo, un binomio specifico disponibile in *Polygonum*, anche per la presenza dell'omonimo anteriore *Polygonum robustum* Meisn.; in questo lavoro abbiamo pertanto mantenuto la combinazione nel genere *Polygonella*.

terminators (BigDye Terminator v3.1 Cycle Sequencing kit from Applied Biosystems, Warrington, Cheshire, UK) was performed in 10- μ L volumes, and the products were then purified by ethanol precipitation. The redissolved samples were run on an Applied Biosystems 3100 Genetic Analyzer automated DNA sequencer following the manufacturer's protocols. Double readings were made and primers for this sequence determination were *rbcL*-up, *rbcL* 2up (5'-GGACATTACTTGAA-TGCTACTG-3'), *rbcL*-down or 1FS and *rbcL* 2-down 5'-GCATGTACCTGCAG-TAGCATT-3').

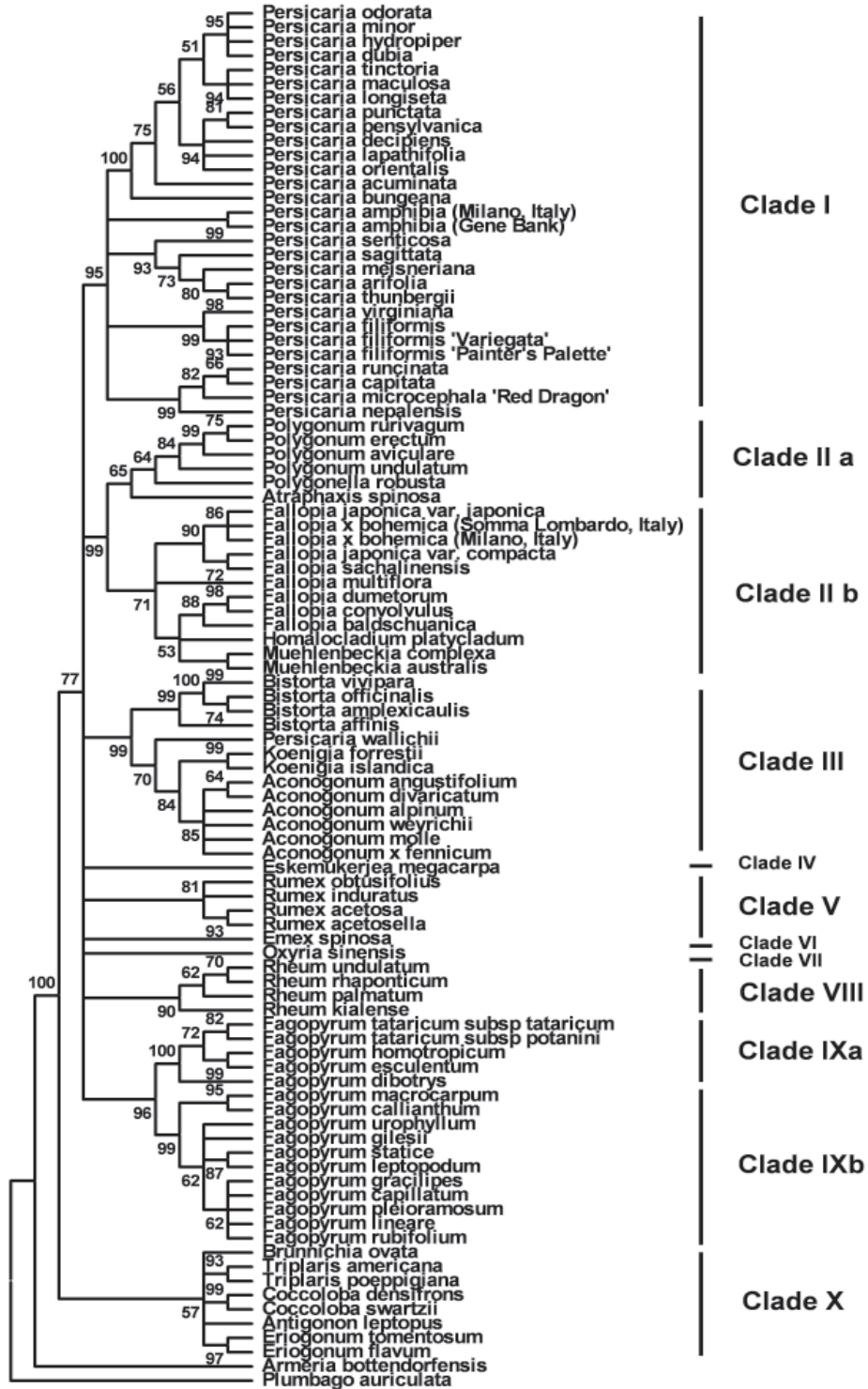
Data analysis - The boundaries of sequences were determined by comparison of the newly obtained sequences with those downloaded from the GenBank. Sequences were aligned using the Clustal X 1.8.1 (Thompson *et al.*, 1997) computer program and were refined manually. Phylogenies were reconstructed by using maximum parsimony optimality criterion using MEGA4 (Tamura *et al.*, 2007) and PAUP version 4.0 (Swofford, 2002). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei & Kumar, 2000) with search level 3 in which the initial trees were obtained with the random addition of sequences (200 replicates). Confidence in nodes was assessed using the bootstrap method with 5000 replicates. The tree was rooted using taxa from *Armeria* and *Plumbago*, whose family had previously been shown to be an appropriate outgroup (Cuénoud *et al.*, 2002).

Results

The evolutionary history was inferred using the Maximum Parsimony method. Of 1,334 total characters in the data matrix, 824 (62%) are constant characters, 253 (19%) are variable characters that are parsimony-uninformative, and 257 (19%) are parsimony-informative variable characters. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. Parsimony analysis of the data yielded 976 most parsimonious trees of 1,027 steps with a consistency index (CI) of 0.595, retention index (RI) of 0.808, and rescaled consistency index (RC) of 0.481. The mean composition of nucleotide bases in this alignment was as follows: T = 28.9%, G = 24.1%, C = 20.3%, A = 26.7%. The majority-rule consensus of the phylogenetic trees obtained by MEGA4 and Paup provided the same topology (Fig. 1). Polygonaceae are confirmed to be a monophyletic family (100% bootstrap support), according to the systematic tradition and the previous molecular analysis (Cuénoud *et al.*, 2002; Lamb Frye & Kron, 2003; Kim *et al.*, 2005; Sanchez & Kron, 2008). The tree illustrates the relationships within Polygonaceae and it indicates 2 blocks, not corresponding to the traditional subdivision in Eriogonoideae and Polygonoideae. Block I includes 9 still unresolved major lineages (clades I-IX). *Fallopia* s.l., *Muehlenbeckia* s.l. and *Persicaria* are not monophyletic genera, justifying their splitting. This is in agreement with previous morphological studies and systematic proposals, suggesting that the *rbcL* phylogram reflects the true phylogeny, even if the full picture of the species phylogeny cannot be concluded solely from a single gene.

Fig 1 - Phylogram based on maximum parsimony analysis of the *rbcL* sequences. *Armeria* and *Plumbago* (Plumbaginaceae) were considered as the outgroup. Bootstrap values > 50% are shown on the branches.

Fig 1 - Filogramma basato sull'analisi della massima parsimonia delle sequenze *rbcL*. Come *outgroup* sono utilizzati *Armeria* e *Plumbago* (Plumbaginaceae). Sopra i rami sono mostrati i valori di *bootstrap* > 50%.



Discussion and systematic-phylogenetic arrangement of *Polygonum* s.l.

The family Polygonaceae, analogously to what has been demonstrated by Lamb Frye & Kron (2003), Kim *et al.* (2005) and Sanchez & Kron (2008), appears to be monophyletic (bootstrap 100%). However, the subfamily Polygonoideae is paraphyletic as it includes the Eriogonoideae, though with a low bootstrap (57%). In Fig. 1 the Polygonaceae are subdivided into two large blocks: the first (block I: clades I-IX; bootstrap 77%) comprises the present tribes Persicarieae, Polygoneae and Rumiceae, the second (block II: clade X; bootstrap 57%) the Eriogonoideae and the remaining wooden tribes of the Polygonoideae. On the basis of these results Sanchez & Kron (2008) proposed a new outline of the subfamily, restricting the Polygonoideae to the only prevalently herbaceous tribe (block I of our tree) and including the wooden tribes in the Eriogonoideae (block II of our tree); the results presented here confirm these conclusions with which the authors agree.

The Polygonoideae s.s. (block I) is not resolved and has 9 clades which on the basis of the rich morphological evidence, may nevertheless be grouped into almost 4 superclades corresponding to 4 tribes: Polygoneae, Persicarieae, Fagopyreae and Rumiceae. Consequently, a new systematic model is put forward for the species belonging to these tribes. Subsequent analyses comprising a greater number of species or sequences may lead to slight modifications of the model. With regard to the nomenclature of the suprageneric taxa the authors have collaborated with Adriano Soldano (Vercelli), and the latter appears as co-author of the new nomenclatural subtribal name.

Subfamilia **Polygonoideae** Eaton (block I: clades I-IX, bootstrap 77%)

Block I, composed of clades I-IX corresponds to the subfamily Polygonoideae, thus as redefined by Sanchez & Kron (2008). It is subdivided into four tribes.

Tribus **Polygoneae** Rchb. (clade II, bootstrap 99%)

Clade II corresponds well to the tribe Polygoneae, as defined by Ronse Decraene & Akeroyd (1988), with the addition of *Muehlenbeckia* and *Homalocladium*. It is characterized by:

- 1) tepals with a principal nervature, more or less branched,
- 2) epidermic cells irregular to elongate, rarely rectangular with mostly sinuate anticlinal walls, cuticles rarely with longitudinal striation but with strong orthogonal to reticulate ridges or striae, often without correlation between cells (type II sensu Hong *et al.*, 1998),
- 3) nectariferous zone generally not well developed externally to form visible nectaries (except, for example, in *Muehlenbeckia*) but being buried in the receptacle or fused with the base of the inner filaments that thus appear thickened proximally; trichomes or papillae absent or range from a few to several.

This clade results as being subdivided into two subclades, assignable to two subtribes: the Polygoninae and the Reynoutriinae.

Subtribus **Polygoninae** Roberty & Vautier (clade II a, bootstrap 65%)

= *Atraphaxidinae* H.Gross

= *Calligoninae* Roberty & Vautier

Genera included: *Atraphaxis*, *Polygonum* (incl. *Polygonella*) and, probably, *Calligonum*, *Oxygonum*, *Parapteropyrum* and *Pteropyrum*.

Polygonum L. (ca. 75 spp.)T: *Polygonum aviculare* L., typ. cons.= *Polygonella* Michx.T: *Polygonella parvifolia* Michx. (= *Polygonella polygama* (Vent.) Engelm. & A.Gray)

The tepal structure in *Polygonella* is very similar to that of *Polygonum* s.s., especially to that of the sect. *Duravia* (Ronse Decraene & Akeroyd, 1988; Hong *et al.*, 1998). This similarity, supported also by the pollen morphology (Hedberg, 1946; Hong *et al.*, 2005), by the floral morphology (Ronse Decraene & Akeroyd, 1988; Ronse Decraene *et al.*, 2004) and by anatomical evidence (Haraldson, 1978), induced Ronse Decraene *et al.* (2004) to propose the union with *Polygonum* s.s. at the level of subsection. It should be noted, however, that the combinations proposed by Ronse Decraene *et al.* (2004) are not valid, as the basionyms are not cited. The present results are in line with a sister relationship between the sect. *Polygonum* and *Duravia*, however, as they are based on only a few species of this genus they cannot fully confirm the theory of Ronse Decraene *et al.* (2004).

In the case of *Polygonella robusta*, sometimes considered a variety of *P. fimbriata* (Horton, 1963) however, it is quite distinct from the latter (Nesom & Bates, 1984; Freeman, 2005), there is no specific binomial available, as far as is known, in *Polygonum*, also due to the presence of the earlier homonym *Polygonum robustum* Meisn. It would be necessary to find a valid synonym within *Polygonum* or to set up a new name; in the meantime in the present study the combination within the genus *Polygonella* has been retained.

Polygonum L. sect. ***Duravia*** S.Watson (ca. 31 spp.)T: *Polygonum californicum* S.Watson= *Polygonella* Michx.T: *Polygonella parvifolia* Michx. (= *Polygonella polygama* (Vent.) Engelm. & A.Gray)= *Gonopyrum* Fisch. & C.A.Mey. [1840] ("1845")≡ *Psammogonum* Nieuwl. [1914]T: *G. americanum* Fisch. & C.A.Mey. (≡ *Polygonella a.* (Fisch. & C.A.Mey.) Small)= *Thysanella* A.Gray [1845]T: *T. fimbriata* (Elliott) A.Gray (≡ *Polygonum f.* Elliott)***Polygonum*** L. sect. ***Polygonum*** (ca. 45 spp.)≡ *Avicularia* (Meisn.) BörnerT: *Polygonum aviculare* L., typ. cons.= *Tephis* Adans.≡ *Polygonum* L. sect. *Tephis* (Adans.) Meisn.T: *Atraphaxis undulata* L. (≡ *Polygonum u.* (L.) P.J.Bergius)***Polygonum*** L. sect. ***Pseudomollia*** Boiss. (1 sp.)T: *Polygonum molliaeforme* Boiss.***Atraphaxis*** L. (ca. 25 spp.)T: *Atraphaxis spinosa* L.***Calligonum*** L. (35-80 spp.)T: *Calligonum polygonoides* L.***Oxygonum*** Burch. ex Campd. (ca. 30 spp.)T: *Oxygonum alatum* Burch.***Parapteropyrum*** A.J.Li (1 sp.)T: *Parapteropyrum tibeticum* A.J.Li

Pteropyrum Jaub. & Spach (4-5 spp.)T: *Pteropyrum aucheri* Jaub. & Spach

The genera *Atraphaxis*, *Calligonum* and *Pteropyrum* are among those which have in common a particular type of tepal nervature (Vautier, 1949), which are petaloid and not proximally fused (Ronse Decraene & Akeroyd, 1988); they are also closely associated by anatomical (Perdrigeat, 1900; Haraldson, 1978) and pollen (Hedberg, 1946) characters. The monotypic genus *Parapteropyrum* is closely related to *Pteropyrum* and has a very similar pollen (Hong, 1995); they are distinguished on the basis of a few characters of the floral morphology (Li, 1981). Ronse Decraene & Akeroyd (1988) also associate *Oxygonum* to *Atraphaxis* and *Pteropyrum*. Nevertheless, this is differentiated on the basis of the type III epidermic cells (sensu Hong *et al.*, 1998), analogously to *Fagopyrum* sect. *Fagopyrum* and *Eskemukerjea*: mostly irregular, mammiliform, papillose, with either longitudinal tightly pressed or randomly ridged cuticles.

The position of these genera with respect to the other Polygonoideae is thus still doubtful, though Ronse Decraene & Akeroyd (1988) relate them to *Polygonum* s.s. Both the present results and those of Lamb Frye & Kron (2003), which includes only one of these genera, clearly place *Atraphaxis* in a sister position with respect to *Polygonum*. Until confirmation is provided by the study of the DNA sequences of the other genera, it seems plausible to the authors to consider them to have morphological affinities with *Atraphaxis* and therefore unite them within the tribe of Polygoninae, even if the pollen characters (Hong, 1995) and the tepal morphology (Hong *et al.*, 1998) place *Oxygonum*, *Parapteropyrum* and *Pteropyrum* closer to the Fagopyrinae.

Subtribus **Reynoutriinae** Roberty & Vautier

(clade II b, bootstrap 71%)

= *Muehlenbeckiinae* Roberty & VautierGenera included: *Fallopia*, *Homalocladium*, *Muehlenbeckia*, ×*Reyllophia*, *Reynoutria*.

The Reynoutriinae and the Muehlenbeckiinae were described simultaneously by Roberty & Vautier (1964) and therefore would have equal priority; in the present study priority is given to Reynoutriinae.

Haraldson (1978) was the first to demonstrate the close relationship between the genera *Fallopia* s.l. (incl. *Reynoutria*) and *Muehlenbeckia* s.l. (incl. *Homalocladium*) and he placed them in the tribe Cocolobeae, together with *Antigonon*, *Brunnichia*, *Coccoloba*, *Harpagocarpus* and *Podopterus*. Nevertheless, analogously to the cladograms of Lamb Frye & Kron (2003) and of Sanchez & Kron (2008) and the results of Kim *et al.* (2005), *Coccoloba* and the majority of these genera (excluding *Harpagocarpus*) show no affinity with *Fallopia* and *Muehlenbeckia* and even fall outside of the Polygonoideae. *Harpagocarpus* could show instead affinities with *Fagopyrum* (Ronse Decraene & Akeroyd, 1988), while *Fallopia* s.l. and *Muehlenbeckia* s.l. fall without doubt within the Polygoneae. The latter two genera are characterized by the synapomorphy of the presence of extrafloral pit nectaries at the base of the petioles and in the node position which attract some ant species that attack the phytophagous insects (Salisbury, 1909; Sukopp & Schick, 1991; Kawano *et al.*, 1999); they may also be ascribed to their own subtribe. It would also be interesting to be able to also verify the similarity of the pollen morphology.

The genus *Fallopia* s.l. is paraphyletic as *Muehlenbeckia* is nested within it. A possible systematic interpretation is therefore to include *Muehlenbeckia* in *Fallopia* (priority name). A second possible solution is to subdivide *Fallopia* into more genera (at least two). Taking

into consideration that *Fallopia* and *Muehlenbeckia* have always been considered as being quite separate entities and that *Fallopia* has often been subdivided into more genera, it is here recommended to take the second option, and also to retain *Homalocladium*.

Reynoutria Houtt. (3-7 spp.)

≡ *Fallopia* Adans. sect. *Reynoutria* (Houtt.) Ronse Decr.

T: *Reynoutria japonica* Houtt.

On the basis of the morphological characters the genus *Reynoutria* has often been included within the *Fallopia* (eg. Hedberg, 1946; Ronse Decraene & Akeroyd, 1988; Bailey & Stace, 1992), also on account of the presence of hybrids (Bailey, 1988; Bailey & Stace, 1992; Bailey, 2001; Bailey & Spencer, 2003); in the past they have been united also under the generic name *Reynoutria* or other names erroneously retained as having priority. Other authors (eg. Nakai, 1926; Roberty & Vautier, 1964; Webb & Chater, 1963; Webb, 1964, 1993; Holub, 1971; Haraldson, 1978; Brandbyge, 1993; Mandák *et al.*, 2004; Padula *et al.*, 2008), instead, have considered them as being autonomous. *Reynoutria* is a quite distinct morphological unit within the *Fallopia* complex (see Kim & Park, 2000) and the present molecular results show how it could, or better still, should be maintained separate at genus level. The traditional differentiating characters are listed below.

Fallopia. Stems scandent; flowers hermaphrodite; styles short; stigmas capitate or peltate, however, in *F. koreana* flowers dioecious and stigmas fimbriate (Kim *et al.*, 2000b)-nevertheless in the protologue (Oh & Kim, 1996) peltate stigmas are mentioned but no reference is made to dioecy-; achenes with beak absent or very short; $x = 10, 11$.

Reynoutria. Stems erect and robust; flowers functionally unisexual (hermaphrodite and female plants); styles long; stigmas fimbriate; achenes with beak evident; $x = 11$.

The genus *Reynoutria* is polyploid and extremely variable, both in the morphological characters and in the chromosome number. Consequently, there is a high degree of taxonomic confusion and difficulty in determining the limits between species (Kim & Park, 2000); furthermore infraspecific hybridization is relatively common (Bailey & Stace, 1992). The two species universally recognized are ***R. japonica*** Houtt. and ***R. sachalinensis*** (F.Schmidt) Nakai, however, there are numerous other taxa, variously synonymized or reduced to variety rank, such as *R. sachalinensis* (F.Schmidt) Nakai var. *intermedia* (Tatew.) Tatew.

Reynoutria japonica Houtt. var. *compacta* (Hook.f.) Moldenke is a dwarf taxon of the high peaks of Japan and Korea (but probably exclusive to Monte Fuji), smaller than the typical var. *japonica* and often with the inflorescence tinged with red (Ohwi, 1965; Stace, 1991, 1997). It is frequently treated at variety rank (eg. Ohwi, 1965; Conolly, 1977; Stace, 1989, 1991, 1997; Bailey & Stace, 1992; Pyšek *et al.*, 2002; Mandák *et al.*, 2004) or, sometimes, synonymized completely with the type of the species (Kim & Park, 2000; Li & Park, 2003). It is the first colonizer of volcanic lavas, where it forms colonies which expand in circles and gradually thin out towards the center (Adachi *et al.*, 1996). It is cultivated in Europe and sometimes is found in nature as a casual alien. Transplanted at lower altitudes it maintains its characteristics (Shiosaka & Shibata, 1993); furthermore, its *rbcL* sequence shows a greater affinity with *R. sachalinensis* rather than with *R. japonica* var. *japonica*; also the attempts of hybridization by Bailey & Stace (1992) show a certain homology between its chromosomes and those of *R. sachalinensis* rather than with those of *R. japonica* var. *japonica*. Consequently, it may merit species rank, for which a valid combination already exists: ***R. compacta*** (Hook.f.) Nakai.

In the 'Flora of China', Li & Park (2003) retain *Reynoutria* and *Fallopia* as distinct; although the choice of retaining *Fallopia forbesii* (Hance) Yonekura & H. Ohashi within *Fallopia* is nevertheless curious. This species has been re-evaluated by Yamazaki (1994) and Yonekura & Ohashi (1997) and corresponds to the majority of the Chinese and Korean populations of *R. japonica* s.l.: it is very similar to *R. japonica* s.s., however, it is clearly distinct due to the shape of the leaves (Kim & Park, 2000) and, above all, due to the presence of thick, rigid hairs on the lower surface of the leaves (personal observations G.G.), strictly absent in *R. japonica* and *R. compacta*. Its correct name therefore is ***R. forbesii*** (Hance) T. Yamaz.

Yonekura & Ohashi (1997) included within the variability of *Reynoutria japonica* two further taxa, both endemic to Japan and never introduced outside of their area of origin: *Fallopia japonica* (Houtt.) Ronse Decr. var. *hachidoensis* (Makino) Yonekura & H. Ohashi and *Fallopia japonica* (Houtt.) Ronse Decr. var. *uzenensis* (Honda) Yonekura & H. Ohashi (\equiv *Reynoutria japonica* Houtt. var. *uzenensis* Honda) (see Bailey, 2003). The former has shiny leaves while the latter has short rigid hairs on the lower surface of the blades, a character which is absent in *R. japonica*. Preliminary studies based on RAPD (Hollingsworth & Bailey, 2000) and on the plastidial sequence (Inamura *et al.*, 2000) show an elevated variability of the native population of *Reynoutria japonica* s.l., which would lead one to think of the existence of further species. While waiting for their correct systematic interpretation the authors think it is better to treat these taxa at species rank: ***R. hachidoensis*** (Makino) Honda and ***R. uzensis*** (Honda) Honda.

R. bohémica Chrtek & Chrtková, pro hybr., originated from the hybridization between *R. japonica* and *R. sachalinensis* while ***R. x mizushima*** Yokouchi ex T. Shimizu (Shimizu, 1997) is the hybrid of *R. uzensis* and *R. sachalinensis*.

Fallopia Adans. (ca. 15 spp.)

T: *Polygonum scandens* L. (\equiv *Fallopia* s. (L.) Holub)
= *Pleuropterus* Turcz.

T: *P. cordatus* Turcz., nom. illeg. (\equiv *Fallopia multiflora* (Thunb.) Haraldson)

Although in this study only a few species of the genus *Fallopia* have been considered, it is nevertheless possible to make some observations on its infrageneric subdivision. The genus is presently articulated in 3 sections (excluding sect. *Reynoutria*), distinguished on the basis of the habit and the shape of the stigma and trichomes (Holub, 1971; Haraldson, 1978). Only future studies, which should include also *F. koreana*, *F. denticulata* and the species of the sect. *Paragonum*, may clarify the real relationships between them.

Fallopia Adans. sect. ***Fallopia*** (8 spp.)

T: *Polygonum scandens* L. (\equiv *Fallopia* s. (L.) Holub)
= *Bilderdykia* Dumort.

\equiv *Tiniaria* (Meisn.) Rchb.

\equiv *Polygonum* L. sect. *Tiniaria* Meisn.

T: *B. convolvulus* (L.) Dumort. (\equiv *Fallopia* c. (L.) Á.Löve)

It appears to be homogeneous both from a morphological (Kim *et al.*, 2000c) and chemical (Kim *et al.*, 2000a) viewpoint and the present tree confirms that it is monophyletic.

Fallopia Adans. sect. ***Paragonum*** Haraldson (2 spp.)

T: *F. cilinodis* (Michx.) Holub

Haraldson (1978) separated the sect. *Paragonum* from the sect. *Fallopia*, on the basis of the presence of filiform, unicellular and papillose trichomes unique within

the genus; it includes two species, the Asian *F. cynanchoides* (Hemsl.) Haraldson and the American *F. cilinodis* (Michx.) Holub. Furthermore, in contrast to the sect. *Fallopia*, characterized by an annual habit, capitate stigma and $x = 10$, it has a perennial habit and, at least in *F. cynanchoides*, a peltate stigma and $x = 11$ (Kim *et al.*, 2000a). The studies on flavonoids by Kim *et al.* (2000a) confirm the separation. However, in the present study it was not possible to include this species.

Fallopia Adans. sect. ***Pleuropterus*** (Turcz.) Haraldson (4-5 spp. or more)

≡ *Pleuropterus* Turcz.

≡ *Polygonum* L. sect. *Pleuropterus* (Turcz.) Benth. & Hook.

T: *P. cordatus* Turcz., nom. illeg. (≡ *Fallopia multiflora* (Thunb.) Haraldson)

= *Fallopia* Adans. sect. *Sarmentosae* Holub, non *Fagopyrum* Mill. sect. *Sarmentosa* I. Grinț. nom. illeg.

T: *F. baldschuanica* (Regel) Holub

This section, composed of plants with a perennial habit, appears to be heterogeneous both from a morphological, chemical and cytological viewpoint: on the basis of the flavonoids and chromosome number two groups may be distinguished (Kim *et al.*, 2000b). The first, with $x = 11$ and capitate and smooth stigmas, includes *F. multiflora* (Thunb.) Haraldson (and its eventual segregates) and *F. ciliinervis* (Nakai) K. Hammer. The second, with $x = 10$, includes *F. baldschuanica* (Regel) Holub (= *F. aubertii* (L. Henry) Holub), with strongly papillose and sometimes peltate stigmas, and *F. koreana* B.U. Oh & J.G. Kim, with fimbriate stigmas and dioecious flowers (characters in common with *Reynoutria*); nevertheless, in the protologue peltate stigmas are mentioned and dioecy is not referred to (Oh & Kim, 1996). Also *F. denticulata* (C.C. Huang) Holub may belong to one of the two groups in this section. On the basis of that data and the present results this section may not be monophyletic and *Pleuropterus* s.s. may merit genus rank, as was already proposed by Nakai (1914, 1926).

The name published by Grințescu is invalid (Haraldson, 1978), as in his new section *Polygonum multiflorum*, the type of *Polygonum* sect. *Pleuropterus*, is also included (ICBN art. 52.1: McNeill *et al.*, 2006); Holub (1971) explicitly excludes *P. multiflorum* and therefore his section is legitimate and is to be considered as new, not a new combination (ICBN art. 58.1: McNeill *et al.*, 2006).

×***Reyllopia*** Holub (1 sp.)

= *Fallopia* Adans. × *Reynoutria* Houtt.

The only hybrid (sterile) recognized in nature between these two genera is that between male individuals of *Fallopia baldschuanica* and female individuals of *Reynoutria japonica*. Every year the plants of *Reynoutria japonica* produce a remarkable quantity of seeds of this hybrid, however, only a negligible quantity manages to germinate (Bailey & Stace, 1992; Bailey, 2001). The seeds have been recognized in Great Britain since 1983 (Bailey & Conolly, 1984), while finding of the first plant germinated in nature, also in Great Britain, dates to 1987 (Bailey, 1988); observations have followed in the Czech Republic, Germany, Hungary and Norway (Bailey, 2001) and again in the British Isles (Bailey & Spencer, 2003).

The following new combination is here proposed in the nothogenus ×*Reyllopia*, appropriately foreseen by Holub (1998).

×***Reyllopia conollyana*** (J.P. Bailey) Galasso, **comb. nov.** (bas.: *Fallopia* × *conollyana* J.P. Bailey, *Watsonia*, 23 (4): 539. 2001) (= *Fallopia baldschuanica* (Regel) Holub ♂ × *Reynoutria japonica* Houtt. ♀)

Muehlenbeckia Meisn. (22 spp.)T: *M. australis* (G.Forst.) Meisn.***Homalocladium*** (F.Muell.) L.H.Bailey (1 sp.)≡ *Polygonum* L. sect. *Homalocladium* F.Muell.T: *Polygonum platycladum* F.Muell. (≡ *Homalocladium p.* (F.Muell.) L.H.Bailey)

The monotypic genus *Homalocladium*, characterized by flattened stems forming cladodes, is often included in *Muehlenbeckia* (eg. Brandbyge, 1993). The present tree, even if based on a few species, does not show it to be linked to *Muehlenbeckia*; it seems appropriate, however, also taking into consideration the particular morphology of the stems, that it should be retained at genus rank.

Incertae sedisGenus included: *Knorringia*.***Knorringia*** (Czukav.) Tzvelev [1987] (1-2 spp.)≡ *Knorringia* (Czukav.) Hong [1989], comb. superfl.≡ *Polygonum* sect. *Knorringia* Czukav.≡ *Aconogonum* sect. *Knorringia* (Czukav.) SojákT: *Knorringia sibirica* (Laxm.) Tzvelev

The genus *Knorringia*, segregated from *Aconogonum* (Tzvelev, 1987; Hong, 1989) and placed by Hong (1989) within the tribe Coccolobeae, also belongs to the Polygoneae (Liu *et al.*, 2007). In fact, it has the same tepal nervature and similar pollen morphology; furthermore it greatly resembles *Fallopia* due to the morphology of the pollen, the structure of the exocarp, the presence of the anthraquinone and the chromosome number; the style and the stigma instead are similar to *Reynoutria* (Hong, 1989; Wang & Feng, 1994; Ronse Decraene *et al.*, 2000; Zhou *et al.*, 2002). In the description of this genus the presence of extrafloral nectaries is never mentioned and this character, if confirmed, could favour its inclusion within the subtribe Polygoninae.

Tribus **Persicarieae** Dumort.

(clades I + III)

Clades I and III taken together correspond well to the tribe Persicarieae, as was proposed by Ronse Decraene & Akeroyd (1988) however, excluding *Fagopyrum* and related genera. It is characterized by:

- 1) tepals with three main nervatures which depart from the base,
- 2) epidermic cells rectangular to elongate with straight or undulating anticlinal walls, cuticles smooth or striate in longitudinal direction and often continuous (type I sensu Hong *et al.*, 1998),
- 3) conspicuous nectaries, free or variously fused; trichomes or papillae usually present.

Actually the tree presented here does not support, nor exclude, a link between clades I and III, however, the morphological affinities, highlighted several times in the literature, are remarkable and also the phylograms by Liu *et al.* (2007), Sun *et al.* (2008), Kim & Donoghue (2008), Sanchez & Kron (2008), as well as the abstract by Kim *et al.* (2005) confirm the monophyletic nature of the Persicarieae. Nevertheless, the two clades are quite distinct and it is proposed here to treat them at subtribe rank.

Subtribus **Persicariinae** (Dumort.) Galasso, Soldano & Banfi, **subtrib. nov.**

Bas.: *Persicarieae* Dumort., *Fl. Belg.*: 17. 1827.

(clade I, bootstrap 95%)

Genus included: *Persicaria*.

The only genus belonging to this subtribe is characterized by a peculiar morphology of the surface of the pollen granules, which is invariably reticulate but has smooth and not granulate muri (Hedberg, 1946; Park, 1988; Hong & Hedberg, 1990; Hong, 1992; Wang & Feng, 1994). The characteristics of the surface of the achenes are also shared by all the subtribes (Ronse Decraene *et al.*, 2000). Furthermore, the tepals are fused for almost 1/3, thus differing from Koenigiinae in which they are free or fused only at the base (Ronse Decraene & Akeroyd, 1988). Finally, the stamens are not constant in number and the nectaries are not themselves fused nor fused with the stamens to form a disc, except in *Persicaria* sect. *Cephalophilon* (Ronse Decraene & Akeroyd, 1988; Ronse Decraene & Smets, 1991; Hong, 1993); the latter section, based on Kim & Donoghue (2008), results as being the sister group of all the genus.

Persicaria (L.) Mill. (ca. 100 spp.)

≡ *Polygonum* L. [unranked] *Persicaria* L.

T: *Persicaria maculosa* Gray (≡ *Polygonum persicaria* L.)

The number and arrangement of the pollen apertures within the genus *Persicaria* allow 4 typologies to be recognized, which are divided into 6 monophyletic groups identified in the dendrogram and here considered at the level of section; these are quite distinct from each other also on the basis of the macromorphology of the inflorescence and the presence of prickles on the stem.

- Typology 1 (“species *virginiana-filiformis* = sect. *Tovara*”): pollen grains 12-porate with pores arranged as the edges of a cube; stem without prickles; inflorescence linear (strictly spicate and interrupted) with two persistent styles, obliquely bent backwards and hardened at maturity; reduced number of tepals and stamens.

- Typology 2 (“species *runcinata-nepalensis* = sect. *Cephalophilon*”): pollen grains 3(-8)-colpate; stem without prickles; inflorescence capitate.

- Typology 3 (“species *odorata-acuminata* = sect. *Persicaria*” + “species *bungeana* = sect. *Truelloides*” + “species *sentiosa-thunbergii* = sect. *Echinocaulon*”): pollen grains polyporate; inflorescence spicate and flexible and stem without prickles in sect. *Persicaria*; inflorescence always spicate but presence of prickles on stem in sect. *Truelloides*; inflorescence capitate and prickles along stem in sect. *Echinocaulon*.

- Typology 4 (“species *amphibia* = sect. *Amphibiae*”): pollen grains 30-porate with pores arranged as the edges of a pentagonal dodecahedron; stem without prickles and inflorescence spicate and relatively rigid, with robust rhizomes.

Persicaria (L.) Mill. sect. ***Persicaria*** (ca. 60 spp.)

(“species *odorata-acuminata*”, bootstrap 75%)

T: *Persicaria maculosa* Gray (≡ *Polygonum persicaria* L.)

= *Polygonum* L. sect. *Amblygonon* Meisn. [1826]

≡ *Persicaria* (L.) Mill. sect. *Amblygonon* (Meisn.) Tzvelev

≡ *Amblygonum* Rchb. [1837]

T: *Polygonum orientale* L. (≡ *Persicaria o.* (L.) Spach)

= *Lagunea* Lour. [1790], non *Laguna* Cav. [1786], nom. illeg.

≡ *Goniaticum* Stokes [1812]

- T: *L. cochinchinensis* Lag. (= *Persicaria orientalis* (L.) Spach)
 = *Persicaria* (L.) Mill. sect. *Hydropiper* M.A.Hassan
 T: *P. hydropiper* (L.) Delarbre
 = *Persicaria* (L.) Mill. sect. *Planocarpon* M.A.Hassan
 T: *P. lapathifolia* (L.) Delarbre

The generally accepted infrageneric classification of *Persicaria* is therefore confirmed in outline by the present cladogram; the differences lie in the inclusion of the sect. *Amblygonon* in the sect. *Persicaria* and in the addition of the sect. *Amphibia* and *Truelloides*. The section *Amblygonon* (not *Amblygonum*!) was established due to the position of the cotyledons, which are incumbent rather than accumbent. Nevertheless, as already noted by Danser (1927) and by Hedberg (1946), this character may vary also within the same individual and therefore is not of taxonomic value. The only species considered in the present study as belonging to this section is *Persicaria orientalis*, which falls in the cladogram within the core of *Persicaria* s.s. (species *odorata-acuminata*) thus confirming the systematic invalidity of this section. Regarding the sections *Amphibiae* and *Truelloides* refer to what is written below.

Other two sections have been described within the genus *Persicaria*, sect. *Hydropiper* and sect. *Planocarpon* (Hassan, 1997), both included within sect. *Persicaria* by the present tree. The latter however, subsequent to further analyses covering a greater number of species may be re-evaluated, together with sect. *Amblygonon*, at the rank of series and with a different boundary.

One species of this section, endemic to the Galápagos Islands (Caruel, 1889; Wiggins, 1971), is still lacking a combination in *Persicaria*. This is proposed below.

Persicaria galapagensis (Caruel) Galasso, **comb. nov.** (bas.: *Polygonum galapagense* Caruel, *Atti Reale Accad. Lincei. Rendiconti*, s. 4, 5 (1): 624. 1889)

Persicaria (L.) Mill. sect. ***Amphibia*** Tzvelev [1987] (1 sp.)
 (“species *amphibia*”, bootstrap 99%)

T: *Persicaria amphibia* (L.) Delarbre
 = *Persicaria* (L.) Mill. [unranked] *Amphibiae* Small [1933]

T: *Persicaria muhlenbergii* (S.Watson) Small (= *Persicaria amphibia* (L.) Delarbre)

Persicaria amphibia has always been related to all the other species of sect. *Persicaria*, nevertheless it has some characteristics (pollen typology and the presence of a robust rhizome) which place it in a particular position. It was Small (1933) that first recognized its isolated position, while Tzvelev (1987) proposed a new section ad hoc. The analyses of some nucleotide sequences by Kim & Donoghue (2005), Kwak *et al.* (2006) and Kim & Donoghue (2008) confirm its isolated position; the further results presented here indicate that it merits its own section.

This taxon, widely spread throughout the boreal hemisphere and naturalized in Mexico, South America and South Africa, is a highly polymorphic species: there are aquatic-adapted forms and terrestrial-adapted forms, each one being highly variable. For instance, in North America two extreme ecotypes are recognized, one aquatic (var. *stipulacea* (N.Coleman) H.Hara, it is not stated that it is the priority epithet) and one emergent or terrestrial (var. *emersa* (Michx.) J.C.Hickman) linked by an almost continuous series of morphological intermediates (Turesson, 1961; Mitchell, 1968, 1976); the Eurasian plants (var. *amphibia*) are intermediate between the two American extremes, however, they are sometimes indistinguishable (Mitchell & Dean, 1978). There are not therefore sufficient biosystematic reasons for recognising the formal value of the various morphotypes, analogously to the recent treatment in the ‘Flora of North America’ (Hinds & Freeman, 2005).

Persicaria (L.) Mill. sect. ***Cephalophilon*** (Meisn.) H.Gross (ca. 15 spp.)

(“species *runcinata-nepalensis*”, bootstrap 99%)

≡ *Polygonum* L. sect. *Cephalophilon* Meisn.

≡ *Cephalophilon* (Meisn.) Spach [1841], non *Cephalophilum* Meisn. ex Börner [1912]

≡ *Truellum* Houtt. sect. *Cephalophilon* (Meisn.) Soják

T: *Polygonum nepalense* Meisn. (≡ *Persicaria n.* (Meisn.) H.Gross)

= *Ampelygonum* Lindl. [1838]

T: *A. chinense* (L.) Lindl. (≡ *Persicaria c.* (L.) H.Gross)

As far as is known a recent systematic revision of this section is lacking. Firstly, the species included in it and for which combinations are lacking are the following; others may be added subsequently.

Persicaria greuteriana Galasso, **nom. nov.** (bas.: *Polygonum wallichii* Meisn., *Monogr. Polyg.*: 83. 1826, non *Persicaria wallichii* Greuter & Burdet, *Willdenowia*, 19 (1): 41. 1989). This species may be included within the variability of *P. microcephala*.

Persicaria malaica (Danser) Galasso, **comb. nov.** (bas.: *Polygonum malaicum* Danser, *Bull. Jard. Bot. Buitenzorg*, ser. 3, 8: 218. 1927). This species may be included within the variability of *P. chinensis*.

Persicaria strindbergii (J.Schust.) Galasso, **comb. nov.** (bas.: *Polygonum strindbergii* J.Schust., *Bull. Herb. Boiss.*, sér. 2, 8: 712. 1908)

Persicaria umbrosa (Sam.) Galasso, **comb. nov.** (bas.: *Polygonum umbrosum* Sam. in *Hand.-Mazz.*, *Symb. Sin.*, 7: 182. 1929)

Persicaria (L.) Mill. sect. ***Echinocaulon*** (Meisn.) H.Gross (ca. 21 spp.)

(“species *senticosa-thunbergii*”, bootstrap 93%)

≡ *Polygonum* L. sect. *Echinocaulon* Meisn.

≡ *Echinocaulon* (Meisn.) Spach [1841], non Kütz. [1843]

T: *Polygonum sagittatum* L. (≡ *Persicaria s.* (L.) H.Gross ex Nakai)

= *Truellum* Houtt.

T: *Truellum japonicum* Houtt. (= *Persicaria senticosa* (Meisn.) Nakai)

= *Chylocalyx* Hassk.

T: *Chylocalyx perfoliatus* (L.) Hassk. (≡ *Persicaria p.* (L.) H.Gross)

The section *Echinocaulon*, sometimes considered at generic rank under the name *Truellum* (Soják, 1974), is characterized by the presence of recurved prickles on the angles of the stems, on the petioles and on the main nervatures of the abaxial surface of the leaves, by the often scandent habit and by the base of the leaves being hastate or sagittate; it appears monophyletic from a macromorphological point of view (Park, 1988). Nevertheless, except for the prickliness, which is sometimes absent as in *Persicaria nogueirae* S.Ortíz & Paiva (Ortíz & Paiva, 1999), its species are very variable with regard to other characters such as the chemistry of the flavonoids (Park, 1987), the trichomes, the flowers and the achenes (Kim *et al.*, 2001); instead, the pollen grains (Hedberg, 1946) and the other characters such as the morphology of the epidermis of the tepals (Hong *et al.*, 1998) are indistinguishable from those of sect. *Persicaria*. Regarding *Persicaria bungeana*, sometimes considered as belonging to this section (Park, 1988), even if atypical, refer to the description in the present study of sect. *Truelloides*. In *Persicaria perfoliata* the perianth becomes fleshy in fruit and for this reason it is sometimes considered as belonging to the genus *Chylocalyx*.

Some species of the section *Echinocaulon* are still lacking a combination in *Persicaria*. These are proposed below.

Persicaria brachypoda (Baker) Galasso, **comb. nov.** (bas.: *Polygonum brachypodum* Baker, *J. Linn. Soc. Bot.*, 20: 239. 1883)

Persicaria clarkei (C.W.Park) Galasso, **comb. nov.** (bas.: *Polygonum clarkei* C.W.Park, *Brittonia*, 38 (3): 217. 1986)

Persicaria rubricaulis (Cham.) Galasso, **comb. nov.** (bas.: *Polygonum rubricaulis* Cham., *Linnaea*, 8: 130. 1833)

Persicaria stelligera (Cham.) Galasso, **comb. nov.** (bas.: *Polygonum stelligerum* Cham., *Linnaea*, 8: 131. 1833)

Persicaria (L.) Mill. sect. *Tovara* (Adans.) H.Gross (3 spp.)

(“species *virginiana-filiformis*”, bootstrap 99%)

≡ *Tovara* Adans., nom. rej.

T: *Tovara virginiana* (L.) Raf. (≡ *Persicaria* v. (L.) Gaertn.)

= *Antenoron* Raf.

T: *Antenoron racemosum* Raf. (= *A. virginianum* (L.) Roberty & Vautier ≡ *Persicaria virginiana* (L.) Gaertn.)

This section, morphologically distinct from the others, is distributed primarily in eastern Asia and eastern North America where it is represented by three species with high affinity but well separated by morphology (Park *et al.*, 1992), by the chemistry of the flavonoids (Mun & Park, 1995) and by the ITS sequences (Youngbae *et al.*, 1997).

Persicaria (L.) Mill. sect. *Truelloides* Tzvelev (1 sp.)

(“species *bungeana*”)

T: *Persicaria bungeana* (Turcz.) Nakai

According to Park (1988) *P. bungeana* could belong to sect. *Echinocaulon* as it has recurved prickles along the stem. Nevertheless, it possesses some characters that differentiate it from the other species of the same section and show greater affinities to that of sect. *Persicaria* (Hedberg, 1946; Park, 1988): the leaves are cuneate at the base (not hastate or cordate), the ocreolas are funnel-shaped, the inflorescence elongate (not capitate), the prickles along the stem are scarce. For this reason many authors, including Haraldson (1978), Hong *et al.* (1998), Ronse Decraene *et al.* (2000) and the same Soják (1974) who was the first to reconsider the genus *Truellum*, ascribed it to sect. *Persicaria*, while Tzvelev (1987, 1989) established a section ad hoc nominated *Truelloides*. Preliminary molecular analyses of the sect. *Echinocaulon*, based on the nucleotide sequences (Yoo & Park, 2001), confirm the isolated position of this species and its sister relationship with sect. *Persicaria*. The results presented here provide further confirmation of this conclusion.

Subtribus **Koenigiinae** Dammer

(clade III, bootstrap 99%)

Genera included: *Aconogonum*, *Bistorta*, *Koenigia*, *Rubrivena*.

The genera belonging to this subtribe share a peculiar morphology of the surface of the pollen grains: spinulose, microspinulose or granulate (Hedberg, 1946; Hong & Hedberg, 1990; Hong, 1991a, 1992, 1993; Hedberg, 1997; Zhou *et al.*, 2004). In *Koenigia* it is spinulose, with spinules (of variable length in diverse individuals) intermingled with microspinules; except for *K. delicatula* (Meisn.) H.Hara subsp. *relicta* Hedberg (see also Hedberg, 1988), which has a reticulate surface without spinules but with granulate muri, analogous to *Rubrivena*. In *Aconogonum* and *Bistorta* it is microspinulose, with the exception of *A. campanulatum* (Hook.f.) H.Hara (see also Hong, 1991b), whose long-style plants have very long microspinules similar to those of *Koenigia*. In *Rubrivena* it is reticulate, as in the Persicariinae, however, in contrast to this, the muri are gra-

nulate; this represents a unique character in the Polygonaceae, shared only by *Koenigia delicatula* subsp. *relicta*. The number and arrangement of the apertures, instead, varies considerably and is not of particular taxonomic significance at genus level, ranging from 3-colpate to polycolpate to polyporate (Hong & Hedberg, 1990); nevertheless, *Bistorta* is distinguished from the others in that it always has 3-colporate pollen.

Furthermore, the tepals of Koenigiinae are free or fused only at the base, while in the Persicariinae they are fused for circa 1/3 (Ronse Decraene & Akeroyd, 1988). Finally, the nectaries fuse with the base of the stamens to form a disc, although incomplete in *Bistorta* (Ronse Decraene & Akeroyd, 1988; Ronse Decraene & Smets, 1991; Hong, 1993). The androecium is constantly octameric (Král, 1985; Ronse Decraene *et al.*, 2000), apart from in *Koenigia* where it is reduced (Ronse Decraene, 1989; Hedberg, 1997).

An alternative systematic solution to that put forward here is that of maintaining united the genera *Aconogonum*, *Koenigia* and *Rubrivena*, with the priority name *Koenigia*. Taking into consideration the fact that *Koenigia* has always been kept distinct from *Aconogonum* and that this union would mean numerous new nomenclatural combinations, it is recommended here that the three genera be retained distinct.

***Aconogonum* Rchb. (ca. 25 spp.)**

≡ *Polygonum* L. sect. *Aconogonon* Meisn.

T: *Polygonum divaricatum* L. (≡ *Aconogonum d.* (L.) Nakai) designated by Roberty & Vautier (1964), non *A. alpinum* (L.) Schur designated by Král (1985)

In relation to the spelling of the name of this genus see Galasso *et al.* (2006).

Its limits are well defined from a macromorphological and palynological point of view and only *Knorringia* and *Rubrivena* must be separated from this genus. Nevertheless, it should be noted that in *A. campanulatum* (Hook.f.) H.Hara, the only species of *Aconogonum* that shows the phenomenon of heterostylism (Conolly, 1977; Hong, 1991b; Hong, 1993), the long-styled plants have pollen with elongate microspinules similar to the spinules of *Koenigia* (Hong & Hedberg, 1990; Hong, 1991b; Hedberg, 1997).

***Koenigia* L. (6 spp.)**

T: *K. islandica* L.

= *Polygonum* L. sect. *Eleutherospermum* Hook.f.

T: *Polygonum delicatulum* Meisn. (≡ *Koenigia delicatula* (Meisn.) H.Hara)

The genus *Koenigia* has long been considered monotypic. It should, however, be extended to include the species that were placed within *Polygonum* L. sect. *Eleutherospermum* Hook.f., that share the same characteristically spinulose pollen type (Hedberg, 1946; Měsíček & Soják, 1973; Hedberg, 1997; Zhou *et al.*, 2004). As already mentioned above, *K. delicatula* (Meisn.) H.Hara is a very particular species, with pollen dimorphism: the nominal subspecies has spinulose pollen, typical for this genus, while the subsp. *relicta* Hedberg has reticulate pollen with granulate muri, typical of the genus *Rubrivena* (Hedberg, 1988; Hong & Hedberg, 1990).

The dendrogram shows *Koenigia* as monophyletic, analogously to the results presented by Liu *et al.* (2007), and sister to *Aconogonum*; in its turn, *Rubrivena* is sister to both the latter genera.

***Rubrivena* M.Král (2 spp.)**

≡ *Persicaria* (L.) Mill. sect. *Rubrivena* (M.Král) S.P.Hong

T: *R. polystachya* (Wall. ex Meisn.) M.Král (≡ *Polygonum polystachyum* Wall. ex Meisn.; ≡ *Aconogonum polystachyum* (Wall. ex Meisn.) Small [1922]; ≡

Aconogonum polystachyum (Wall. ex Meisn.) M.Král [1969], comb. superfl.; ≡ *Aconogonum polystachyum* (Wall. ex Meisn.) Haraldson [1978], comb. superfl.; ≡ *Persicaria wallichii* Greuter & Burdet, ≡ *Persicaria polystachya* (Wall. ex Meisn.) H.Gross [1913], non Opiz [1852])

The species *Polygonum polystachyum* has been variously attributed to the genera *Aconogonum* (see Král, 1969; Haraldson, 1978), *Persicaria* (see Hong & Hedberg, 1990; Ronse Decraene & Smets, 1991; Hong, 1993; Hong *et al.*, 1998; Ronse Decraene *et al.*, 2000) or to its own genus *Rubrivena* (Král, 1985). Actually, as highlighted in the studies listed and as has been stated previously, it shows macromorphological aspects typical of *Aconogonum*, while the pollen characters show affinities to *Persicaria*. Nevertheless, it is differentiated from *Persicariinae* as the crests of the muri are granulate and not smooth; also the epidermic cells of the achenes differ from those of *Persicaria* (Hong *et al.*, 1998). Furthermore, it differs from *Aconogonum* s.s. in having long styles (Král, 1985): *Rubrivena* is heterostylous, as is *Aconogonum campanulatum* (Conolly, 1977; Hong, 1991b; Hong, 1993), nevertheless, it differs from the latter species as even the short-styled plants have styles longer than *Aconogonum*.

On the basis of the present results, this species does not appear to be related to *Persicaria*, but rather to *Aconogonum*; in particular it results as being sister of the pair *Aconogonum/Koenigia*. The retention of the genus *Rubrivena* appears more than justified. *R. pinetorum*, strictly related to *R. polystachya* (Hong, 1993), is added to the genus, as already suggested by Král (1985); the new combination is proposed below.

Rubrivena pinetorum (Hemsl.) Galasso, Labra & F.Grassi, **comb. nov.** (bas.: *Polygonum pinetorum* Hemsl., *J. Linn. Soc. Bot.*, 26: 345. 1891)

Bistorta (L.) Scop. (ca. 50 spp.)

≡ *Polygonum* L. [unranked] *Bistorta* L.

T: *Bistorta officinalis* Delarbre (≡ *Polygonum bistorta* L.)

On the basis of the cladograms by Lamb Frye & Kron (2003), Kim & Donoghue (2008) and the results presented here the genus *Bistorta*, being homogeneous from a morphological and palynological point of view, appears as sister of the (*Aconogonum/Koenigia*)/*Rubrivena* group.

Tribus **Fagopyreae** Yonek.

= cohors Fagopyrastrae Roberty & Vautier [According to Roberty & Vautier (1964) the cohors is a rank between subtribe and genus]

(clades IV + IX)

Genera included: *Fagopyrum*, *Harpagocarpus* and, probably, *Eskemukerjea* and *Pteroxygonum*.

The genus *Fagopyrum* and the genera correlated with it have morphological particularities that differentiate them both from the *Persicarieae* and the *Polygoneae*. They were traditionally related to *Fallopia* due to the superficial similarity (Gross, 1913a); nevertheless, on the basis of some floral characters (stamen morphology, tepal nervation and nectary typology) Ronse Decraene & Akeroyd (1988) included them in the *Persicarieae* (at the base), while, again on the basis of the nectaries, Ronse Decraene & Smets (1991) placed them at the base both of the *Persicarieae* and the *Polygoneae*. It should also be noted that the tepal nervation is not constant in all of the species but in some it is more similar to *Polygoneae* and in others to *Persicarieae*.

The morphology of the epidermic cells of the tepals does not help in clarifying the situation: *Fagopyrum* sect. *Polygonopsis*, *Harpagocarpus* and *Pteroxygonum*

have type I cells (sensu Hong *et al.*, 1998) analogously to Persicarieae, while type III (as in *Oxygonum*) are found in *Fagopyrum* sect. *Fagopyrum* and *Eskemukerjea*. It is probable that this similarity between *Fagopyrum* sect. *Fagopyrum* and *Oxygonum* is only due to homoplasy (Hong *et al.*, 1998), as both these genera show significant differences in other characters, such as the tepal vascularization and the typology of the nectary. The morphology and anatomy of the achenes (Ronse Decraene *et al.*, 2000) also show particularities not shared by the other tribes.

The present results do not correlate *Fagopyrum* to any of the other genera of the Polygonoideae; therefore, the best systematic solution seems to be to consider an own tribe, according to Yonekura (Iwatsuki *et al.*, 2006), which probably should be placed at the base of the Polygoneae + Persicarieae as was already suggested by Ronse Decraene & Smets (1991), or only the Persicarieae (Kim & Donoghue, 2008; Sun *et al.*, 2008). Marek (1958) previously suggested placing this genus in its own subfamily, while Ronse Decraene *et al.* (2000) seemed more inclined to assign it to its own tribe.

The genera *Eskemukerjea*, *Harpagocarpus* and *Pteroxygonum* are, according to the authors, variously retained as distinct or included in *Fagopyrum* s.l. Among the numerous phylogenetic studies on *Fagopyrum* based on DNA sequences (Yasui & Ohnishi, 1996; Ohnishi & Matsuoka, 1996; Ohsako & Ohnishi, 1998; Yasui & Ohnishi, 1998a, 1998b; Ohsako & Ohnishi, 2000; Ohsako *et al.*, 2001, 2002; Yamane *et al.*, 2003; Sun *et al.*, 2008), only Ohsako *et al.* (2001) and Sun *et al.* (2008) have to date taken into consideration two of these satellite genera, respectively *Eskemukerjea* and *Pteroxygonum*. Their results reveal that neither of these genera are strictly related to *Fagopyrum* s.s., however, the results do not allow them to be collocated precisely within the Polygoneae. In addition the present tree confirms the autonomy of *Eskemukerjea*, which may also not belong to this tribe. It would be useful in future research to include also the other genera segregated from *Fagopyrum* utilizing the same sequences in order to clarify their reciprocal relationships and verify the monophyly of Fagopyreae, which is nevertheless most probable on the grounds of morphological similarities.

***Eskemukerjea* Malick & Sengupta (1 sp.)**

(clade IV)

T: *Eskemukerjea nepalensis* Malick & Sengupta (= *Eskemukerjea megacarpa* (H.Hara) H.Hara ≡ *Fagopyrum megacarpum* H.Hara)

A monotypic genus from Nepal, not recognized by Hong *et al.* (1998) but clearly distinct from *Fagopyrum* s.s. on the basis of the present tree and the previous study by Ohsako *et al.* (2001); as stated it may also not belong to this tribe.

***Fagopyrum* Mill. (ca. 19 spp.)**

(clade IX, bootstrap 96%)

T: *Fagopyrum esculentum* Moench, typ. cons. (≡ *Polygonum fagopyrum* L.)

As shown both by the morphology and by the isozymes and DNA (Ohnishi & Matsuoka, 1996; Yasui & Ohnishi, 1998a, 1998b; Ohsako *et al.*, 2001), the genus *Fagopyrum* s.s. is monophyletic and composed of two groups of species: “*cymosum*-group” and “*urophyllum*-group”. The first is characterized by large and dull achenes, only partially covered by the perianth which is persistent, characterized by epidermic cells of type III (sensu Hong *et al.*, 1998) and with three main nervatures analogously to Persicarieae (Sun *et al.*, 2008); the second has shiny achenes of smaller dimension, completely covered by the perianth, which is persistent, characterized by epidermic cells of type I (sensu Hong *et al.*, 1998) and has only one main

nervature analogously to Polygoneae (Sun *et al.*, 2008). These two groups, clearly recognizable also in the present results, may be considered at the rank of section, analogously to what has been already proposed by Roberty & Vautier (1964).

Fagopyrum Mill. sect. **Fagopyrum** (4 spp.)

(clade IX a, bootstrap 100%)

≡ *Helxine* L.

≡ *Phegopyrum* Peterm.

T: *Fagopyrum esculentum* Moench, typ. cons. (≡ *Polygonum fagopyrum* L.)

= *Kunokale* Raf. [1837] (“1836”)

T: *K. carneum* Raf., nom. illeg. (≡ *Polygonum emarginatum* Roth = *Fagopyrum esculentum* Moench)

Fagopyrum Mill. sect. **Polygonopsis** Roberty & Vautier (14 spp.)

(clade IX b, bootstrap 99%)

T: *F. gilesii* (Hemsl.) Hedberg

Also *F. suffruticosum* Schmidt, to date not considered in the phylogenetic studies may belong to this section.

Harpagocarpus Hutch. & Dandy (1 sp.)

T: *Harpagocarpus snowdenii* Hutch. & Dandy (≡ *Fagopyrum snowdenii* (Hutch. & Dandy) S.P.Hong; = *Fagopyrum ciliatum* Jacq.-Fél.)

A monotypic genus that, differently to the other species of *Fagopyrum* s.l., is distributed in eastern Africa. Based on the suggestions by Ronse Decraene & Akeroyd (1988) and the palynological studies by Hong (1988), Ronse Decraene & Smets (1991) and Hong *et al.* (1998) proposed not considering it valid and including it in *Fagopyrum*. Until further in depth studies are carried out the authors prefer to retain it at genus rank, also due to its different geographical distribution.

Pteroxygonum Dammer & Diels (1 sp.)

≡ *Fagopyrum* Mill. sect. *Pteroxygonum* (Dammer & Diels) Haraldson

T: *Pteroxygonum giraldii* Dammer & Diels (≡ *Fagopyrum giraldii* (Dammer & Diels) Haraldson)

Chinese monotypic genus, distinguished from *Fagopyrum* s.s. due to the winged achene with three sharp horns at the base and an elongated floral tube in the fruiting stage; furthermore it has a basal chromosome number $x = 10$ instead of $x = 8$. Haraldson (1978), Ronse Decraene & Akeroyd (1988) and Hong *et al.* (1998) do not consider these characters sufficient for segregating it from *Fagopyrum* and they distinguish it only at section level; nevertheless, on the basis of the studies by Sun *et al.* (2008) it is clearly distinct from *Fagopyrum* s.s. and therefore also in the present study it is retained at genus rank, analogously to the treatment in the ‘Flora of China’ (Li & Grabovskaya-Borodina, 2003).

Tribus **Rumiceae** Dumort.

Genera included: *Emex*, *Oxyria*, *Rheum*, *Rumex*.

(clades V + VI + VII + VIII)

The present results do not support (but also do not refute) the monophyly of this tribe; instead, according to the phylogenetic analyses by Kim *et al.* (2005) and the phylogram by Sanchez & Kron (2008) the Rumiceae result as being monophyletic. It should be considered that the main aim of this research was *Polygonum* s.l. (Polygoneae and Persicarieae) and that the genera belonging to other groups are

poorly represented. Only further analyses, focused on their species, may verify their homogeneity, to date well supported by the morphological characters.

Emex Campd. (2 spp.)
(clade VI)

T: *Emex spinosa* (L.) Campd., typ. cons.

Certainly a monophyletic genus, composed of very similar species sometimes considered as subspecies. According to Sanchez & Kron (2008) it may be included within *Rumex*.

Oxyria Hill (1-4 spp.)
(clade VII)

T: *Oxyria digyna* (L.) Hill

Certainly a monophyletic genus, composed of very similar species sometimes considered as subspecies.

Rheum L. (ca. 60 spp.)
(clade VIII, bootstrap 90%)

T: *Rheum rhaponticum* L.

A genus confirmed as monophyletic from recent studies based on plastidial sequences (Wang *et al.*, 2005).

Rumex L. (ca. 200 spp.)
(clade V, bootstrap 81%)

T: *Rumex patientia* L.

Although on the basis of the results by Navajas-Pérez *et al.* (2005) based on nuclear and plastidial sequences the genus *Rumex* appears monophyletic, according to Sanchez & Kron (2008) it may include *Emex*.

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