Can subgenus *Rubus* (bramble) classification rely on morphological characters?

Introduction

Numerous species concepts have been debated since Linnaeus (1753, 1758) and by 1997, 24 species concepts were reviewed (Mayden, 1997; de Queiroz, 2005). Ranging from biological species, evolutionary species to ecotypes and micro-species, plant phenotypic plasticity and varied reproduction adds more complexity for defining plant species (Quinn, 1978; Rieseberg et al., 2006; Lowry, 2012). Although DNA barcoding has revolutionised plant species identification (Li et al., 2015; Kress et al., 2015), polyploidy, hybridisation and asexual reproduction poses challenges to species-level identification (Stebbins, 1969; Hörandl and Paun, 2007; Soltis et al., 2007). Unreliable morphology-based species identification can have an enormous impact on ecological studies, habitat management, and conservation (Hufford and Mazer, 2003; Ouborg et al., 2006).

The genus *Rubus* consists of many commercially important species (e.g. raspberries, blackberries) (Stirk et al., 2007), while subgenus *Rubus* (brambles) is ecologically important and known to include noxious and highly invasive environmental weeds worldwide (Figure 1, Table 1) (Amsellem et al., 2000; Caplan and Yeakley, 2013). Brambles are also known to be taxonomically challenging (Newton, 1980; Holub 1997; Haveman and Ronde, 2013). They have been described as biotypes (Taylor, 1958), morphotypes (Holub, 1997), species (Sochor et at., 2015) and micro-species (Weber, 1999; Edees et al., 1988). The complexity of their classification is mainly caused by (i) nature of polyploidy (Sochor et al. 2015; Sochor and Travnicek, 2016), (ii) variable reproduction (sexual, asexual, rapid hybridization; Kraft and Nybom, 1995; Šarhanová et al., 2012) and (iii) geographic parthenogenesis (Hörandl et al., 2008; Krutto et al., 2010; Haveman et al., 2016).

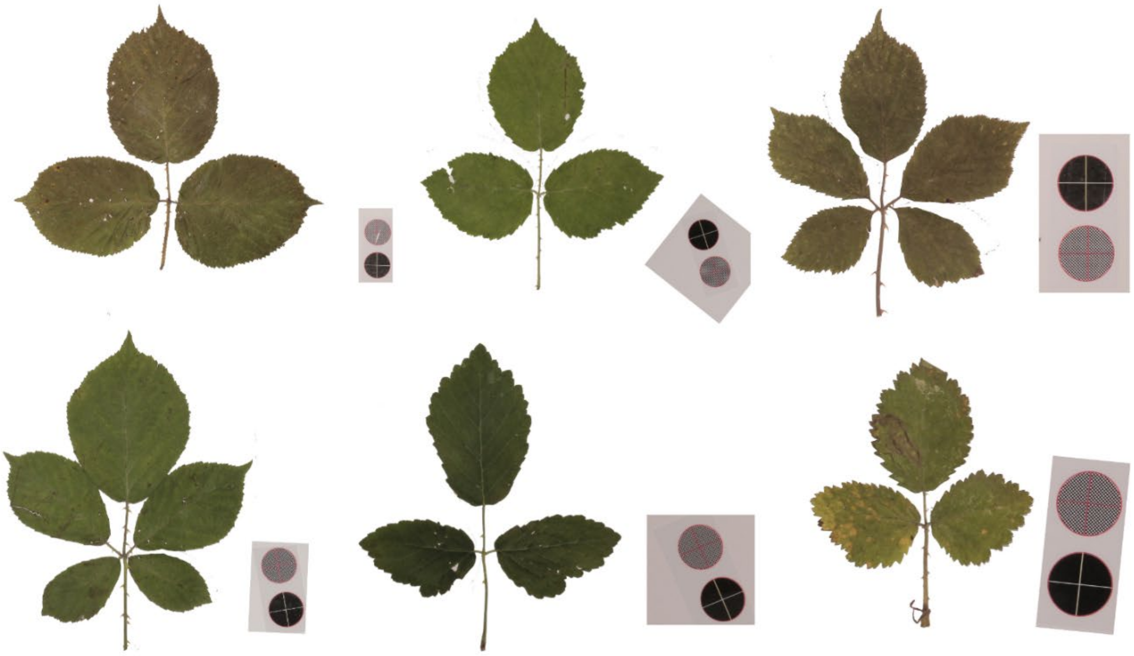


Figure 1. Diversity of bramble leaflet morphology shown of three European haplotypes (Sochor et al., 2015). Pictures taken by author in the UK.

Table 1. The four main reasons for genus *Rubus* research is due to their commercial value, invasiveness and their ecological importance, especially for pollinators.

Motivation	Study	Species	Reference
Commercial value	- Blackberry production: globally expanding: 13,958 ha (1995) to 20,036 ha (2005)	*R. fruticosus*	Stirk et al., 2007
	- Raspberry production in Serbia: 84,309 tons in 2002, pest infestations	*R. idaeus*	Nikolić et al., 2008
	- Tea production: no strong alkaloids, health potential	*R. caucasicus*	Melkadze et al., 2008
Invasiveness	- Asian micro-species in Australia	*R. alceifolius*	Baret et al., 2004
	- European micro-species in California: native oak *(Quercus)* establishment	*R. discolor*	Williams et al., 2006
	- Asian micro-species in America: competition with native brambles around tree falls	*R. phoenicolasius*	Gorchov et al., 2011
Pollinators	- Different pollinators attracted to different raspberry species	*R. idaeus, R. pubescens*	Whitney, 1984
	- Pollen quality assessed with cotton blue staining in Sweden	20 taxa	Nybom, 1985
	- Number of flowers, quantity of pollen and 332 insect pollinators in Canada	*R.chamaermorus*	Brown and McNeil, 2009
Ecology	- Role of herbivory, shade, fertiliser around a woodland on bramble growth	*R. vestitus*	Bazely et al., 1991
	- Tree seedling establishment under thickets	*R. fruticosus*	Harmer et al., 2010
	- Groundcover under canopy gap monitoring for eight years	*R. hirtus*	Pancer-Koteja et al., 1998

To avoid confusion, this essay uses the term micro-species and follows Edees et al.'s (1998) classification system. In their morphology-based classification system, the micro-species are grouped into sections, subsections, and series. Their identification focuses on characters of their leaves, prickles, flowers, and root systems. Sochor et al. (2015) suggested that although this is a pragmatic approach, however it does not reflect phylogenetic relationships. The origin of brambles is unknown but suspected it to be either Asia (Alice and Campbell, 1999), America or Europe (Darrow, 1920). In Europe, the number of taxa are estimated to be around 200-750 (Krutto et al., 2010; Sochor et al., 2015), however this depends on how a micro-species is defined.

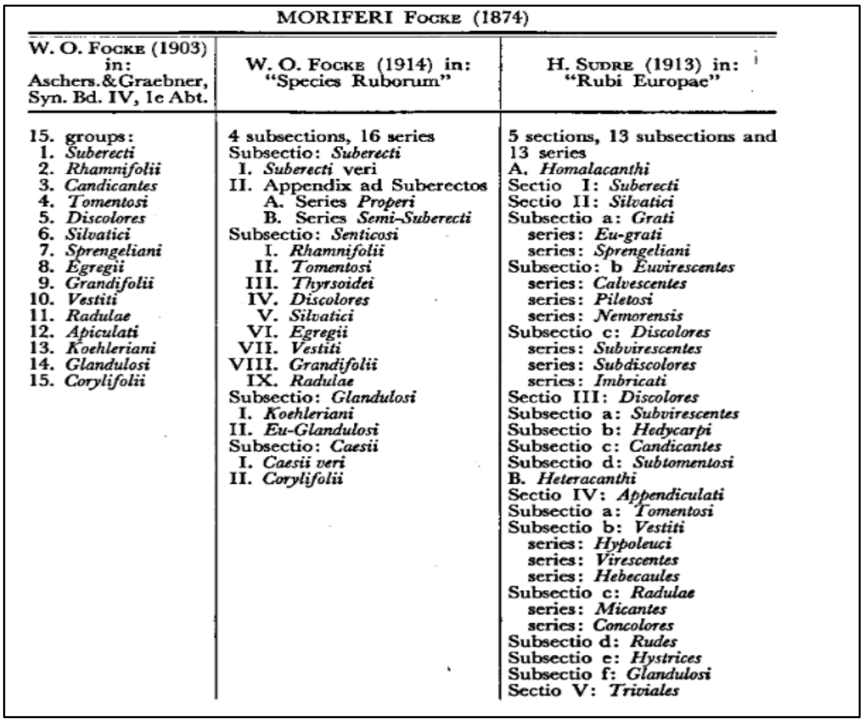
This essay reviews the brief history of bramble taxonomy, discusses studies focusing on morphology and briefly mentions recent molecular work. It aims to highlight the need for future molecular work and relying less on morphology-based identification.

Brambles life history and taxonomy

Knowledge of bramble life history is crucial for correct identification. Plant growth of *Hiemales* subsection is characterised by biennial shoots (Taylor, 2005; Bull, 2010). The first year shoot, the primocanes are the fastest growing developmental phase of brambles while the second year canes produce flowers and are called floricanes. Characters from both the primocane and the floricanes are needed for micro-species level identification. The majority of *Rubus* are tetraploid (with some triploid, pentaploid and hexaploid; Crane, 1940; Šarhanová et al., 2012) and only four are sexual diploid *(R. ulmifolius, R. canescens, R. incanescens and R. sanctus;* Kurtto et al., 2010). Their reproduction can vary from obligate sexuality to obligate apomixis (clonal reproduction through seeds, Asker and Jerling, 1992; Pratt and Einset, 1955) and rapid hybridisation leads to several genotypes even within a bramble shrub patch.

Brambles were under the name *R. fruticosus* agg. until Focke (1910) and Sudre (1908-13) first attempted to group European *Rubi* under 'circle species' terms (Figure 2). This concept was abandoned and replaced by grouping into sections and series in Watson's (1958, 1996), Weber's (1972; 1985) and Edees et al.'s (1988) classification systems.

Weber (1972) introduced the importance of distribution of micro-species, which was the 'Weberian reform' (Holub, 1997). The four groups were i) individual biotypes, (ii) locally distributed biotypes (<20 km), (iii) regionally distributed biotypes (50-250 km) and (iv) widely distributed biotypes (>500 km). This system helped scientist to describe national distributions of brambles and Haveman (2013) described this as the pragmatic species concept of brambles (Figure 3). In the UK, eight regional florulas were proposed by Newton (1980) with additional micro-florulas and regional endemic complexes. Krutto et al. (2010) mapped the distribution of 750 European micro-species and altered Weber's grouping slightly. Their five groups based on their distribution were (i) local (<50 km), (ii) regional (50-250 km), (iii) supra regional (250-500 km), (iv) widely distributed (500-1,500 km) and very widely distributed (> 1,500 km). Although these approaches were widely accepted in Europe (Holub, 1997, Haveman, 2013; Sochor and Travnicek, 2016), there are debates (Loos, 2008; Ryde, 2011).



MORIFERI Focke (1874)

W. O. Focke (1903) in: Aschers.&Graebner, Syn. Bd. IV, 1e Abt.	W. O. Focke (1914) in: "Species Ruborum"	H. Sudre (1913) in: "Rubi Europae"
15. groups: 1. *Suberecti* 2. *Rhamnifolii* 3. *Candicantes* 4. *Tomentosi* 5. *Discolores* 6. *Silvatici* 7. *Sprengeliani* 8. *Egregii* 9. *Grandifolii* 10. *Vestiti* 11. *Radulae* 12. *Apiculati* 13. *Koehleriani* 14. *Glandulosi* 15. *Corylifolii*	4 subsections, 16 series Subsectio: *Suberecti* I. *Suberecti* veri II. Appendix ad Suberectos A. Series *Properi* B. Series *Semi-Suberecti* Subsectio: *Senticosi* I. *Rhamnifolii* II. *Tomentosi* III. *Thyrsoidei* IV. *Discolores* V. *Silvatici* VI. *Egregii* VII. *Vestiti* VIII. *Grandifolii* IX. *Radulae* Subsectio: *Glandulosi* I. *Koehleriani* II. *Eu-Glandulosi* Subsectio: *Caesii* I. *Caesii veri* II. *Corylifolii*	5 sections, 13 subsections and 13 series A. *Homalacanthi* Sectio I: *Suberecti* Sectio II: *Silvatici* Subsectio a: *Grati* series: *Eu-grati* series: *Sprengeliani* Subsectio: b *Euvirescentes* series: *Calvescentes* series: *Piletosi* series: *Nemorensis* Subsectio c: *Discolores* series: *Subvirescentes* series: *Subdiscolores* series: *Imbricati* Sectio III: *Discolores* Subsectio a: *Subvirescentes* Subsectio b: *Hedycarpi* Subsectio c: *Candicantes* Subsectio d: *Subtomentosi* B. *Heteracanthi* Sectio IV: *Appendiculati* Subsectio a: *Tomentosi* Subsectio b: *Vestiti* series: *Hypoleuci* series: *Virescentes* series: *Hebecaules* Subsectio c: *Radulae* series: *Micantes* series: *Concolores* Subsectio d: *Rudes* Subsectio e: *Hystrices* Subsectio f: *Glandulosi* Sectio V: *Triviales*

Scheme of Classification by Edees and Newton, 1988

Genus ***Rubus*** L
Subgenus ***Rubus***
1. **Chamaemorus** (Hill) Focke
2. **Cylactis** (Raf.) Focke
3. **Anaplobatus** (Focke)
4. **Idaeobatus** (Focke)
5. ***Rubus***
    Section A. ***Rubus***
        Subsection 1. ***Rubus***
        Subsection 2. **Hiemales** E.H.L. Krause
        Series
        1. **Sylvatici** (Mueller) Focke
        2. **Rhamnifolii** (Babington) Focke
        3. **Spreneliani** Focke
        4. **Discolores** (Mueller) Focke)
        5. **Vestiti** (Focke) Focke
        6. **Mucronati** (Focke) Weber
        7. **Micantes** Sudre ex Bouvet
        8. **Anisacanthi** Weber
        9. **Radulae** (Focke) Focke
        10. **Hystrices** Focke
        11. **Glandulosi** (Wimmer and Grab.) Focke
    Section B. **Corylifolii** Lindley
    Section C. **Caesii** Lej. And Courtois

Figure 2. Classification by Focke, and Sudre from 1903 (left; Edees et al., 1988). The number of subsections and series grew as Sudre focused on morphological characters. Morphology-based classification used today (right) is based on Edees et al. (1988). This system has been widely accepted in Europe (Holub; 1997; Haveman et al., 2013; Sochor et al., 2015)

The distribution-based approach neglects to acknowledge hybrids, stabilised apomictic biotypes and unstablisied 'swarms' and capture the variation of a micro-species on large distribution range (Loos, 2008; Ryde, 2011). The light and soil conditions have also been used to describe *Rubus* distribution. Weber grouped them as thamnophilus (growing in open areas) and nemophilus (growing is sheltered areas) and according to temperature (e.g. series *Discolores* is xeromorphic, with waxy cuticle on stems and felted leaves) and soils (e.g. clay is associated with *Corylifolii*). These pragmatic approaches led to the current, highly artificial classification system.

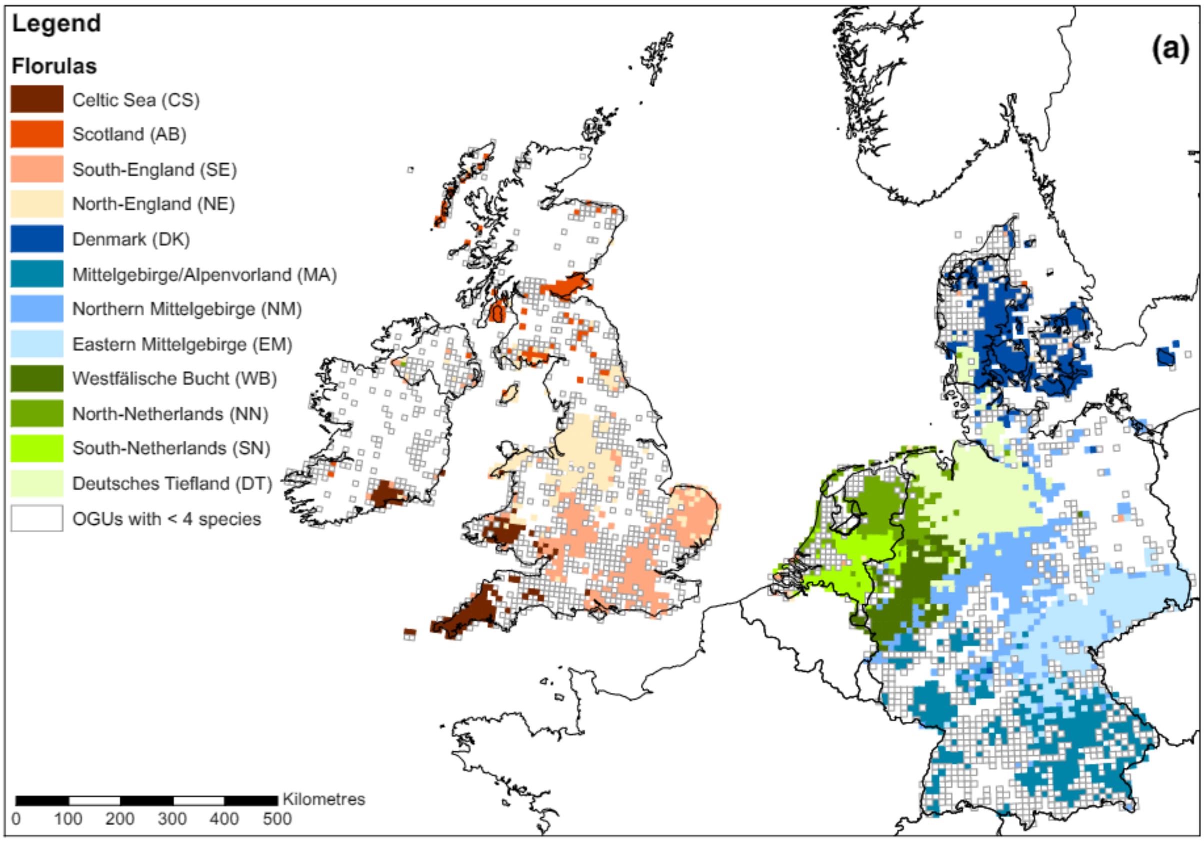


Figure 3. European florulas mapped by Haveman et al. (2013), using Krutto et al. (2010) distribution-based groupings. The reproduction of these groups can be physically or reproductively isolated.

Morphology manipulation and comparison

Only a few detailed observations have been reported for brambles (Beijernick, 1953; Heslop-Harrison, 1959; Taylor, 1980). These ambiguously described high variability of leaf size, petiole length, prickle number and size, stamen length and petal size and colour.

Quantitative, manipulative studies were first conducted in and around woodlands (Taylor, 1980; Bazely et al., 1991). Differences of leaf dry weight stem width and leaf numbers were found between *R. vestitus* primocanes, floricanes and lateral shoots at shaded and open locations (Taylor, 1980). *R. ulmifolius* showed different responses to different light conditions, fertiliser application, leaf and apical meristem cut back around a woodland (Bazely et al., 1991). There were significantly less number of internodes and prickles were at shaded areas compared to open canopies. There were more prickles and longer internodes with fertiliser application and more prickles due to leaf and apical meristem cut back. These studies were the first to show that there are differences in morphology of single micro-species at different locations of woodlands (Table 2).

Later studies compared the physiology and biomass of invasive and noninvasive micro-species (McDowell and Turner, 2002; Lambrecht-McDowell and Radosevich, 2005; Caplan and Yeakley, 2013).

These studies found the specific leaf area measurements to be lower for invasive species suggesting that thicker and denser leaves can outcompete the noninvasive micro-species. Plasticity and elasticity calculations were also significantly higher for invasive species especially for stem width. Photosynthetic rate was reported to be higher for *R. discolour* and *R. lacuniatus, two* invasive micro-species compared to *R. ursinus* and *R. leucodermis* noninvasive micro-species (McDowell and Turner, 2002). In terms of statistical stringency, the study by Caplan and Yeakley (2013) used principal component analysis (PCA) and data points clustered according to micro-species and different water regimes. This method of morphological differences is the most robust. They also found increased water use efficiency for the highly invasive *R. armeniacus* (Caplan and Yeakley, 2006) and different biomass allocations in response to drought conditions. The morphology of *R. alceifolius,* invasive micro-species on a volcanic island, varied greatly throughout different developmental stages (Baret et al., 2003). The morphometric analysis showed that while the plant is rapidly growing upwards, the stem girth increases more than the leaf volume until it reaches a tree and enters a non-self-supporting stage. Throughout this development, significant difference were found in leaf and stem measurements. In Poland, *R. hirtus* showed a sevenfold growth within eight years of two 30cm^2 plots, which suggests that space availability, has an enormous impact on bramble growth (Pancer-Koteja et al., 1998).

However, when Kraft and Nybom (1995) combined a morphometric analysis (Figure 4a) with genetic analysis, four pairs of micro-species were significantly different in terms of their morphology. Using DNA microsatellites, no genetic difference was found (Kraft and Nybom, 1995).

Table 2. Overview of studies, focusing on bramble morphology by either manipulating abiotic and biotic factors or comparing different species and their plasticity.

References	Comparison/ Manipulation	Morphology	Species
Bazely et al., 1991	Light, fertiliser addition, herbivory imitation in woodland	Internode length and width, prickle number	*R. ulmifolius*
Taylor, 1980	Density and canopy around woodland	Number and length of nodes, dry leaf weight, number of lateral shoots,	*R. vestitus*
McDowell and Turner, 2002	Invasive and noninvasive micro-species	Photosynthetic capacity, specific leaf area, construction cost, Nitrogen cc. per unit leaf mass	*R. discolor, R. laciniatus, R. ursinus, R. leucodermis*
Lambrecht-McDowell and Radosevich, 2005	Invasive and noninvasive micro-species, floral bud removal	Seedling mortality, cane length, cane growth, specific leaf, cane survival area, leaf area produced	*R. discolor, R. ursinus*
Caplan and Yeakley, 2013	Invasive and noninvasive micro-species, water regiment	Leaf area, plant mass, root mass, cane tissue density, length of canes, Huber value, specific cane length, specific root length, shoot water content, shoot growth rate	*R. armeniacus, R. spectabilis, R. parviflorus, R. ursinus, Rosa nutkana*
Baret et al., 2003	Five plant developmental stage	Number of nodes, internode lengths, pith diameter, leaf area, leaf volume, length of midrib	*R. alceifolius*
Pancer-Koteja et al., 1998	Forest canopy gap	Plant density and height	*R. hirtus*
Harmer et al., 2010	Fenced/unfenced plots, scarified/unscarified, vegetation control/no control	Survival of oak and birch seedlings, bramble height and density	*R. fruticosus*
Tomaszewski et al., 2014	Abaxial leaf surface with scanning electron microscope	Trichome branching, length, hair coverage	98 micro-species
Kellogg et al., 2011	Prickle and glandular trichome growth with scanning electron microscope	Prickle growth, prickle base width, prickle height	24 micro-species
Kraft and Nybom, 1995	Biometry of leaflets and DNA micro-satellites	Figure 4	10 micro-species

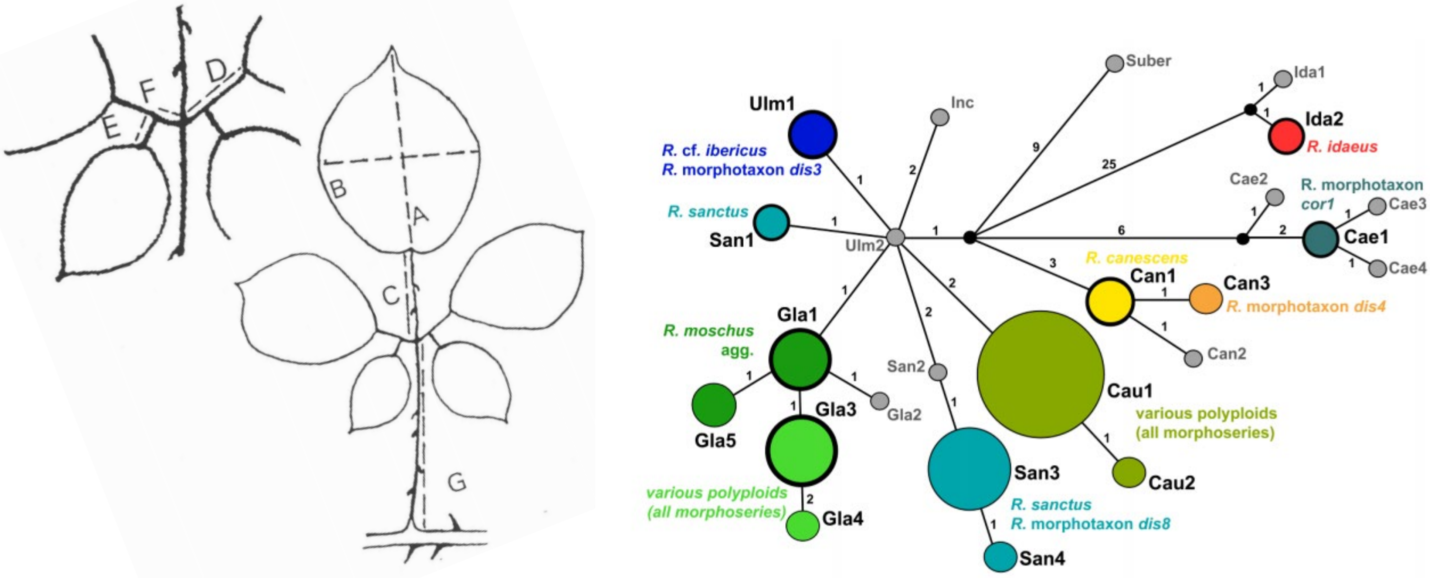


Figure 4. a) The lengths measured in the biometry analysis of 10 micro-species by Kraft and Nybom (1995). b) Median joining network of European haplotypes of *R. canescens,* which is a micro-species from Caucasus (Sochor et al., 2015). This micro-species is suggested to be one of the original haplotypes that hybridised in Europe.

Molecular results briefly

It was suggested that brambles could be targeted for shape difference studies, and to determine whether there is genetic or a non-genetic component of their phenotypic plasticity (Kessler and Sinha, 2004). Molecular studies (Alice and Campbell, 1999; Eriksson et al., 2003; Yang and Pak, 2006; Alice et al. 2001) combined maternally inherited cpDNA and biparentally inherited nrDNA markers (Wang et al., 2016) for phylogenetic analysis. Several new methods have also been used recently (Table 3).

The most recent molecular work using *trnL-trnF* and *matK* regions combined with nrDNA ITS (Figure 4b) (Šarhanová et al., 2012; Sochor et al., 2015; Sochor et al., 2017; Šarhanová et al., 2017; Kiraly et al., 2017) has shown that European haplotypes originate from a hybridisation event of seven diploid micro-species, and some of them are now extinct. However, morphology characters used for identification have not been consistent with phylogenetic studies (Alice and Campbell, 1999; Wang et al, 2016).

Table 3. Increasing number of molecular methods have been used to attempt to reconstruct bramble phylogeny. However, these methods can only be used for haplotype and ribotype studies and these lead to gene trees rather than species trees (Doyle, 1992).

Methods	- Microsatellites for *Discolores, Radula* and *Glandulosi* series	*10 taxa*	Šarhanová et al., 2017
	- SSR analysis on diploid and polyploid *Discolores* series from 11 European regions	*R. ulmifolius, R. sanctus*	Sochor et al., 2017
	- Flow cytometry for ploidy level determination	234 genotype	Meng and Finn, 2002
Phylogeny	- Korean bramble phylogeny using three loci	21 taxa	Yang and Pak, 2006
	- European bramble phylogeny with three loci and role of apomixis	145 microspecies	Sochor et al., 2015
	- Phytogeorgraphical analysis in Ireland, Great Britain, the Netherlands, Denmark and Germany	618 microspecies	Haveman et al., 2016

Conclusion

Subgenus *Rubus* has numerous taxonomical problems. From a morphological perspective, this is partly due to their phenotypic plasticity; e.g. plants in shaded areas compared to open sites have different morphologies (e.g. prickle numbers) in woodlands. Invasive micro-species have been shown to have different patterns of resource allocation (e.g. low specific leaf area) compared to noninvasive micro-species and their plasticity can explain their colonisation ability. It is important to consider their developmental stages when it comes to morphology and the effect of rapid resource availability (e.g. canopy gaps). The recent molecular results showed how complex is the European haplotype evolution but no relationship has been found in terms of bramble morphology and their phylogeny (Figure 5). Future molecular work is needed to understand gene expression in *Rubus.* With quantitative data on their phenotypic plasticity, we will be able to redefine what is a bramble micro-species which could ultimately lead to a new classification system.

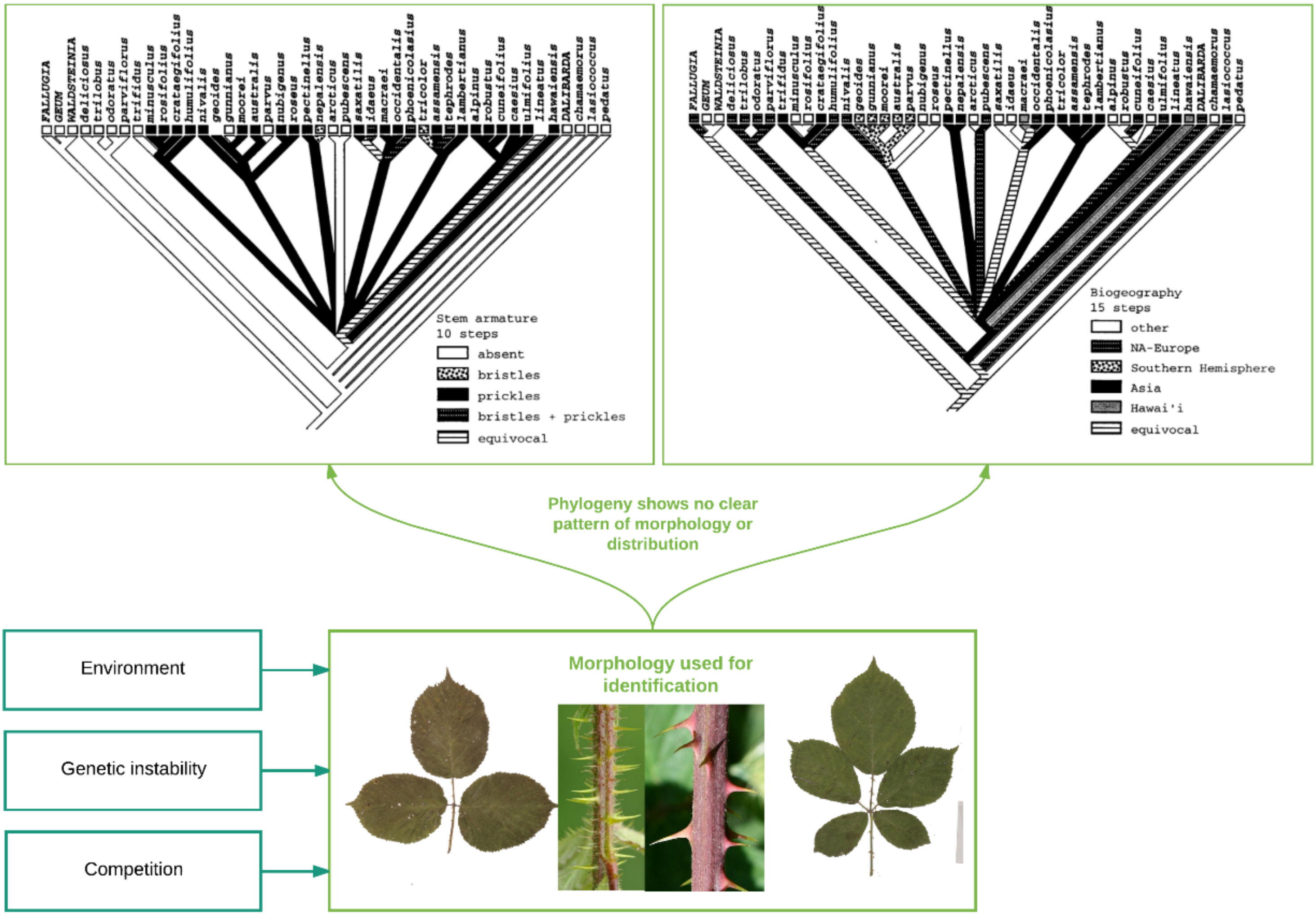


Figure 5. Phenotypic plasticity of bramble leaves, stems, prickles and overall growth enables them to thrive worldwide. However, defining the number of micro-species has to consider this plasticity and their numbers could be highly exaggerated. This could explain the unsuccessful molecular work looking for phylogenetic signal in their morphology (Alice and Campbell, 2009).

Word count: 1621

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