

Variability of venation patterns in extant genus *Salix*: Implications for fossil taxonomy

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The extant genus *Salix* Linnaeus (1753) represents one of the most diverse groups of woody plants. Leaf areas vary from a few mm² in arctic or high alpine habitats to more than 100 cm² in humid subtropical zones. *Salix* leaves are represented across the range of possible leaf shapes, from circular, obovate, and ovate, to lanceolate and linear with a length-to-width ratio of up to 30. Leaf venation may be eucamptodromous, eucamptodromous with occasional brochidodromous or semicraspedodromous arches, or brochidodromous. Because brochidodromous and semicraspedodromous arches may occur on the same leaf, the more inclusive term brochoid is introduced here. This study gives an overview of venation patterns within extant genus *Salix* and sorts leaves into five venation-defined morphotype groups. In some species of subgenera *Protitea* and *Salix*, individuals in hot, dry environments develop long brochoid chains over most of the blade length or intramarginal veins with only tertiary-gauged connections to the secondary vein framework. These unusual venation patterns correlate with high mean monthly temperature (MMT) and low mean monthly precipitation (MMP) of the hottest month. This study also discusses possible reasons as to why intramarginal veins seem to be absent or at least rare in fossil *Salix* specimens. These findings aid in both distinguishing between fossil *Salix* species and in separating fossil *Salix* remains from those of other genera.

Keywords: Salicaceae, *Salix*, leaf, venation, fossil, climate

INTRODUCTION

The extant genus *Salix* Linnaeus (1753) includes about 450 species worldwide (Argus 2010). Its species are native to all continents, but are not found in Australia, New Zealand, Oceania, and Antarctica (Skvortsov 1999). Willows are most widespread in temperate, boreal, and arctic-alpine climates but do extend into subtropical and tropical regions. The modern genus *Salix* represents one of the most diverse groups of woody plants and includes large trees, shrubs, and trailing or mat-forming plants. Leaf areas vary from a few mm² in arctic or high alpine habitats to more than 100 cm² in humid subtropical zones. *Salix* leaves are represented across the range of possible leaf shapes, from circular, obovate, and ovate, to lanceolate and linear with a length-to-width ratio of up to 30.

Salix is one of the few woody genera with a large number of polyploid species (Wilkinson 1944), and has ploidy levels from diploid ($2n=2x=38$) to dodecaploid ($2n=12x \approx 228$) (Argus 1997). Hybridization, introgression and allopolyploidy are the most important causes for high morphological variability in some groups of extant willow species (Argus 2010). Linnaeus (1753), Endlicher (1841), Schneider (1919), and others attributed problems with identifying certain species and distinguishing them from others to the wide variation in some character states. Classification of modern species is mainly based on floral characteristics and traits of the leaf surface (hairiness, glaucousness, and luster). While identification keys for modern species use up to 176 floral and leaf characters, only 19 can be found in even well-preserved fossil leaves (Argus 2005, Buechler 2008). The use of venation pattern, tooth forms, and cuticular characteristics, traits that are usually neglected in descriptions of modern species, may help to characterize and distinguish fossil *Salix* species.

Leaf venation and marginal tooth characteristics are important traits in botanical and paleobotanical species and specimen descriptions. While early nineteenth century botanists (e.g., De Candolle 1813) only used traits that are easily recognizable in the field (first order vein character states), paleobotanists soon recognized the importance of higher order veins. Ettingshausen (1861) established the first comprehensive classification of venation patterns and illustrated his work with 1267 leaf venation images printed in *Natur Selbstdruck*. His key and catalog were used extensively by paleobotanists into the 1950s when paleobotanists began to move away from simple picture matching to the use of fine venation and cuticular analysis for the description of fossil species (Dilcher 1974). Hickey (1973) published a new classification of dicotyledonous leaf traits. More recently, revised and refined editions have been published by Wing et al. (1999) and Ellis et al. (2009).

Leaf venation variability within individual plants and among members of the same species or genus was first addressed by Ettingshausen (1880). In modern species of *Myrica*, *Castanea*, and *Fagus* he found atypical leaf shapes and venation patterns in both cultivated plants growing in a greenhouse and plants growing naturally but under extreme climatic conditions. He called these forms “regressive” and used their similarity to traits in fossil specimens of the same genera as evidence for a close evolutionary relationship. Ettingshausen and Krašan (1890) found an individual of *Quercus sessiliflora* Smith [Synonym of *Q. petraea* (Mattuschka) Lieblein 1784], a species with normally deeply lobed leaves, produced new unlobed leaves with a weak brochidodromous venation after it had lost all its previous leaves in a severe frost.

Information regarding venation pattern in *Salix* is scarce.

Glatfelter (1894) studied the leaf venation of 24 native or introduced North American *Salix* species. He found considerable variation within a species that is chiefly dependent on the season in which a leaf was initiated. He grouped the species into three categories: (1) regularly veined leaves, e.g., *S. alba* Linnaeus (1753) and *S. fragilis* Linnaeus (1753); (2) leaves with partly irregular veining, e.g., *S. nigra* Marshall (1785) and *S. amygdaloides* Andersson (1858); and (3) species with almost invariably irregular secondary venation, e.g., *S. longifolia* Muehlenberg (1803) (= *S. exigua* Nuttall 1842) and *S. herbacea* L. (1753). This classification system uses character states similar to the leaf ranks (rank numbers 1r, 2r, 3r, etc. corresponding to the highest order of veins that is well organized) in the Manual of Leaf Architecture (Ellis et al. 2009).

Singh et al. (1976) investigated 15 *Salix* species, most of them native to North America, but also *S. acmophylla* Boissier (1846) from the near East and *S. babylonica* Linnaeus (1753) from Asia. They found that the venation is typically pinnate-ecumptodromous in all species investigated, but mentioned that secondary loops are present in some species. In Singh et al. (1976) an image of a *S. humilis* Marshall (1785) leaf shows loops of the weak brochidodromous type.

Weber (1978) created a database with finely graded and illustrated leaf traits of 28 extant *Salix* species growing in Switzerland. Data were then used for the identification of late glacial fossil willow leaves from that country. He found that the classification system proposed by Hickey (1973) was not optimal because Hickey's approach aimed at a much wider range of leaf forms (all angiosperms) and the character definitions were neither fine nor precise enough for separating his fossil species and comparing them to modern ones. Weber was astonished by the great variability in *Salix* leaves. He found that leaves from the same herbarium sheet may show different aspects, especially in their form and size, and that perfectly stable characteristics within a species were rare (Weber 1978).

The purpose of this study is to draw attention to the large inter- and intra-specific variation of venation patterns within the extant genus *Salix*. I provide an overview of venation patterns in modern *Salix*, investigate intra-specific variations of venation patterns as a function of environmental parameters, and discuss possible implications for fossil species definition. These findings may help to better distinguish between fossil *Salix* species and to separate them from fossil remains of other genera.

MATERIALS AND METHODS

Unless otherwise noted, terms describing leaf venation are used in accordance with the Manual of Leaf Architecture (Ellis et al. 2009). The new term *brochoid*, modified from *brochidodromous* (Ellis et al. 2009), is introduced here to describe intermediate forms between *semicraspedodromous* (secondary veins divided into equally gauged parts) and *brochidodromous* (exmedial veins departing from the arch

are of tertiary gauge). The term *brochoid* is used for leaves with semicraspedodromous arches occurring together with intermediate forms on the same specimen but not for brochidodromous patterns if they are consistently present in a species. The term is also used to describe single arches of brochoid character. A secondary vein loop is called a *brochoid arch* if the vein is of a distinctly larger gauge than neighboring (tertiary) veins and its overall course is curved. The term also includes patterns in which the distal vein changes course at the junction of the two veins. Note that this definition is similar to *weak brochidodromous* included in the 1999 version of the Leaf Architecture Manual (Wing et al. 1999).

The term *brochoid chain* is used when marginal sections of secondary arches (exmedial of the junction) form a line parallel to the margin. Note that this situation is not covered by the definition of *simple brochidodromous* in the new version of the Manual, because in this case secondaries change course at junctions. Because a *brochoid chain* is composed of several secondary vein sections, the term *intramarginal vein*, suggesting a single independent vein, is not adequate to describe this morphology. Further justification for the introduction of these new terms is provided in the Discussion.

To quantify the degree to which a leaf is brochoid, I calculated the total length of brochoid arches or intramarginal veins (TLBA) as a percent of whole margin length, with only segments of more than two consecutive arches counted.

Climate data were obtained from the US National Oceanic and Atmospheric Administration (NOAA) website (database US Climate Normals, Monthly Station Climate Summaries) and from the World Meteorological Organization (WMO) website.

Leaves were chemically bleached and stained using modifications of standard sodium hydroxide (NaOH)—sodium hypochlorite (NaHClO₂) methods following Dilcher (1974). Specimens were stained in a safranin O alcohol solution and temporarily or permanently mounted in cedar oil or immersion oil between Teflon or acetate foils. Cleared leaves were then scanned on a flatbed scanner. To soften shadows from leaf venation, a glossy white piece of photo paper was installed 5 mm above the specimen. Venation details and tooth forms were studied with a light microscope. Short descriptions of alternative clearing and mounting procedures used in this study are given in Buechler (2004). A pdf file with more detailed instructions is available from the author or may be downloaded.

Leaves were collected from naturally growing or cultivated plants, or as dried specimens from the author's herbarium (Table 1). Additional herbarium material was used from the National Herbarium of Canada (CAN) and from the Herbarium of the Federal Institute of Technology in Zürich (Z+ZT). Unless otherwise noted, specimens listed in Table 1 or pictured in this publication are the largest mature leaves, situated in the medial part of a shoot. In some cases early (performed) leaves and leaves from second generation (syleptic) shoots were examined in their natural (uncleared) state

Table 1. List of specimens figured or referred to in this study. Abbreviations: **SRP**, Snake River Plain Herbarium, Boise, Idaho; **CAN**, National Herbarium of Canada, Ottawa; **MO**, Missouri Botanical Garden Herbarium, St. Louis, MO.

Specimen no.	Fig. no.	Subgenus/section, species	Leaves taken from plant grown in/Original site of growth (if different) coordinates, elevation	Herbarium specimen no. Other comments
<i>Protitea/Humboldtianae</i>				
1	1a, 1b	<i>S. acmophylla</i> Boiss.	Idaho, Boise, 900 m / Bot. Garden, Kairo, Egypt, ~sea level	SRP 47664
2	15a, 15b	<i>S. humboldtiana</i> Willd.	Argentina, swamp near Buenos Aires, 34° 35' S; 58° 29' W, ~25 m	SRP 47653
3		<i>S. humboldtiana</i> Willd.	Switzerland, Wetzikon, greenhouse, 550 m/grown from seeds of [2]	SRP 47654
4		<i>S. humboldtiana</i> Willd.	Idaho, Boise, greenhouse, 900 m/grown from cuttings of [3]	SRP 47655
5		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Warnes, 17° 32' S, 63° 12' 30" W, 350 m	CAN 541107
6		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Pando, Prov. Madre de Dios, 11° 12' S, 66° 41' W, 125 m	CAN 531047
7		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Pando, Prov. Madre de Dios, 11° 12' S, 66° 41' W, 125 m	CAN 531037
8		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Gutierrez, 17° 23' S, 63° 32' W, 295 m	CAN 541097
9		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Santiesteban, 17° 19' S, 63° 18' W, 300 m	CAN 541865
10		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Santiesteban, 17° 19' S, 63° 18' W, 300 m	CAN 541869
11		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Sara, 17° 13' S, 64° 24' 30" W, 260 m	CAN 586754
12		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Santiesteban, 17° 19' S, 63° 18' W, 300 m	CAN 541867
13		<i>S. humboldtiana</i> Willd.	Bolivia, Depto. Santa Cruz, Prov. Warnes, 17° 32' S, 63° 12' 30" W, 350 m	CAN 541106
14		<i>S. nigra</i> Marsh.	Arkansas, Shirley, Van Buren Co., 35° 39' N, 92° 18' W, 150 m	CAN 496176 (early leaf)
15		<i>S. nigra</i> Marsh.	Minnesota, Kasson, Dodge County, 43° 54' N, 92° 44' W	CAN 580627 (early leaf)
16		<i>S. nigra</i> Marsh.	Illinois, Fort Kaskaskia, Randolph County	CAN 438636 (early leaf)
17		<i>S. nigra</i> Marsh.	Michigan, Union Pier, Berrien County	CAN 464837 (early leaf)
18		<i>S. nigra</i> Marsh.	Canada, Ontario, Carleton Regional Municipality, Ottawa, 45° 21' N 75° 48' W	CAN 589606 (early leaf)
19	18	<i>S. nigra</i> Marsh.	Canada, Ontario, Carleton Regional Municipality, Ottawa, 45° 21' N 75° 48' W	CAN 589608
20	12	<i>S. nigra</i> Marsh.	Oklahoma, Cedar Creek, Heavener, Ouachita National Forest, 34° 58' N, 94° 38' W, 150 m	CAN 498913
21		<i>S. nigra</i> Marsh.	Texas, Lake Creek, Munday, Foard County, 33° 25' N, 99° 34' W, 439 m	CAN 517193
22		<i>S. nigra</i> Marsh.	Florida, Cypress, Jackson County, 30° 42' N, 85° 04' W, 470 m	CAN 48490 (early leaf)
23	2a, 2b	<i>S. nigra</i> Marsh.	Ohio, Oberlin, 41° 17' N 82° 13' W, 248 m	SRP 47656
24	13, 19	<i>S. nigra</i> Marsh.	Mexico, Hacienda La Babia, Municipio de Muzquiz, Estado de Coahuila, 28° 33' N, 102° 03' W, ~490 m	CAN 512292
<i>Protitea/Floridanae</i>				
25		<i>S. floridana</i> Chapman	Ocala National Forest, Florida	SRP 47657

Table 1. (cont.). List of specimens figured or referred to in this study.

Specimen no.	Fig. no.	Subgenus/section, species	Leaves taken from plant grown in/Original site of growth (if different) coordinates, elevation	Herbarium specimen no. Other comments
26		<i>Protitea/Wilsonia</i> <i>S. chaenomeloides</i> Kimura	cultivated in Boise, Idaho; cultivar, orig. from Japan	SRP 47665
27	14	<i>Salix/Subalbac</i> <i>S. babylonica</i> L.	Idaho, Boise, 900 m / Nepal, Langtang village, 3450 m	SRP 47666 (early and late leaves)
28		<i>Salix/Salix</i> <i>S. alba</i> L.	Volketswil, Kt. Zuerich, Switzerland, 460 m	SRP 47658
29		<i>Salix/Magnifacae</i> <i>S. magnifica</i> Hemsl.	Cultivated in Boise, Idaho; cultivar, orig. from China	SRP 47667
30	7a, 7b	<i>S. moupinensis</i> Franch	Cultivated in Boise, Idaho; cultivar, orig. from Szechwan, China	SRP 47668
31		<i>Longifoliae/Longifoliae</i> <i>S. columbiana</i> (Dorn) Argus	Cultivated in Boise, Idaho; cultivar <i>S. fluviatilis</i> "Multnova", orig. from lower Columbia River, Oregon	SRP 47672
32	8a, 8b	<i>S. exigua</i> Nutt.	New Mexico, Bosque del Apache NWR, banks of Rio Grande	SRP 47659
33		<i>Chamaetia/Chamaetia</i> <i>Salix reticulata</i> subsp. <i>reticulata</i> L.	Alaska, Broad Pass, near road from Fairbanks to Anchorage	SRP 47660
34	11	<i>Chamaetia/Myrtilloides</i> <i>S. myrtilloides</i> L.	Cultivated in Arboretum Oberli, Wattwil, Switzerland/orig. from Gamperfin, Toggenburg, Switzerland	SRP 47661
35	5	<i>Chamaetia/Diplodictyae</i> <i>S. cascadenis</i> Cockerell	Cultivated in Boise, Idaho 900 m/Bear Tooth Pass, Wyoming, 3500 m	SRP 47669

Table 1. (cont.). List of specimens figured or referred to in this study.

Specimen no.	Fig. no.	Subgenus/section, species	Leaves taken from plant grown in/Original site of growth (if different) coordinates, elevation	Herbarium specimen no. Other comments
36		<i>Chamaetia</i> /Herbella		
37	10	<i>S. polaris</i> Wahlenb. <i>S. rotundifolia</i> Trautv. subsp. <i>dodgeana</i> (Rydb.) Argus	Kola Peninsula, Russia Sable Pass, McKinley Nat. Park, 1190 m	SRP 47662 MO 103 4967
38		<i>Vetrix</i> /Glabrella <i>S. crataegifolia</i> Bertolini	Cultivated in Wattwil, Switzerland/Italy, Orto di Donna, Apuanian Alps, 1400–1500 m	SRP 47663
39	4	<i>Vetrix</i> /Nigricantes <i>S. myrsinifolia</i> Salisb.	Cultivated in Boise, Idaho/Scheffenu, Wattwil, Switzerland	SRP 47670
40	3	<i>Vetrix</i> /Cinerella <i>S. appendiculata</i> Villars	Cultivated in Boise, Idaho/Wattwil, Switzerland	SRP 47673
41		<i>Vetrix</i> /Subviminalis <i>S. gracilistyla</i> Miq. subsp. <i>melanostachys</i> Makino	cultivated in Boise, Idaho; cultivar, orig. from Japan	SRP 47671
42	9	Fossil specimens <i>S. payettensis</i> Ax. sensu nov. Buechler	Miocene Purple Mountain Flora, Fernley, Nevada	UCMP 166657
43	6	<i>S. molesta</i> MacGinitie	Middle Eocene, Wind River Basin	UCMP 5608
44		<i>S. hausruckensis</i> Kovar-Eder	Late Miocene, Hinterschlagen, Austria	See Kovar-Eder and Wójcicki (2001)

in through-light under a dissecting microscope. Data gained from the examination of cleared leaves were in general verified with untreated herbarium material. In addition to my own material, I studied cleared leaf specimens published on the University of California Museum of Paleontology (UCMP) [photo database](#) and specimens from Jack Wolfe's cleared leaf collection at UCMP. Overall more than 250 cleared leaves from 90 taxa were examined.

I followed the subgeneric classification of [Argus \(2010\)](#) for New World species, with five subgenera: *Protitea* [Kimura \(1928\)](#), *Salix* [Linnaeus \(1753\)](#), *Longifoliae* (Andersson) [Argus \(1997\)](#), *Chamaetia* (Dumortier) [Nazarov \(1936\)](#), and *Vetrix* [Dumortier \(1862\)](#). The small Asian subgenus *Pleurodenia* [Kimura \(1988\)](#) (only two species) is not included in this study because specimens were unavailable. For sectional assignments of European and Asian species, I follow [Skvortsov \(1999\)](#) and [Zhenfu et al. \(1999\)](#).

RESULTS

Specimens studied here are listed in Table 1 with specimen number, taxonomic affiliation, origin of plant material, and herbarium specimen number. The specimen number is used in the text and in figure captions for reference to plant specifications given in Table 1, e.g., [1], [2, 7], or [7–12]. Images of specimens not figured in this study are available on request from the author.

All Groups—The underlying secondary vein framework in most *Salix* species is eucamptodromous with a varying tendency to form brochoid arches. Rare exceptions include some species of subgenus *Chamaetia* that have a brochidodromous venation pattern. Areole development is usually moderate, sometimes good (sensu [Ellis et al. 2009](#), for exceptions see group 3). Because venation patterns may vary greatly within a species, specimens examined in this study have been arranged in five venation-defined morphotype groups, starting with the most common patterns. Note that a species may be a member of more than one group, depending on the geographical or seasonal origin of its leaves.

Group 1—Venation is eucamptodromous with or without occasional weak brochoid arches; major secondary veins turn towards apex for approximately one to two times the half-width of the leaf. This venation type is predominantly found in species of subgenera *Salix* and *Vetrix* and includes different leaf shapes, sizes, and margin types. Examples for this group include *S. acmophylla* [Boissier \(1846\)](#) [1] (Figs. 1a, 1b), *S. alba* [28], *S. myrsinifolia* [Salisbury \(1796\)](#) [39] (Fig. 4), and *S. appendiculata* [Villars \(1789\)](#) [40] (Fig. 3). *Salix myrtilloides* [Linnaeus \(1753\)](#) [34] (Fig. 11) illustrates eucamptodromous, brochoid, and festooned brochoid venation on the same leaf. Some species with brochoid chains or intramarginal veins (characteristics of group 5) may produce leaves with eucamptodromous venation in spring or early summer, and at the climatically cold border of their geographical distribution: e.g., *S. nigra* [15–17] and [23] (Figs. 2a, 2b). An example of a fossil specimen of this morphotype

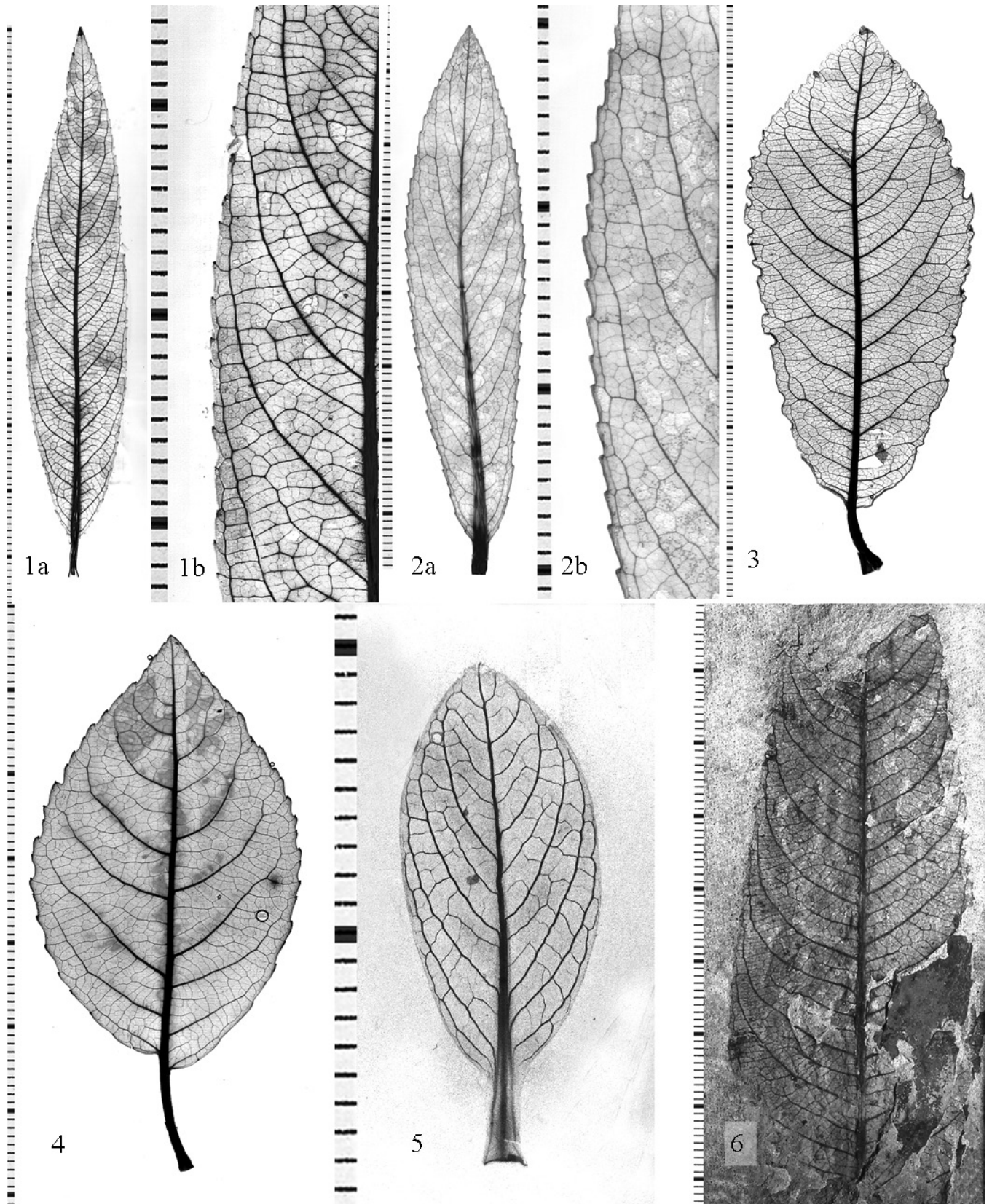
group is *S. molesta* [MacGinitie \(1974\)](#) [43] (Fig. 6) from the early middle Eocene Wind River Basin.

Group 2—Venation is eucamptodromous and usually with some weak brochoid arches near the apex. The spacing of major secondaries is regular. Intersecondaries are rare or absent; tertiary veins are predominantly opposite percurrent; leaf rank ([Hickey 1973](#)) is 3r or 4r. Examples of Group 2 include some Asian species of subgenus *Salix* and subgenus *Vetrix* like *S. moupinensis* [Franchet \(1887\)](#) [30] (Figs. 7a, 7b), *S. chaenomeloides* [Kimura \(1928\)](#) [26], *S. gracilistyla* Miquel subsp. *melanostachys* [Makino \(1914\)](#) [41], *S. magnifica* [Hemsley \(1906\)](#) [29], and to a lesser extent the European *S. crataegifolia* [Bertolini \(1813\)](#) [38]. *Salix floridana* [Chapman \(1860\)](#) [25] of North America and the Late Miocene *S. hausruckensis* [Kovar-Eder \(2001\)](#) [44] from Austria also belong in this group.

Group 3—Venation is eucamptodromous with occasional brochoid arches that do not form brochoid chains or intramarginal veins. The major secondary veins turn towards the apex for a distance of about 3–5.5 times the half-width of the leaf. The leaf rank is 1r–2r per [Hickey \(1973\)](#) and the areole development is poor. The intersecondary frequency is one to several per intercoastal area. This pattern is restricted to *Longifoliae* and occurs in most species of this subgenus. The only exception found is a leaf of *S. columbiana* (Dorn) [Argus \(2010\)](#) (previously *S. fluviatilis* [Nuttall, 1842](#)) from the lower Columbia River that has a eucamptodromous venation pattern [31]. In a specimen of *S. exigua* from New Mexico [32] (Figs. 8a, 8b) the secondaries extend to the margin, turn sharply towards the apex following the margin for a distance up to 5.5 times the half-width of the leaf, thus mimicking a group of short intramarginal veins. A fossil specimen of *S. payettensis* [Axelrod sensu nov. Buechler \(2008\)](#), from the Miocene Purple Mountain flora shows the same venation characteristics as modern leaves of subgenus *Longifoliae* [42] (Fig. 9).

Group 4—Venation is brochidodromous or festooned brochidodromous with secondaries originating near the base or in the proximal half of the blade. The leaves are small and of more or less circular shape. This pattern is restricted to some low growing mat-forming arctic species of subgenus *Chamaetia*: e.g., *S. rotundifolia* [Trautvetter \(1832\)](#) [37] (Fig. 10) and *S. polaris* [Wahlenberg \(1812\)](#) [36]. In *S. reticulata* [Linnaeus \(1753\)](#) [33] and other members of this subgenus, secondaries typically originate in the proximal half of the blade. Secondaries may be eucamptodromous in the proximal part of the blade and turn brochidodromous near the apex or be festooned brochidodromous throughout. Fossil specimens of *S. polaris* and other members of subgenus *Chamaetia* have been found in many Pleistocene and Holocene floras (see e.g., [Heer 1879](#) and [Tralau and Zagwijn 1962](#)).

Group 5—Secondary veins form long brochoid chains or intramarginal veins. This venation is predominant in some narrow leaved species of subgenus *Protitea* such as *S. nigra* [14, 20–22, 24] (Figs. 12, 13) and *S. humboldtiana*



Figures 1-6. Specimens of group 1, scale: mm. 1a, b. *S. acmopylla* [1]. 2a, b. *S. nigra* [23]. 3. *S. appendiculata* [40]. 4. *S. myrsinifolia* [39]. 5. *S. cascadiensis* [35]. 6. *S. molesta* [43].

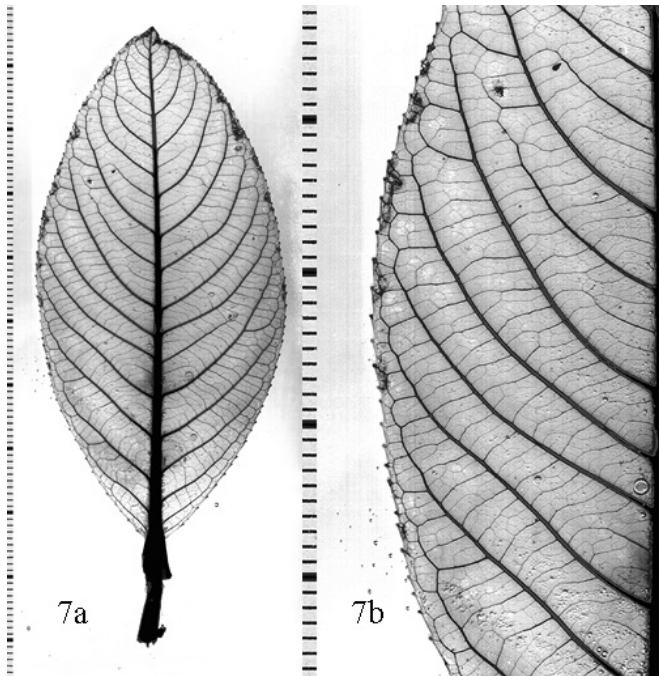
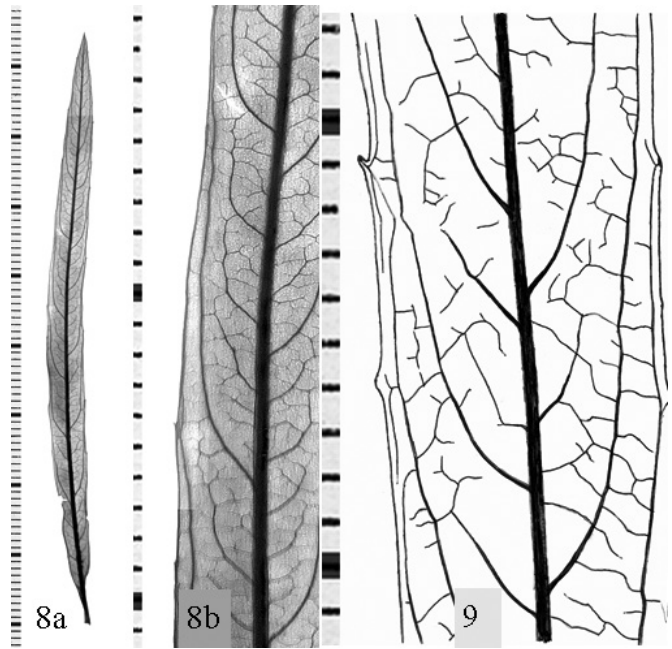


Figure 7. Specimen of group 2, scale: mm. 7a, b. *S. moupinensis* [30].



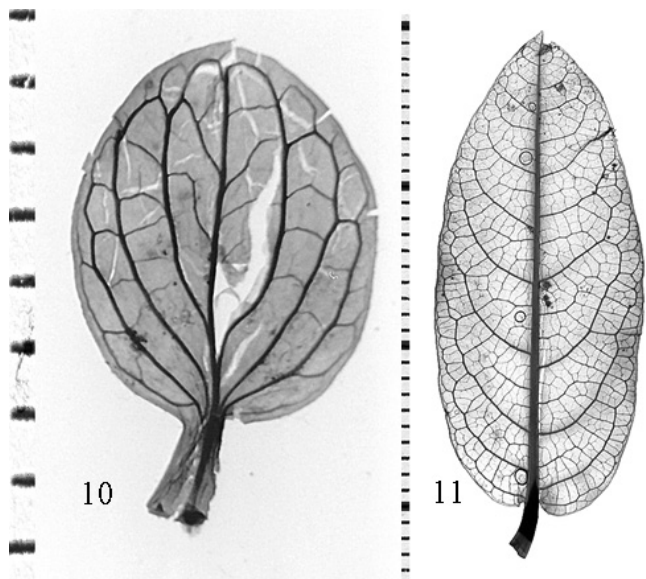
Figures 8, 9. Specimens of group 3, scale: mm. 8a, b. *S. exigua* [32]. 9. Line drawing of specimen UCMP 166657, *S. payettensis* [42].

Willdenow (1806) [2–13] (Figs. 15a, 15b), and in *S. babylonica* [27] (Fig. 14) (subgenus *Salix*). The defining characteristics of this group, brochoid chains and intramarginal veins, are not present in all leaves of a species or a plant. In *S. nigra* venation pattern changes from eucamptodromous with or without occasional brochoid arches (characteristics of group 1) in leaves from high latitudes [15–19] to brochoid chains or intramarginal veins in plants from lower latitudes [20–22, 24] (Figs. 12, 13). No fossil specimens with this venation pattern have been found to date.

Figure 16 shows TLBA or intramarginal veins as a percent of whole margin length of 11 *S. nigra* leaves [14–24] from a north-south transect between Quebec (Canada) and Muzquiz (Mexico) as a function of July mean monthly temperature (MMT). Below 26°C, TLBA is relatively low (8–22%) but increases sharply towards 100% between 26–30°C. The same pattern is seen when comparing TLBA to degree northern latitude. Venation patterns change from eucamptodromous (with or without occasional brochoid arches) at low temperatures to partial or complete intramarginal veins above 26°C July MMT. TLBA correlates best with the combined June, July, August MMT (correlation coefficient = 0.86) and with the lowest mean monthly precipitation (MMP) during the same time period (correlation coefficient = -0.49), suggesting that the formation of long brochoid chains and intramarginal veins may be induced by a hot-arid environment.

In *S. humboldtiana*, a species of subgenus *Protitea* with a natural range from Chile to northern Mexico (Hauenstein et al. 2005, Argus 2010), venation pattern also varies from eucamptodromous with occasional brochoid arches [3] to

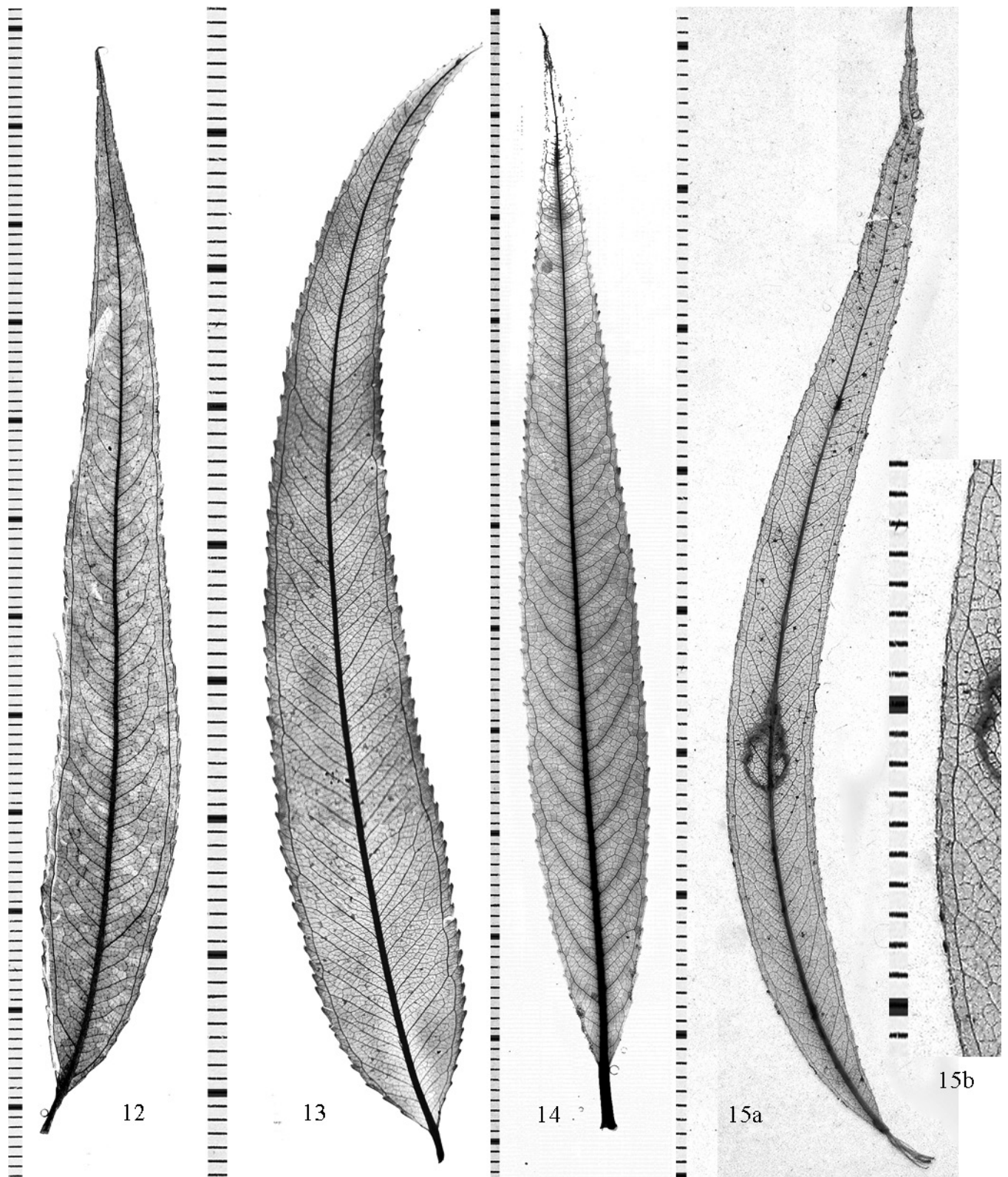
long brochoid chains and intramarginal veins [2] (Figs. 15a, 15b) and [6, 7]. Although leaves from 10 natural habitats in South America (Bolivia and Argentina) and leaves from plants cultivated in greenhouses in Boise, Idaho, and Wetzikon, Switzerland, have been examined during this study, no clear correlation between TLBA and climate parameters could be found. This may be in part due to the fact that no specimens



Figures 10, 11. Specimens of group 4: *S. rotundifolia* subsp. *dodgeana* [37], scale: mm. 11. Specimen of group 1: *S. myrtilloides* [34], scale: mm.

from the southern, and hence, cold border of the range in Chile were available, and in part because no climate data were

available for some collection sites in Bolivia. However, three leaves with 100% TLBA and almost straight intramarginal



Figures 12–15. Specimens of group 5, scale: mm. 12. *S. nigra* [20]. 13. *S. nigra* [24]. 14. *S. babylonica* [27]. 15a, b. *S. humboldtiana* [2].

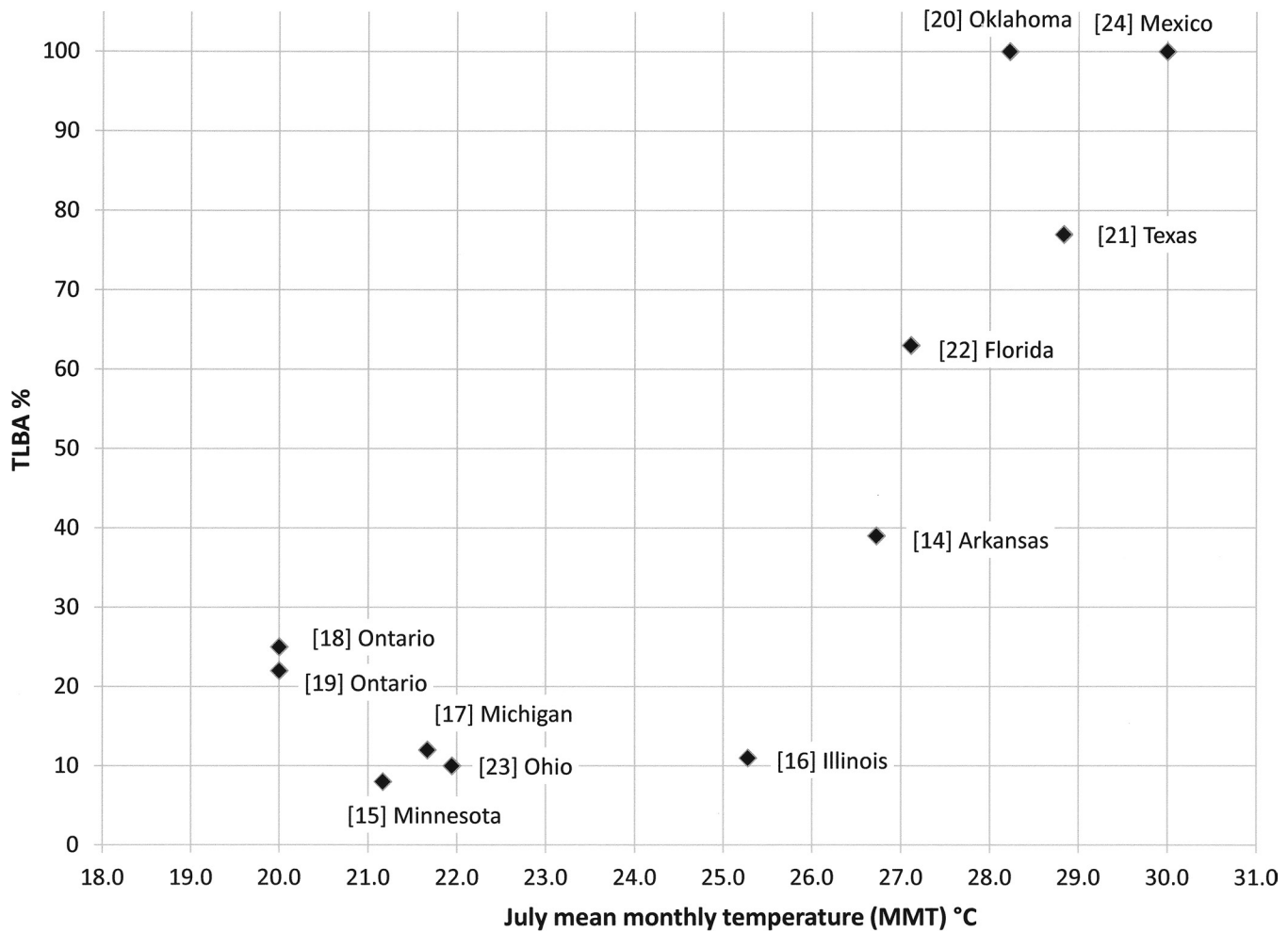


Figure 16. Total Length of Brochoid Arches (TLBA) or intramarginal veins in relation to July mean monthly temperature of 11 *S. nigra* leaves [14–24] from a north-south transect between Quebec (Canada) and Muzquiz (Mexico). Data labels show specimen number [] and the state of origin.

veins came from Buenos Aires [2] (Figs. 15a, 15b) (WMT = 25.4°C, WMP = 121 mm) and from the Province Madre de Dios, Bolivia [6, 7] (WMT = 28.2°C, WMP = 74 mm).

Only one individual of *S. babylonica* (subgenus *Salix*) was available for the study: a plant from Nepal, cultivated in Boise, Idaho [27]. Figure 17 shows TLBA as a function of leaf position on two yearly shoots, one from a low branch (1.5 m above ground) and one from a branch located at approximately 75% of the tree height (5 m above ground). On both shoots early leaves show almost no brochoid arches. TLBA then increases, and reaching more than 90% at leaf no. 13 on the lower branch and leaf no. 30 on the higher branch. The last two leaves on both shoots were immature and probably were initiated in late July or early August. Data suggest that the expression of long brochoid chains and intramarginal veins in *S. babylonica* may also be initiated by climate parameters.

Of the three species found in this study with long brochoid chains or intramarginal veins two belong to subgenus

Protitea (*S. nigra* and *S. humboldtiana*) and one to subgenus *Salix* (*S. babylonica*). While searching for other species with these rare venation patterns, I examined herbarium material of some species in the same taxonomic sections as *S. nigra*, *S. humboldtiana*, and *S. babylonica*. Leaves of the following species showed only eucamptodromous venation with few brochoid arches: *S. acmophylla*, *S. amygdaloides* Andersson (1858), *S. bonplandiana* Kunth (Humboldt et al. 1817), *S. caroliniana* Michaux (1803), *S. goodingii* Ball (1905), *S. laevigata* Bebb (1874), *S. tetrasperma* Roxburgh (1798) (all subgenus *Protitea*, sect. *Humboldtianae*), and *S. matsudana* Koidzumi (1915). (subgenus *Salix*, section *Subalbae*). Long brochoid chains or intramarginal veins were not found in any specimens of this group.

DISCUSSION

The most surprising result is the great variability of some characters within a leaf, plant, species, and the whole genus. The secondary vein framework may change from

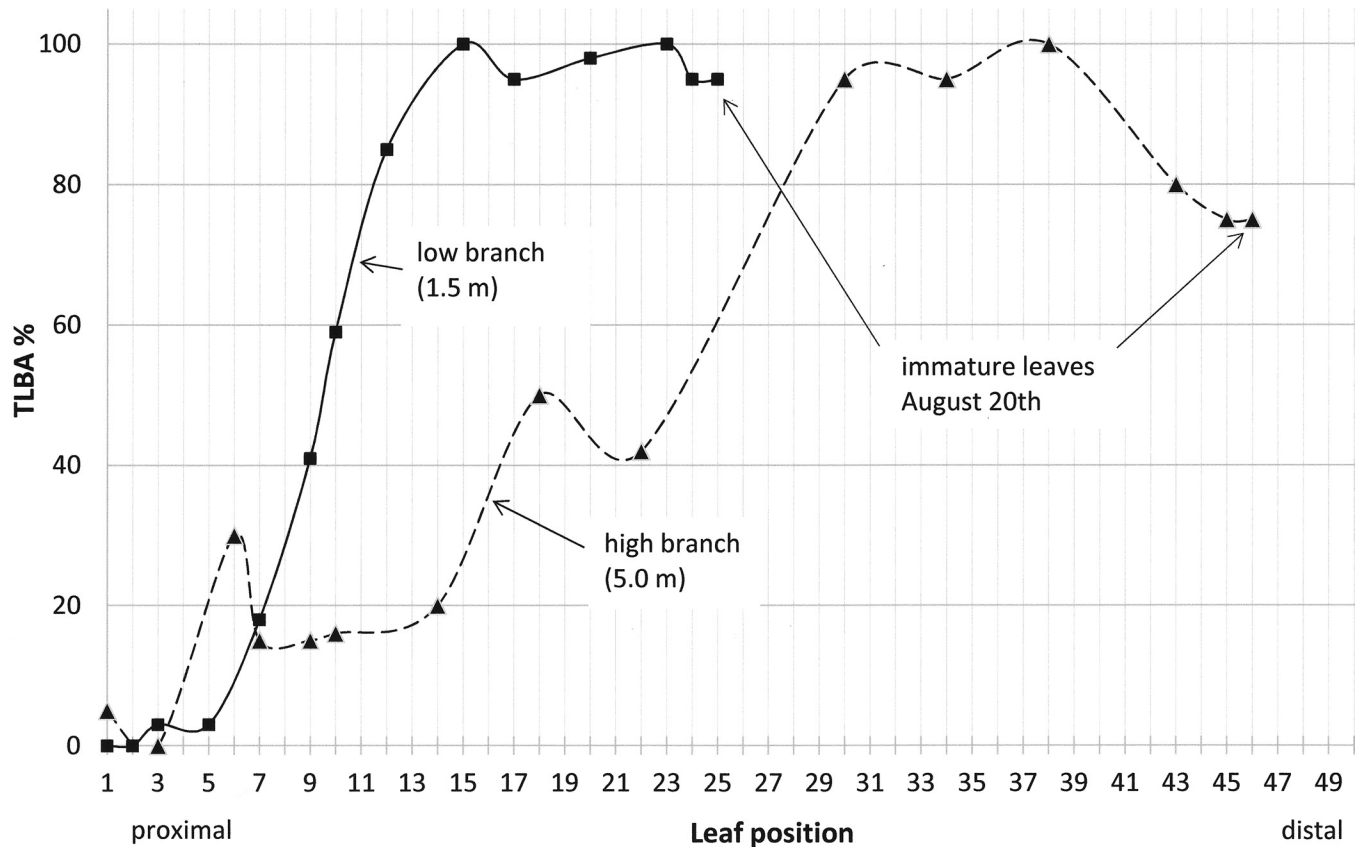


Figure 17. Total Length of Brochoid Arches (TLBA) or intramarginal veins in relation to leaf position on two yearly shoots of *S. babylonica* [27] from a low branch (1.5 m above ground) and from a branch at about 75% of the tree height (5 m above ground).

eucamptodromous to eucamptodromous with interspersed semicraspedodromous (secondary vein divided into equally gauged parts) or brochidodromous (exmedial veins departing from the arch are of tertiary gauge) arches within a plant or between clones of a plant grown under different environmental conditions. Semicraspedodromous arches and brochidodromous forms as well as intermediate forms were sometimes found on the same leaf or plant. An assignment to a predominant secondary venation type was often impossible or seemed at least highly subjective. To avoid establishing non-essential and potentially confusing terminology the more inclusive term *brochoid* was introduced here for the purpose of this study (see definition in Methods).

Differences between open (e.g., eucamptodromous) and closed (brochoid or brochidodromous) secondary venation pattern may have a functional explanation. In a numerical model study comparing closed and open venation patterns, Roth-Nebelsick et al. (2001) found that closed venation patterns guarantee a better water supply to the marginal region which is especially prone to high water stress (Zwieniecki et al. 2002). Therefore, it seems likely that the observed change from an open to a closed secondary venation pattern in *S. nigra* (Fig. 16) and *S. babylonica* (Fig. 17) may be due to water stress.

It is not immediately clear which environmental parameters are responsible for these changes in venation patterns. TLBA data for *S. nigra* and *S. babylonica* (Group 5) suggest that high summer temperatures combined with drought conditions are the driving forces for the formation of long brochoid chains and intramarginal veins. Climate parameters may, however, also be responsible for seemingly random variations in secondary venation pattern in groups 1–4.

For *S. nigra* (Fig. 16), the correlation between TLBA and July temperatures may result from a direct influence of temperature on each leaf, or from a genetically fixed adaptation of local populations to climate parameters. In *S. babylonica* (Fig. 17), however, where all data are drawn from the same plant, this correlation is likely a direct response to high temperatures and the notorious summer drought of southern Idaho. An examination of the buds in *S. babylonica* [27] showed an average of four preformed leaves per bud. In leaves from the low branch (Fig. 17) TLBA begins to increase after the 5th or 6th leaf, i.e., with leaves that have been initiated in spring with rising temperatures.

Only a limited number of early and late leaves have been available for TLBA analysis in *S. nigra* (Table 1). As such, we have no information regarding whether venation patterns in early and late leaves react differently to warm-month mean

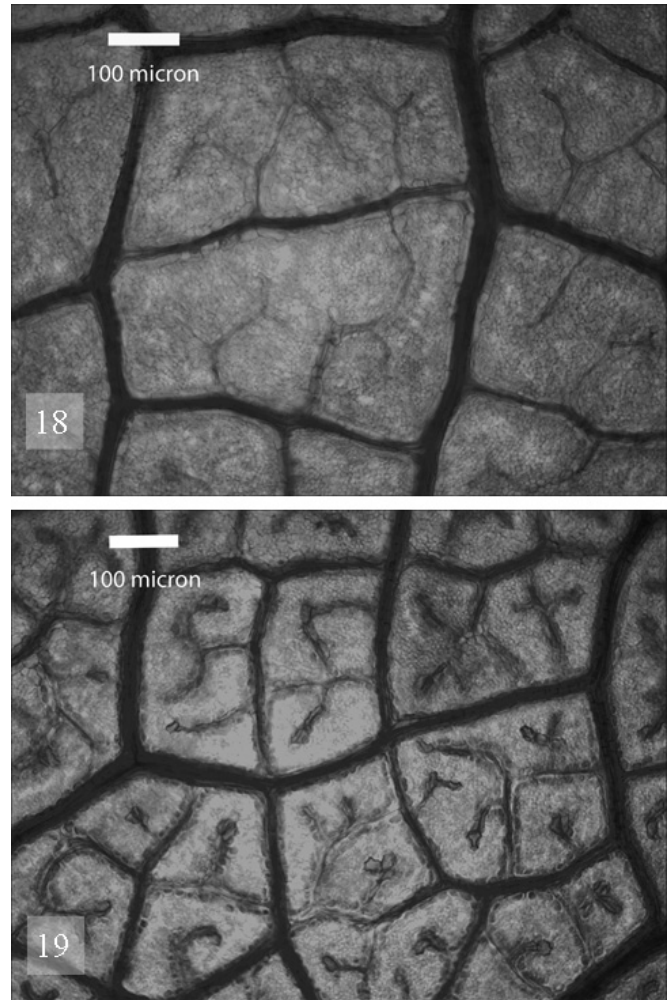
temperature (WMT) and warm-month precipitation (WMP). Early leaves are initiated in fall and expand after bud break in spring. It is, however, unclear at which stage of development the final venation pattern of the leaf is determined. Near the northern border of the *S. nigra* distribution (e.g., Quebec) both early [18] and late leaves [19] exhibit low TLBA venation patterns. Near the southern edge of the distribution near Musquiz, Mexico [24], where the duration of the vegetation period is close to 12 months (average cold-month high temperature = 20.2°C), early leaf TLBA may be elevated if not equal to late leaf TLBA. Between these two extreme locations, late leaf TLBA will be higher than early leaf TLBA.

The WMT and WMP levels beyond which high TLBA values occur vary from species to species. For *S. humboldtiana* a WMT of 25.4°C and a WMP of 121 mm results in a straight intramarginal vein over the whole length of the leaf [2] (Figs. 15a, 15b). In *S. babylonica*, summer leaves show the same pattern at 23.7°C and 10 mm WMP (Boise, Idaho).

Vein density is known to vary in response to several external parameters, e.g., soil water availability, air humidity (review in Roth-Nebelsieck et al. 2001) and to the leaf economics spectrum (Blonder et al. 2011). Leaves of *S. nigra* from their northern and southern range qualitatively confirm this trend, in that secondary vein spacing is distinctly narrower and areolation field size is smaller in the south [24] (Figs. 13, 19) than in northern locations [19, 23] (Figs. 2a, 2b, 18).

Brochoid venation patterns and intramarginal veins in *Salix* predominantly occur in subgenus *Protitea*. Skvortsov (1999) considered members of that subgenus to be evolutionarily delayed by which he meant that they have retained certain characteristics developed under past climate conditions. In some members of subgenus *Protitea* the hypodermis (protective cell layer below the epidermis) is either indistinct or entirely missing, as in *S. nigra* (Skvortsov and Golysheva 1966). A hypodermis would have had a limited function in the early Neogene when the climate was predominately warm and humid. With increasing summer droughts in middle and late Miocene, however, these species may have developed the ability to produce closed venation patterns to minimize water loss and to secure water supply to the marginal zone of the leaf blade. *Longifoliae* have narrow leaves with a bilateral hypodermis (Skvortsov and Golysheva 1966) and secondaries running along the margin for up to 5.5 times the half leaf width, which may have been an adaptation to dry air conditions. Species of subgenus *Vetrix* and *Chamaetia* have thicker leaves and protective features of the leaf surface like wax layers, reset stomata, or a dense indumentum instead, which are also likely a response to aridity.

Venation pattern variability in *Salix* is greater than in many, if not most, other genera. In *Salix*, leaf ontogenesis begins inside the bud during the fall. Leaf development stalls during winter and resumes in early spring. Except for some arctic species with a very short vegetation period, initiation of new leaves begins again in spring and continues throughout the growing season (Moore 1909, Neumann 1981, Skvortsov 1999). Moore (1909) examined size and content of winter



Figures 18, 19. Areolation fields. 18. *S. nigra* from Ontario [19]. 19. *S. nigra* from Mexico [24].

buds in 22 woody plant species of 17 genera. In 19 of these species, the number of initiated leaves in buds on average matched the number of nodes counted on previous years' shoots or showed only slight deviation. For *Betula populifolia*, *S. fragilis*, and *S. alba* Moore found that new additions of leaves take place during the subsequent growing season. For *S. fragilis* and *S. alba*, the nodes on primary shoots varied from 7 to 36, while the number of leaves in any winter bud did not exceed 13 (Moore 1909). These findings are in accordance with results from a *S. babylonica* tree [27] growing in Boise, Idaho, where there were 25 mature leaves and between three and six preformed leaves in winter buds. At the time of initiation *Salix* leaves are therefore exposed to a much wider range of climatic influences than leaves of genera in which most leaves are preformed in fall. The wide geographic range also exposed this genus to greater climatic variation. *Salix* species have been able to adapt to many different climate conditions as a result of their ability to develop different leaf forms and venation patterns.

The regularity of vein systems varies widely in both fos-

sil and extant *Salix* species. A survey of Miocene material from Pickett Creek (Buechler et al. 2007), *Clarkia* (Rember 1991), and the *Salix* modern cleared leaf specimens at UCMF revealed leaves with well-organized and poorly-organized venation patterns (leaf ranks 1r to 3r). As far as preservation of fossil specimens allowed, leaf ranks were consistent with those of extant species with the same overall leaf shape. Four fossil specimens of the *Longifoliae* group (Buechler 2008) with a leaf rank of 1r had the same unorganized appearance as those of modern taxa of that group. In three specimens of *S. wildcatensis* Axelrod (1944) (Late Miocene of Pickett Creek, Buechler et al. 2007), leaf ranks were 1r while ranks of their extant counterpart *S. lasiolepis* Benth (1857) were 1r or 2r. Nine specimens of the *S. hesperia*-*S. inquirenda* complex (Buechler 2008) and leaves of morphologically similar extant *S. floridana* Chapman and *S. laevigata* Bebb had leaf ranks of 2r or 3r. The correspondence between morphologically similar fossil and extant leaves suggests that regularity of vein systems has not significantly changed since Miocene times. Images of some of these specimens not figured in this study are available from the author.

Leaves of *S. magnifica* [29] and *S. moupinensis* [30] (Figs. 7a, 7b) are not typical of *Salix* leaves because of their large size (upper mesophyll range) and high leaf ranks (3r to 4r). Both species have catkins up to 30 cm long and are native to the cloud forests of Sichuan, China, which has a subtropical to temperate climate and an annual mean precipitation of approximately 1000 mm. Leaf areas in *Salix* vary by a ratio of about 500, i.e., far more than the size of areolation fields (the smallest areas of the leaf tissue that are completely surrounded by veins). Therefore, it is obvious that large wide leaves need a more hierarchically structured vein system than small leaves for an equable water supply to all parts of the leaf blade. Because leaf size is strongly correlated with mean annual temperature, length of the growing season, and growing season precipitation (Wolfe 1993), as evident in other tropical-subtropical plant taxa, large *Salix* leaves with leaf ranks of 3r or 4r are indicators for a humid subtropical or warm temperate environment. *S. hausruckensis* Kovar-Eder [44], a Late Miocene species from Hinterschlagen, Austria (Kovar-Eder and Wójcicki 2001) has leaves similar to those of *S. floridana* [25] with leaf ranks of 2r or 3r. Both occur together with *Taxodium* sp. suggesting a swamp and floodplain environment with shallow open waters (Kovar-Eder and Wójcicki 2001).

No fossil *Salix* leaves with intramarginal veins have been found to date. Findings of this study suggest that the development of long brochoid chains and intramarginal veins may be an adaptation to high temperatures and drought stress. Due to overall climate cooling since Eocene times, one would expect to find more brochoid *Salix* leaves in the fossil record than in modern *Salix* from similar latitudes. However, this does not appear to be the case. A thorough search of European and American paleobotanical literature found no undoubted fossil *Salix* specimens with this venation type.

Possible explanations are: (1) *Salix* leaves decay faster than leaves of most other woody genera (personal observation) and secondary veins may rarely be sufficiently preserved to permit distinction between eucamptodromous and brochoid patterns; (2) willow-shaped leaves with brochoid venation pattern or intramarginal veins may have been wrongly assigned to species or genera which are known for such traits, e.g., *Eucalyptus*, *Eugenia*, *Nerium*, *Sapindus*, and *Decodon* (Lythraceae); (3) species of subgenus *Protitea*, section Humboldtianae are xeromorphic and prefer arid climates (Skvortsov 1999); and (4) the adaptation of some members of subgenera *Protitea* and *Salix* to a dry climate may be a relatively recent phenomenon. A search of the NECLIME (Neogene Climate Evolution in Eurasia) database revealed that hot-arid conditions like those in locations where TLBA values $\geq 63\%$ were found for *S. nigra* have not been reported for the Neogene of Eurasia. The closest match (WMT = 28.2°C and WMP = 124 mm) was found for the early Miocene fossil site Fushan I in Southern China (Yao et al. 2011). These data were established using the Coexistence Approach (CA) (Mosbrugger and Utescher 1997) using pollen. Similar data for the New World are not available because the Climate Leaf Analysis Multivariate Program (CLAMP) method (Wolfe 1993), which is predominantly used in North America, does not provide WMT and WMP data. The apparent absence of hot-arid climate fossil localities does not necessarily mean that *Salix* habitats of that climate did not exist. Like the sites in which modern *S. nigra* with high TLBA values grows, hot-arid adapted species may not have been near a lake or other suitable site of deposition. Because the fossil record is strongly biased towards aquatic environments, chances for drought-adapted *Salix* species to be deposited in a lacustrine environment may be smaller than for other willows.

Changes of secondary venation pattern as a response to water stress are also seen in other genera. Denk (1999) found that venation in *Fagus sylvatica* L. (1753) changes from craspedodromous to brochidodromous with entire margins in dry stands of western Asia. Typical examples for variable venation pattern are extant *Quercus chrysolepis* Liebm (1854), an evergreen oak from California and southern Oregon, and its Miocene counterpart *Q. pollardiana* Axelrod (1940). *Quercus chrysolepis* shows craspedodromous, semi-craspedodromous, eucamptodromous, and brochidodromous venation patterns, and often up to three different patterns on leaves of the same plant (see cleared leaves on UCMF webpage). However, the cause for the variations in leaves of these oaks has not been explored.

The species that exhibited high TLBA values, *S. nigra* and *S. babylonica*, have leaves with a toothed margin. The combination of a brochidodromous secondary venation and a toothed margin is rare in woody plants and seemingly contradictory. While brochidodromous patterns prevail in tropical floras (Bailey and Sinnott 1915), toothed margins are more common in cooler and humid climates (Wilf 1997). Toothed margins have been associated with water loss (Wolfe

1993), which would clearly be disadvantageous in a hot-arid environment. Most toothed *Salix* species secrete a clear liquid from tooth glands during a few days or weeks in early spring (Curtis and Lersten 1980, Buechler, *personal observation*). Because closed secondary venation improves the water supply to the margin (Roth-Nebelsick et al. 2001), water loss through margin glands is potentially higher in these leaves with an open secondary venation. To determine whether water loss occurs later during the growing season, I cut off a 1.0 m long twig of *S. babylonica* in late summer and attached it pressure-tight to a plastic tube. The tube was supplied with water from a reservoir 7.20 cm higher than the twig, producing a calculated pressure of 71 kPa higher than the pressure of the surrounding air. Due to transpirational pull, the xylem water pressure close to the leaves is usually negative under high evaporation conditions. The experimental setup represents therefore a worst case situation concerning water loss through gutation. While leaves on the twig did not wilt during the week long experiment, no water loss was observed from marginal teeth. Remnants of the previously secreted and dried-out solution may have clogged the tooth glands. The result of the experiment suggests that during the hottest time of the year, a high TBLA venation does not lead to increased water loss through marginal glands.

CONCLUSIONS

This study reveals high variability of venation pattern in the extant genus *Salix*. Patterns may vary within species, individual plants, and even across a single leaf. The underlying secondary vein framework in most species is eucamptodromous with a varying tendency to form brochoid arches. Under hot-arid environmental conditions, this tendency extends into the formation of long brochoid chains or intramarginal veins in some species of subgenera *Protitea* and *Salix*. The examination of Miocene fossil specimens reveals no systematic differences in venation pattern between modern and fossil *Salix* leaves of the same general morphotype, but did not find examples of long brochoid chains or intramarginal veins in the fossil record. These venation patterns are likely rare or absent in the fossil record because: (1) leaf deposition is strongly biased towards aquatic environments and chances for fossilization of drought-adapted *Salix* species to be deposited in a lacustrine environment are smaller than for other willows; or (2) because Miocene climates were on average more humid than modern climates and hot-dry habitats of the kind that produces long brochoid chains or intramarginal veins may have been even more uncommon than today. However, one should not a priori assume that fossil leaves with long brochoid chains or intramarginal veins belong to a genus other than *Salix*. Species descriptions based on a single *Salix* specimen are unlikely to include all possible variants. Conversely, because proximal and the largest mature leaves sometimes have different venation patterns, they may be interpreted as separate species. As such, descriptions of fossil *Salix* species must account for the large variability in venation patterns discussed here.

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