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## Molecular phylogeny of *Populus* (Salicaceae, Salicales) with focus on inter- and intrasectional relationships

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**Abstract:** The relationships among sections and species within each section in the genus *Populus* have attracted much attention from taxonomists and biologists. In this paper, we provide an overview of the current state of *Populus* phylogenetics and discuss the origin of some hybrid species. There has been some controversy surrounding the origin of the genus *Populus* in North America and subsequent dispersal and differentiation in other parts of the world. We present the most plausible scenario based on phylogenetic analysis and fossil records. Sections Abaso, Turanga and *Populus* are all monophyletic, whereas sections *Aigeiros*, *Tacamahaca* and *Leucoides* show some degree of polyphyly. Sections Abaso and Turanga are relatively primitive lineages in *Populus*. With the accumulation of sequencing data and new analytical tools, it will be possible to investigate more complicated issues concerning genus *Populus* within a phylogenetic framework.

**Keywords:** molecular phylogeny, section *Populus*, section *Tacamahaca*, section *Aigeiros*

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## Introduction

Exploring the phylogenetic relationships among different organisms is a prerequisite for many aspects of evolutionary biology (Delsuc et al., 2005). Phylogenetic reconstruction is a key step in revealing relationships among species and genes and accurate phylogenies are vital for assessing population demographic changes and migration patterns. With the rapid development of sequencing technologies, analytical tools for phylogenetic analyses (e.g. some comprehensive software and models) have also progressed rapidly (Yang & Rannala, 2012). Phylogenetic analyses are widely used in many fields

of evolutionary biology, for instance, inferring the relationship within gene families (Barakat et al., 2009; Jiang et al., 2013; Sui et al., 2017), revealing population histories, and in comparative genomics (Edwards, 2009; Ma, 2011; Paten et al., 2008). Over the past decades, phylogenetic analyses have been applied in many organisms, and substantial achievements have been gained (Chaw et al., 2000; Drew et al., 2014; Mao et al., 2012; Wang et al., 2022; Wang & Ran, 2014). Currently, for plant species phylogeny reconstruction, transcriptome and genome data have been widely utilized and some enlightening conclusions have been revealed. For instance, Ding et al. (2022) found bottlenecks and asymmetric

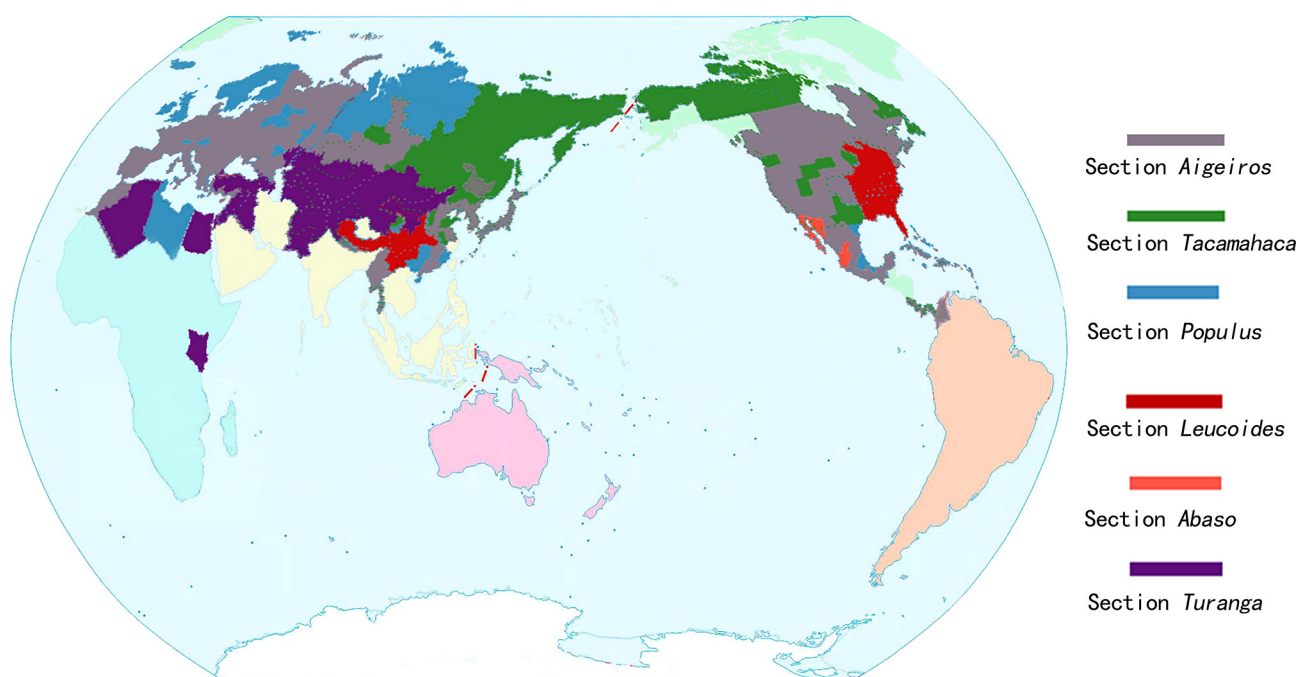


Fig. 1. Distribution pattern of each section in *Populus*

introgression from Persian into iron walnut during domestication with whole-genome resequencing data. Zhang et al. (2021) investigated the species diversity, polyploidy, and morphological innovation in Asteraceae with phylotranscriptome method.

The genus *Populus* is distributed throughout the Northern Hemisphere, from subtropical to boreal forests (Fig. 1). Many species in the genus are ecologically and economically important forest tree species (DiFazio et al., 2011; Stettler et al., 1996). Members of the genus *Populus* are also well known for their rapid growth, stress tolerance, profuse vegetative propagation and multiple usages of wood (Eckenwalder, 1996). Since the release of a complete genomic sequence for *P. trichocarpa* (Tuskan et al., 2006), *Populus* has become an excellent research model in many fields of plant biology (DiFazio et al., 2011; Dickmann & Kuzovkina, 2014).

Eckenwalder (Eckenwalder, 1996) used 76 morphological characters to classify 29 *Populus* species into six sections: *Abaso*, *Populus*, *Aigeiros*, *Tacamahaca*, *Turanga*, and *Leucoides*. Eckenwalder's (1996) result has generally been accepted by many poplar taxonomists (Cervera et al., 2005; Hamzeh & Dayanandan, 2004). However, Chinese taxonomists have identified as many as 62 species, including six hybrid species and a number of varieties and forms (<http://www.iplant.cn/frps/>) (Wu, 1999) (Supporting information Table 1). The differences in the number of species recognised in the genus *Populus* can be attributed to two main reasons. First, hybrid species have occasionally

been recognized as valid species, such as *P. tomentosa* and *P. hopeiensis* (discussed below), which results in tremendous difficulties for phylogenetic reconstruction in *Populus*. Second, different studies have used varying species concepts. Taxonomists that adhere to a broad and general species concept usually accept a broader range of phenotypic variation within species, whereas taxonomists opting for a more narrow species concept tend to emphasize such variation and use this to name different variants as new species (Eckenwalder, 1996). From a phylogenetic perspective, a broad species concept makes it easier to address interspecific or even intersectional relationships, especially in genus *Populus*, where both intra- and intersectional hybridisation is common. Furthermore, to make sense of adaptive evolution within an ecological context in *Populus*, a broad species concept is more applicable since it explicitly acknowledges geographical variation within a species. To conclusively determine the number of species in the genus *Populus*, further analysis that combines broad sampling across geographic distributions with abundant molecular data is needed. While some progress has been made, relationships among sections and within different sections remain controversial and poorly resolved in genus *Populus* due to large species number, high morphological variation within species and substantial interspecific and intersectional hybridization (Cronk, 2005; Eckenwalder, 1996). In this paper, we provide an overview of the current state of *Populus* phylogenetics and discuss the origin of some hybrid species.

Table 1A. The species in genus *Populus* recognized by Eckenwalder (1996)

Section	Species	Distribution area
<i>Abaso</i>	<i>P. mexicana</i> Wesmael	Mexico
<i>Turanga</i>	<i>P. euphratica</i> Olivier	Africa, Asia
	<i>P. ilicifolia</i> (Engler) Rouleau	Africa
	<i>P. pruinosa</i> Schrenk	Asia
<i>Leucoides</i>	<i>P. glauca</i> Haines, s.l.	China
	<i>P. heterophylla</i> L.	North America
	<i>P. lasiocarpa</i> Olivier	China
<i>Aigeiros</i>	<i>P. deltoides</i> Marshall	North America
	<i>P. fremontii</i> S. Watson	North America
	<i>P. nigra</i> L.	Eurasia, North Africa
<i>Tacamahaca</i>	<i>P. angustifolia</i> James	North America
	<i>P. balsamifera</i> L.	North America
	<i>P. ciliata</i> Royle	Himalayas
	<i>P. laurifolia</i> Ledebour	Eurasia
	<i>P. simonii</i> Carr.	East Asia
	<i>P. suaveolens</i> Fischer	China, Japan
	<i>P. szechuanica</i> Schneider	Eurasia
	<i>P. trichocarpa</i> T. & G.	North America
	<i>P. yunnanensis</i> Dode	Eurasia
<i>Populus</i>	<i>P. adenopoda</i> Maximowicz	China
	<i>P. alba</i> L.	Eurasia, Africa
	<i>P. gamblei</i> Haines	Eurasia
	<i>P. grandidentata</i> Michaux	North America
	<i>P. guzmanantlensis</i> Vazquez & Cuevas	Mexico
	<i>P. monticola</i> Branddgee	Mexico
	<i>P. sieboldii</i> Miquel	Japan
	<i>P. simaroa</i> Rzedowski	Mexico
	<i>P. tremula</i> L.	Eurasia, Africa
	<i>P. tremuloides</i> Michaux	North America

Table 1B. The species in genus *Populus* documented in Flora of China (Wu, 1999)

Section	Species	Varieties and forms	Distribution area in China
<i>Turanga</i>	<i>P. euphratica</i> Oliv.		Xinjiang, Gansu, Qinghai, Inner Mongolia
	<i>P. pruinosa</i> Schrenk		Xinjiang
<i>Leucoides</i>	<i>P. glauca</i> Haines s.l.		Tibet, Sichuan, Yunnan
	<i>P. lasiocarpa</i> Oliv.		Hubei, Sichuan, Shaanxi, Guizhou, Yunnan
	<i>P. pseudoglauca</i> C. Wang et P. Y. Fu *		Tibet
	<i>P. violascens</i> Dode #		
	<i>P. wilsonii</i>	f. <i>wilsonii</i> Schneid. f. <i>brevipetiolata</i> C. Wang et Tung. f. <i>pedicellata</i> C. Wang et Tung.*	Shaanxi, Gansu, Hubei, Sichuan, Yunnan, Tibet Shaanxi
<i>Aigeiros</i>	<i>P. × beijingensis</i> W. Y. Hsu		
	<i>P. × berolinensis</i> Dipp.		
	<i>P. × canadensis</i> Moench.		
	<i>P. × jrtyschensis</i> Ch. Y. Yang		
	<i>P. × xiaohei</i> T. S. Hwang et Liang		
	<i>P. afghanica</i>	var. <i>afghanica</i> Schneid. * var. <i>tadishistanica</i> (Kom.) C. Wang et Ch. Y. Yang *	South of Xinjiang Xinjiang
	<i>P. manshurica</i> Nakai #*		Areas between Inner Mongolia and Liaoning
<i>P. nigra</i>		var. <i>nigra</i> L. var. <i>italica</i> (Moench.) Koehne.	Xinjiang Areas along the Yangtze River and the Yellow River
		var. <i>thevestina</i> (Dode) Bean	Northwest and Northern China

<i>Tacamahaca</i>	<i>P. × xiaozhuanica</i> W. Y. Hsu et Liang	
	<i>P. alachanica</i> Kom. *	Inner Mongolia
	<i>P. amurensis</i> Kom. *	North of Heilongjiang
	<i>P. candicans</i> Ait.	Xinjiang
	<i>P. cathayana</i>	Liaoning, Sichuan, Northwest and Northern China
	var. <i>cathayana</i> Rehd.	South Gansu, East Qinghai
	var. <i>latifolia</i> (C. Wang et C. Y. Yu) C. Wang et Tung *	
	var. <i>pedicellata</i> C. Wang et Tung *	Hebei
	var. <i>schneideri</i> Rehd. *	Yunnan
<i>P. charbinensis</i>	var. <i>charbinensis</i> C. Wang et Skv. *	Heilongjiang
	var. <i>pachydermis</i> C. Wang et Tung *	Heilongjiang and North Liaoning
<i>P. ciliata</i>	var. <i>ciliata</i> Wall.	Tibet, Sichuan
	var. <i>aurea</i> Marq. et Shaw #	Tibet
	var. <i>gyirongensis</i> C. Wang et Tung *	Tibet
	var. <i>weixi</i> C. Wang et Tung *	Northwest Yunnan
<i>P. gansuensis</i> C. Wang et H. L. Yang *		Gansu
<i>P. girinensis</i>	var. <i>girinensis</i> Skv. *	Heilongjiang, Jilin
	var. <i>ivaschkevitchii</i> Skv. *	Heilongjiang, Jilin
<i>P. haoana</i>	var. <i>haoana</i> Cheng et C. Wang *	Northwest Yunnan
	var. <i>macrocarpa</i> C. Wang et Tung *	Northwest Yunnan and Southeast Sichuan
	var. <i>megaphylla</i> C. Wang et Tung *	West Yunnan
	var. <i>microcarpa</i> C. Wang et Tung *	Northwest Yunnan
<i>P. hinganica</i> C. Wang et Skv. *		Inner Mongolia, Liaoning
<i>P. iliensis</i> Drob. *		Xinjiang
<i>P. kangdingensis</i> C. Wang et Tung *		South Sichuan
<i>P. koreana</i> Rehd.		Northeast China
<i>P. laurifolia</i> Ledeb.		Xinjiang
<i>P. mainlingensis</i> C. Wang et Tung *		Tibet
<i>P. maximowiczii</i> Henry		Northeast China, Hebei, Shaanxi, Inner Mongolia
<i>P. nakaii</i> Skv. *		Areas between Heilongjiang and Hebei
<i>P. pamirica</i> Kom. *		Xinjiang
<i>P. pilosa</i>	var. <i>pilosa</i> Rehd. *	Xinjiang
	var. <i>leiocarpa</i> C. Wang et Tung *	Xinjiang
<i>P. przewalskii</i> Maxim.		Qinghai, Gansu, Inner Mongolia
<i>P. pseudomaximowiczii</i>	f. <i>pseudomaximowiczii</i> C. Wang et Tung *	Hebei, Shaanxi
	f. <i>glabrata</i> C. Wang et Tung *	
<i>P. pseudo-simonii</i> Kitag.		North Hebei
<i>P. purdomii</i>	var. <i>purdomii</i> Rehd.	Northeast and North China
	var. <i>rockii</i> (Rehd.) C. F. Fang et H. L. Yang *	Hebei, Henan, Shaanxi, Gansu, Hubei, Sichuan
		Southeast Gansu
<i>P. qamdoensis</i> C. Wang et Tung *		Tibet
<i>P. shanxiensis</i> C. Wang et Tung *		West Shanxi
<i>P. simonii</i>	var. <i>simonii</i>	
	f. <i>simonii</i> Carr.	Northeast, North, Central, Northwest and Southwest China
	f. <i>fastigiata</i> Schneid.	Liaoning, Hebei, Shandong, Beijing
	f. <i>robusta</i> C. Wang et Tung *	Inner Mongolia
	f. <i>pendula</i> Schneid.	Hubei, Gansu
	f. <i>rhombifolia</i> (Kitag.) C. Wang et Tung	Liaoning, Gansu, Shaanxi
	var. <i>latifolia</i> C. Wang et Tung *	
	var. <i>liaotungensis</i> (C. Wang et Skv.) C. Wang et Tung *	Liaoning
	var. <i>rotundifolia</i> S. C. Lu ex C. Wang et Tung *	Liaoning, Hebei, Inner Mongolia
	var. <i>tsinlingensis</i> C. Wang et C. Y. Yu *	Inner Mongolia
<i>P. suaveolens</i> Fisch. *		Shaanxi
		Inner Mongolia

<i>Tacamahaca</i>	<i>P. szechuanica</i>	var. <i>szechuanica</i> Schneid. var. <i>tibetica</i> Schneid. *	Sichuan, Yunnan, Gansu, Shaanxi Sichuan, Tibet
	<i>P. talassica</i> Kom. *		Xinjiang
	<i>P. trinervis</i> C. Wang et Tung *		Sichuan
	<i>P. ussuriensis</i> Kom.		Northeast China
	<i>P. wuana</i> C. Wang et Tung *		East Tibet
	<i>P. xiangchengensis</i> C. Wang et Tung *		Sichuan
	<i>P. yatungensis</i>	var. <i>yatungensis</i> (C. Wang et P. Y. Fu) C. Wang et Tung *	Tibet
		var. <i>crenata</i> C. Wang et Tung *	Tibet
		var. <i>trichorachis</i> C. Wang et Tung *	Tibet, Southwest Sichuan, Northwest Yunnan
	<i>P. yuana</i> C. Wang et Tung *		Northwest Yunnan
	<i>P. yunnanensis</i>	var. <i>yunnanensis</i> Dode * var. <i>pedicellata</i> C. Wang et Tung * var. <i>microphylla</i> C. Wang et Tung *	Yunnan, Guizhou, Sichuan Yunnan, Sichuan Yunnan
<i>Populus</i>	<i>P. × pseudo-tomentosa</i> C. Wang et Tung		
	<i>P. adenopoda</i>	var. <i>adenopoda</i> f. <i>adenopoda</i> Maxim.	Shaanxi, Henan, Anhui, Jiangsu, Zhe- jiang, Fujian, Jiangxi, Hubei, Hunan, Guangxi, Sichuan, Guizhou, Yunnan Sichuan, Guizhou
		f. <i>microcarpa</i> C. Wang et Tung *	Yunnan
		f. <i>cuneata</i> C. Wang et Tung *	Yunnan
		var. <i>platyphylla</i> C. Wang et Tung *	Yunnan
	<i>P. alba</i>	var. <i>alba</i> L.  var. <i>bachofenii</i> (Wierzb.) Wesm. var. <i>pyramidalis</i> Bge.	Liaoning, Shandong, Henan, Hebei, Shanxi, Shaanxi, Ningxia, Gansu, Qinghai Xinjiang North China
	<i>P. canescens</i> (Ait.) Smith.		Xinjiang
	<i>P. davidiana</i>	var. <i>davidiana</i> f. <i>davidiana</i> Dode f. <i>laticuneata</i> Nakai f. <i>ovata</i> C. Wang et Tung f. <i>pendula</i> (Skv.) C. Wang et Tung * var. <i>tomentella</i> (Schneid.) Nakai	North to Southwest China Liaoning, Hebei, Shaanxi, Gansu, Qinghai Gansu, Shanxi, Shaanxi, Hebei Heilongjiang Gansu, Sichuan, Yunnan
	<i>P. hopeiensis</i> Hu et Chow		Northern and Northwest China
	<i>P. ningshanica</i> C. Wang et Tung *		Shaanxi, Hubei
	<i>P. rotundifolia</i>	var. <i>rotundifolia</i> Griff. # var. <i>duclouxiana</i> (Dode) Gomb. var. <i>bonati</i> (Levl.) C. Wang et Tung *	Shaanxi, Gansu, Sichuan, Yunnan, Guizhou, Tibet Yunnan
	<i>P. tomentosa</i>	var. <i>tomentosa</i> Carr. var. <i>truncata</i> Y. C. Fu et C. H. Wang * var. <i>fastigiata</i> Y. H. Wang *	North China, Henan, Anhui, Jiangsu Shaanxi Hebei, Henan, Shandong
	<i>P. tremula</i> L.		Xinjiang

Note: \* indicates 'narrow species' described in the manuscript, # indicates species documented in Flora of China but can not find in field investigation.

## Origin of the genus *Populus*

The most ancient *Populus-Salix*-like species currently recognized is *Pseudosalix handleyi* Boucher, Manchester & Judd. Its fossil twig with attached flowers, fruits and foliage was found in North America, and dated back to the Mid-Eocene, 48 million years ago (Boucher et al., 2003). The dating of this fossil corresponds well with the widely accepted divergence time between *Populus* and *Salix*. It has been used as an important calibration point for dating in subsequent phylogenetic analyses (Davis et al., 2005; Du

et al., 2015; Huang et al., 2014). Phylogenetic analyses of *Populus*, based on morphological traits and sequencing data, support the monophyly of the genus (Eckenwalder, 1996; Hamzeh & Dayanandan, 2004; Hamzeh et al., 2006; Huang et al., 2017; Liu et al., 2017; Wang et al., 2014; Wang et al., 2000; Wang et al., 2022). A reconstructed phylogeny of *Populus* based on 151 AFLP markers from 25 natural species and three hybrid species showed that *P. mexicana*, which is distributed exclusively in southern North America and the only extant species of section *Abaso*, was the most different from the other species of *Populus* and



*Salix* and clustered as a single clade (Cervera et al., 2005). Cervera et al. (2005) suggested that *P. mexicana* should be classified in a separate monotypic genus. However, *P. mexicana* is similar in some morphological traits to the fossil species, *P. tidwellii* and *P. cinnamomoides* that have been found in the Green River Formation of North America (Manchester et al., 2006), and also to extant species of the section *Aigeiros* (Eckenwalder, 1996). The conclusion of Cervera et al. (2005) was based mainly on the presence of unique AFLP fragments in *P. mexicana*. Liu et al. (2017) reconstructed the phylogeny of 31 species, representing all six recognised sections, with 23 single-copy nuclear markers and reported that *P. mexicana* occupied a basal position in the phylogenetic tree. However, a chloroplast phylogeny reconstructed using 34 chloroplast DNA (cpDNA) fragments, placed *P. mexicana* in a clade with *P. balsamifera*, *P. trichocarpa* and *P. deltoides*. These four species are all naturally distributed exclusively in North America. Recently, phylogenomics of *Populus* was constructed using re-sequencing genomic data of various species representing all the extant sections; meanwhile, plastome phylogeny of this genus was simultaneously reconstructed (Wang et al., 2020; Wang et al., 2022; Zhang et al., 2018). Inconsistent placement of *P. mexicana* in nuclear and plastid phylogeny was also revealed. Since chloroplast capture is common in many poplar species (see below), the discordance in the placement of *P. mexicana* between nuclear and chloroplast phylogenies could consequently be explained by chloroplast capture in *P. mexicana* from the common ancestor with other related species (Huang et al., 2014; Liu et al., 2017; Wang et al., 2020; Wang et al., 2022).

According to the phylogenetic analyses, combined with the fossil records of *Populus* found in North America, Liu et al. (2017) inferred that *Populus* originated in North America and has subsequently radiated into other continents in Northern Hemisphere through the North Atlantic Land Bridge (NALB) and the Bering Land Bridge (BLB). This scenario is further supported by the phylogeny of *Populus* reconstructed using morphological traits (Eckenwalder, 1996) and genomic data (Wang et al., 2020; Wang et al., 2022). However, others have suggested that Salicaceae originated in northeast East Asia, based on fossil records and modern distribution patterns (Ding, 1995; Gong, 2004). Nevertheless, the centre of modern distribution and diversity does not necessarily equate to the location of origin of a genus, as has been established in many molecular phylogenetic analyses (Liu et al., 2017). Consequently, based on the integration of molecular phylogenetics and fossil evidence, a North American origin of genus *Populus* is most likely.

To further analyse the biogeography of *Populus*, it would be valuable to collect genetic and ecological

data from an area encompassing the current distribution of all species. For example, Du et al. (2015) investigating the biogeographic history of *P. tremuloides*, *P. tremula* and *P. davidiana* (section *Populus*) showed that the North American species *P. tremuloides* was the most basal. In contrast, *P. davidiana* and *P. tremula* dispersed and differentiated further in Eurasia. However, to what extent these results can be generalized to other sections in the genus *Populus* is unknown. There is currently little data available on the biogeography and phylogeography of other sections. This highlights the need for further investigations into the biogeography of *Populus* and the need to combine data from the fossil record with morphological, geographic and genetic information.

## The intersectional phylogeny of *Populus*

Evolutionary relationships among different sections in *Populus* are still controversial due to intersectional hybridization and introgression, and the varying utility of molecular markers across different studies. For example, it was found that section Turanga clustered as a single clade separated from all other sections in a phylogenetic analysis based on AFLP data (Li et al., 2007). However, in another AFLP data set (Cervera et al., 2005), section Turanga (represented by one species, *P. euphratica*) showed a close relationship with *P. wilsonii*, a species from section Leucoides, creating a sister clade to all other species from sections *Tacamahaca*, Leucoides and *Aigeiros*. Similarly, section Turanga was closely associated with section *Aigeiros* in a phylogenetic analysis based on nuclear ITS data (Shi, 2001). However, it showed a closer affinity to section *Tacamahaca* based on cpDNA phylogeny (Wei et al., 2010). The relationship between sections *Tacamahaca* and *Aigeiros* is also complex. It has still yet to be clarified, even though phylogenetic analyses have been conducted using morphological traits and molecular markers. Likewise, some species of section Leucoides have shown a closer affinity to section *Aigeiros* and *Tacamahaca*, and it has thus been suggested that these species should be separated from section Leucoides (Cervera et al., 2005).

All of these above results suggest that there are substantial incongruences in the intersectional relationships in *Populus* in different phylogenetic studies that are based on varying methods and/or data sets. However, phylogenetic resolution at the intersectional level has been greatly improved with the introduction of single-copy nuclear markers and genomic data into phylogenetic studies of *Populus*. Using multiple single-copy nuclear markers, it has been established that sections *Populus*, Turanga and Abaso are

all monophyletic, and their evolutionary positions in genus *Populus* are clear (Liu et al., 2017; Wang et al., 2014). These results also show that sections Abaso and Turanga are basal clades and relatively primitive lineages within the genus. It is further supported by heteroblasty (willow-like juvenile leaves), which is a trait that is thought to have developed early in the evolutionary history of *Populus* (Eckenwalder, 1996). The section Leucoides appears to have diverged later than section Turanga and Abaso (Eckenwalder, 1996; Wang et al., 2014; Liu et al., 2017). Section *Populus* is a monophyletic lineage in the phylogenetic tree of multiple single-copy nuclear markers (Liu et al., 2017; Wang et al., 2014); however, it showed sister relationship to the clade comprising sections Leucoides, *Tacamahaca* and *Aigeiros* in a nuclear genomic tree (Wang et al., 2020). Also, Wang et al. (2022) provided evidence that section *Populus* showed sister relationship to section Turanga in another genomic tree. This discrepancy resulting from the analysis of different regions of the nuclear genome is mainly related to relationships among different sections (Wang et al., 2020). Results show that sections *Aigeiros*, *Tacamahaca* and Leucoides are polyphyletic and have a close affinity with each other. The origin and differentiation of sections *Aigeiros* and *Tacamahaca* involve species from other sections, and this will be further addressed below. The evolutionary relationships of different sections in *Populus* inferred from the molecular phylogenetic analysis is largely consistent with results based on morphological data and with data from the fossil records. The divergence of the sections in the genus is thought to involve phases of ecological radiation and geographical vicariance (Eckenwalder, 1996). However, the key factors that have promoted intersectional differentiation, either intrinsic events such as adaptive radiation or extrinsic factors such as climatic and geological events, are still unclear.

## Intrasectional phylogenies of *Populus*

Intrasectional relationships in *Populus* have thus far been addressed for sections *Populus*, *Aigeiros* and *Tacamahaca* as the number of species in sections Abaso, Turanga, and Leucoides are relatively small (i.e. only one species in Abaso and two species in Turanga). The number of species and the intrasectional phylogenetic position of some species in different studies vary substantially, so only some general conclusions are drawn here.

### Section *Populus*

Compared to other sections, such as *Aigeiros* and *Tacamahaca*, section *Populus* is a monophyletic lineage

in morphological and molecular phylogenetic trees (Eckenwalder, 1996; Hamzeh & Dayanandan, 2004; Leskinen & Alström-Rapaport, 1999; Liu et al., 2016; Liu et al., 2017; Wang et al., 2014; Wang et al., 2020; Wang et al., 2022; Zong et al., 2019). Furthermore, complete natural reproductive isolation exists between section *Populus* and other sections in the genus (Zsuffa, 1975). However, some ancient hybridization between ancestors of section *Populus* and other sections can not be excluded entirely when reproductive isolation was not yet complete. In phylogenetic analyses based on single-gene sequencing data, species' relationships within section *Populus* are mostly poorly resolved (Hamzeh & Dayanandan, 2004; Shi, 2001; Wei et al., 2010) and data from multiple single-copy nuclear sequences and cpDNA fragments as well as genomic data are used to resolve interspecific relationships within this section (Liu et al., 2017; Wang et al., 2014; Wang et al., 2015; Wang et al., 2020; Wang et al., 2022). Although it has allowed for increased resolution, inconsistent results are still observed among different studies. For example, *P. tremuloides* was placed as basal species of section *Populus* in a phylogenetic analysis based on 12 cpDNA fragments (Wang et al., 2014). In contrast, this species was clustered with *P. grandidentata* in other studies based on 34 cpDNA fragments and plastome data (Liu et al., 2017; Wang et al., 2015; Wang et al., 2022). The unusual placement of *P. tremuloides* in Wang et al. (2014) could be the result of long-branch attraction (Li et al., 2007). The sister relationship between *P. tremuloides*, and *P. grandidentata* is more convincing considering the sympatric distribution of these two species in North America and possible chloroplast capture occurring between both species. Support values for terminal nodes have been relatively low compared with internal nodes within the section, highlighting the general low levels of differentiation among species and hence low resolution offered by sequencing data. Interspecific relationships within this section should be further evaluated using more extensive morphological and/or molecular data from relatively "pure species" and ignoring putative hybrid species.

To address various factors driving species differentiation in section *Populus*, Du et al. (2015) investigated interspecific relationships among *P. tremuloides*, *P. tremula* and *P. davidiana* using multilocus sequencing data. The results show that historical tectonism, such as the sundering of BLB and the rapid uplift of the Qinghai-Tibetan Plateau (QTP) and climate oscillations have driven allopatric speciation among these species. Vicariance has thus played an important role in the divergence of these species in the section *Populus* (Du et al., 2015; Wang, 2016). Furthermore, genome re-sequencing data has been applied in the evolution and speciation study of these three closely

related aspen species, and how the genomic landscape was built up during speciation was clearly deciphered (Wang et al., 2016a; Wang et al., 2016b; Hou & Li, 2020; Wang et al., 2020). Divergence of some parapatric or sympatric distributed species of section *Populus* or species within genus *Populus* may be even more complicated, and the second contact can not be entirely excluded.

The origin of hybrid species in section *Populus* has also attracted substantial attention from taxonomists and biologists. It has been confirmed that *P. × canescens* is a natural hybrid species between *P. alba* and *P. tremula*, which provides a bridge for gene flow between the two species (Lexer et al., 2005; Yin et al., 2016). The origin of *P. tomentosa* has also generated some controversy. Based on analyses of RAPD and AFLP data, *P. tomentosa* was hypothesised to be closely related to *P. adenopoda* and possibly arose from a hybridization event between *P. alba* and *P. adenopoda* (Li et al., 1997). It was in line with the results of phylogenies derived from nuclear sequence data. In a maternally inherited plastid phylogeny, *P. tomentosa* clustered with *P. davidiana* and *P. hopeiensis* (Wang et al., 2014). According to these results, it is inferred that the hybridization event giving rise to *P. tomentosa* may involve the ancestor of *P. davidiana* or *P. hopeiensis* in a maternal role and *P. adenopoda* as a paternal role. As further sequencing data have accumulated, the complicated origin of *P. tomentosa* has been studied in greater detail (Wang et al., 2019). It suggests that *P. tomentosa* consists of two genetic lineages with different maternal parents. Initially, *P. adenopoda* hybridized with *P. alba* forming the first hybrid lineage. Then, the first hybrid lineage hybridized with *P. davidiana* to generate the second one (Wang et al., 2019). However, the evolutionary pattern of these two genetic lineages needs further evaluation to explain, for instance, the formation of morphological consistency despite repeated hybridisation among some species. Recently, high quality haplotype-resolved genome assemblies of *P. tomentosa* is generated and phylogenomic analysis revealed that *P. tomentosa* is comprised of two distinct subgenomes, which is likely to have resulted from hybridization between *P. adenopoda* as the female parent and *P. alba* var. *pyramidalis* as the male parent (An et al., 2021). This genomic analysis definitely clarifies the hybrid origin of *P. tomentosa*. *P. hopeiensis* is thought to be a domesticated form of *P. tomentosa*, based on their sympatric distributions. However, molecular phylogenetics and population genetic studies suggest that *P. hopeiensis* originated by unidirectional pollination from *P. alba* to *P. davidiana* (Sun et al., 2020; Wang et al., 2014; Wang, 2016). The lack of evidence for recent hybridisation with other sections and the relatively small number of extant species in section *Populus* makes it challenging to investigate the origin of some hybrid

species. Now large-scale sequencing data has made it possible to establish the origin of some hybrid species in this section and the parental contributions to these hybrids. These results illustrate that intrasectional hybridization has played a significant role in the origin of these hybrid species and has also excluded contributions from species in other sections in these hybridization events. Due to the evolutionary independence of section *Populus* within the genus *Populus*, species in this section are sound model systems for addressing factors driving evolutionary divergence within the genus.

## Section *Tacamahaca*

Section *Tacamahaca* was shown to be polyphyletic based on morphological characters (Eckenwalder, 1996). This hypothesis has received further support in subsequent molecular phylogenetic analyses (Hamzeh & Dayanandan, 2004; Liu et al., 2017; Wang et al., 2014; Wang et al., 2020; Wang et al., 2022). However, the origin of this polyphyletic lineage has rarely been addressed. Molecular phylogenetics based on biparental and maternal inherited datasets all show that species from sections *Aigeiros* and *Leucoides* play vital roles in the origin of section *Tacamahaca* (Liu et al., 2017; Wang et al., 2014; Wang et al., 2020; Wang et al., 2022; Zhang et al., 2018). This complicated origin may directly contribute to the polyphyly of section *Tacamahaca*.

Section *Tacamahaca* is rich in species, and includes many microspecies or ecotypes, such as *P. haoana*, *P. kangdingensis* and *P. gonggaensis*, which all have limited distributions in southwestern China. These microspecies are all likely the result of a “narrow” species concept. They would be subsumed under other more established species in the section. The possible origin and phylogenetic relationships among a number of these microspecies, such as *P. yunnanensis*, have been explored using data from multiple molecular markers (Chen et al., 2007), while the origin of other microspecies or ecotypes has still to be evaluated with high-resolution genetic markers. The similarities in morphological traits between microspecies and well-established species have resulted in further difficulties in the delimitation and phylogenetic establishment of these microspecies. On the other hand, the relatively narrow and concentrated distributions of these microspecies have led to applying population genetics and phylogeographic methods to investigate their origin and migration history. For example, Jiang et al. (2016) showed that *P. fjrtyshensis*, which is mainly distributed in Xinjiang, China, arose through the hybridization between two distantly related species, *P. nigra* and *P. luarifolia*, and that further hybridization has contributed to its expansion. Besides difficulties in the delimitation of microspecies



based on phylogenetic analyses in section *Tacamahaca*, the rationale for the delimitation may be dubious. For example, *P. schneideri* can be considered as a variety of *P. kangdingensis* based on morphological characteristics, rather than as a natural hybrid between *P. cathayana* and *P. kangdingensis* (Wan et al., 2013; Zhao & Liu 1994). Furthermore, the morphological characteristics used to distinguish these microspecies from traditional well-established species often emphasize differences in morphology of leaf and crown, which are known to display high levels of morphological variation. The recognition of these microspecies results in difficulties for molecular phylogenetic studies within section *Tacamahaca*. It raises further questions concerning their origin and evolutionary history, which require extensive field investigation combined with molecular data to resolve. As mentioned above, ‘pure’ species or well-established species should be considered when reconstructing the phylogenetic relationship within this section.

Although there are many species with broad distributions in section *Tacamahaca*, research on the geographic and climatic factors driving population differentiation and speciation has been relatively rare (Wang & Ran, 2014). Using coalescent-based approach of data from 32 nuclear loci, Levsen et al. (2012) showed that the divergence between the two closely related North American species, *P. balsamifera* and *P. trichocarpa*, occurred approximately 75 Ka, corresponding to the late Pleistocene glaciation. However, investigation using whole-plastome sequencing of these two species placed the divergence to at least the Pliocene, 6–7 Ma (Huang et al., 2014). The discordance between these estimates can be attributed to an ancient partial plastome capture event from a possibly extinct taxon (ghost lineage) and subsequent lineage sorting, recombination and backcrossing of the two species. Whole-genome sequencing from multiple individuals from both species is needed to identify the putative ‘ghost’ lineage and to ascertain further evolutionary factors driving the differentiation between these two species, and even other species in *Tacamahaca*.

## Section *Aigeiros*

Species from section *Aigeiros* clustered into a single clade based on nDNA data but were scattered into several separate clades in the cpDNA phylogeny (Wang et al., 2014), indicating that origin of section *Aigeiros* may be complicated and involve species from other sections. This phylogenetic pattern has received further support in subsequent phylogenetic analyses (Liu et al., 2017; Wang et al., 2022; Zhang et al., 2018; Zong et al., 2019). For instance, *P. nigra* clustered with *P. alba*, while *P. deltooides* clustered with *P. balsamifera* and *P. trichocarpa*, and *P. afghanica*

clustered with *P. lasiocarpa*, suggesting substantial polyphyly of this section. Although chloroplast capture can not be completely ruled out as an explanation for these phylogenetic patterns, hybridization events between species in section *Aigeiros* and other sections are common in nature (Chhatre et al., 2018; Meirmans et al., 2010).

Initial studies on the hybrid origin of *P. nigra* using RFLP markers suggested that *P. alba* possibly contributed maternally to *P. nigra* (Smith & Sytsma, 1990). This observation was not confirmed by a subsequent phylogenetic analysis using sequencing data from a single nuclear locus (Hamzeh & Dayanandan, 2004). Further analyses of multiple nDNA and cpDNA markers established that *P. alba* was the maternal parent of *P. nigra* (Liu et al., 2017; Wang et al., 2014), which was later supported by phylogenetic analysis based on poplar plastome data (Wang et al., 2020; Wang et al., 2022; Zhang et al., 2018; Zhou et al., 2021; Zong et al., 2019). This ancient hybridization event between the ancestors of *P. alba* and section *Aigeiros* giving rise to *P. nigra* may have occurred before establishing reproductive isolation between section *Populus* and other sections as mentioned above. *Populus nigra* was subsequently implicated in the origin of some microspecies in other sections, which contributed to difficulties in elucidating the evolutionary history of these microspecies. *Populus deltooides* showed close affinity to species of section *Tacamahaca* in cpDNA phylogenetic analyses, such as *P. balsamifera* and *P. trichocarpa* (Cervera et al., 2005; Hamzeh et al., 2006; Rajora & Dancik, 1995; Wang et al., 2014; Wang et al., 2022). These species have partly sympatric distributions across North America, where they are known to hybrid freely (Chhatre et al., 2018; Hamzeh et al., 2007; Meirmans et al., 2010; Thompson et al., 2010), so chloroplast capture among these species may occur. Two representative species in section *Aigeiros*, *P. nigra* and *P. deltooides*, have allopatric distributions in Eurasia and North America, respectively. The distribution of *P. nigra* in China is restricted to Xinjiang (Wu, 1999). The factors driving the formation of this allopatric distribution, such as the break of the land bridges and the reasons for the limited expansion of *P. nigra* in China deserves further investigation.

The phylogenetic position of section *Aigeiros* has resulted in much confusion regarding the taxonomy. For instance, Rajora and Dancik (1995) have argued, based on the peculiar phylogenetic position of *P. nigra* and results from an RFLP analysis, that *P. nigra* should be removed from section *Aigeiros* to a separate section *Nigrae*. Furthermore, there have also been suggestions to move *P. deltooides* to a new separate section (Cervera et al., 2005). Also, due to the relatively close affinity between section *Tacamahaca* and *Aigeiros* based on morphology (Eckenwalder,

1996), some researchers proposed that the two sections should be merged. This suggestion also received support from molecular phylogenetic analysis (Hamzeh & Dayanandan, 2004). However, these hypotheses have all been invalidated based on phylogenetic analyses that provide higher resolution to these questions. Species in section *Aigeiros* cluster within a single clade in the nDNA phylogeny, and their morphology differs from species from other sections (Morin, 2000). Consequently, maintaining the taxonomic position of section *Aigeiros* is currently warranted and species in this section would likely serve as excellent model systems for further research into the hybrid origin of plant species.

## Conclusion

*Populus* is one of the most significant genera of forest trees with broad economic use and high ecological value. Therefore, it is paramount to understand the phylogenetic relationships among species in the genus to facilitate future breeding and genetic improvement of *Populus*. However, the large-scale patterns of introgression among species and the hitherto lack of nuclear markers with sufficiently high resolution has hindered detailed phylogenetic studies of the genus, which confounds the reconstruction of the reticulate relationships among species and results in much uncertainty at both inter- and intrasectional levels. Although the hybrid origin of some species, such as *P. tomentosa*, *P. jrtyschensis* and *P. nigra*, have been investigated in detail, the roles that these hybrid species are playing in the evolutionary history of *Populus* have still to be clarified using a combination of ecology, biology and genomic data.

Recently, a set of single-copy nuclear markers for Salicaceae have been developed (Du et al., 2014), and more than 10 million nuclear single nucleotide polymorphisms (SNPs) among different *Populus* species have also been mined (Wang et al., 2022). These data have been successfully applied to phylogenetic reconstruction and speciation research in *Populus* (Du et al., 2015; Liu et al., 2017; Wang et al., 2014; Wang et al., 2022). The results show that *Populus* as a whole is monophyletic and the genus arose in North America. It has subsequently dispersed and differentiated into other parts of the world. Sections Abaso and Turanga represent relatively primitive sections in the genus, whereas sections *Aigeiros*, *Tacamahaca* and *Leucoides* are polyphyletic, and several other sections are implicated in their origin. Hybridization, introgression and chloroplast capture are common in *Populus*, especially some of these occurred during the early evolutionary history of this genus. Incomplete lineage sorting is likely to have persisted widely in the long-time generation tree species (Zhang et al., 2018). A

relatively long time (about 9–12 generations) is necessary to sort two descendant species into reciprocally monophyletic clades at most genomic loci, even in a simple and ideal allopatric speciation scenario according to the pure drift hypothesis (Hudson & Coyne, 2002). Therefore, genetic diversity is shared between species that recently diverged, which conflicts with phylogenetic analysis in most circumstances when different loci or individuals are used. However, it is often difficult to distinguish whether incomplete lineage sorting or gene flow caused by hybridization results in conflicting phylogenies (Zhang et al., 2018). It highlights the utility of megadata and ‘pure’ species for phylogenetic reconstruction in lineages with complicated evolutionary history. Furthermore, geographical and climate factors have played significant roles in the adaptive evolution of *Populus*. Thus far, few studies have utilized transcriptomic or genomic data to reconstruct the phylogenetics of *Populus* or to investigate the origin of putative hybrids (Duvall et al., 2016; Huang et al., 2017; Pease et al., 2016; Wang et al., 2020; Wang et al., 2022). With decreasing costs of DNA and RNA sequencing and rapid development of bioinformatic analyses, available genomic data in *Populus* should grow rapidly and allow for phylotranscriptomic and phylogenomic approaches to address phylogenetic relationships among species and sections in the genus. Questions, such as the origin of *Populus*, the adaptive evolution history of species, including genes playing key roles, as well as the hybrid origin of some species, should therefore be possible to be addressed in the future within a phylogenetic framework.

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