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Guard cell sizes and ploidy levels in Polylepis (Rosaceae)

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Abstract: The Andean tree genus Polylepis (Rosaceae) has recently been recognized to include polyploid species, but their occurrence within the genus is still incompletely known, especially in light of a forthcoming taxonomic treatment based on a narrow species concept including morphological, climatic and biogeographic distinctness that recognizes 45 species. We obtained guard cell measurements as proxies of ploidy level from 114 individuals of 33 species of Polylepis, including all species for which no previous measurements were available. In combination with previously published data, also on nucleus mass and chromosome counts, we infer that on current knowledge 19 (42%) species are probably purely diploid, 15 (33%) purely tetraploid, and one (2%) purely octoploid. The remaining eight (18%) species have mixed ploidy levels, with three (7%) being di- and tetraploid, two (4%) di- and hexaploid, and one each tetra- and hexaploid, tetra- and octoploid, and di-, tri-, tetra- and hexaploid. Based on our understanding of the evolutionary relationships in Polylepis, it would appear that polyploidy has originated at least about eight times independently in the genus, sometimes as autopolyploidy, sometimes as a result of interspecific hybridization, and sometimes in relation to cultivation. The taxonomic implications of the ploidy levels are complex, in some cases supporting species-level distinction and in others posing the question whether different ploidy levels within a species should better be treated as distinct species. Ploidy level needs to be taken into account for the conservation of the genus, as for example if different populations of a species have different ploidy levels, mixing these origins in reforestation schemes may lead to the formation of sterile hybrids. Guard cell measurement is a low cost and simple technique that can be readily used on both live and dried plant material for such applications, but it has limitations and further data on chromosome counts and nucleus mass are also needed to fully understand the evolution of ploidy levels in Polylepis and its implications.

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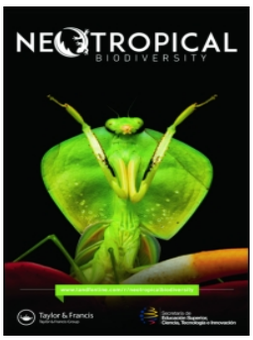

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ABSTRACT

The Andean tree genus *Polylepis* (Rosaceae) has recently been recognized to include polyploid species, but their occurrence within the genus is still incompletely known, especially in light of a forthcoming taxonomic treatment based on a narrow species concept including morphological, climatic and biogeographic distinctness that recognizes 45 species. We obtained guard cell measurements as proxies of ploidy level from 114 individuals of 33 species of *Polylepis*, including all species for which no previous measurements were available. In combination with previously published data, also on nucleus mass and chromosome counts, we infer that on current knowledge 19 (42%) species are probably purely diploid, 15 (33%) purely tetraploid, and one (2%) purely octoploid. The remaining eight (18%) species have mixed ploidy levels, with three (7%) being di- and tetraploid, two (4%) di- and hexaploid, and one each tetra- and hexaploid, tetra- and octoploid, and di-, tri-, tetra- and hexaploid. Based on our understanding of the evolutionary relationships in *Polylepis*, it would appear that polyploidy has originated at least about eight times independently in the genus, sometimes as autopolyploidy, sometimes as a result of interspecific hybridization, and sometimes in relation to cultivation. The taxonomic implications of the ploidy levels are complex, in some cases supporting species-level distinction and in others posing the question whether different ploidy levels within a species should better be treated as distinct species. Ploidy level needs to be taken into account for the conservation of the genus, as for example if different populations of a species have different ploidy levels, mixing these origins in reforestation schemes may lead to the formation of sterile hybrids. Guard cell measurement is a low cost and simple technique that can be readily used on both live and dried plant material for such applications, but it has limitations and further data on chromosome counts and nucleus mass are also needed to fully understand the evolution of ploidy levels in Polylepis and its implications.

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Introduction

Changes in ploidy level are an important process in plant evolution, impacting both the diversification of major clades as well as microevolutionary processes at the species level [1,2]. In many plant groups, one can find different ploidy levels, resulting from either auto- or allopolyploidization, the latter linked to hybridization, where it allows for the stabilization of genomes of mixed origin [3–5]. Besides its evolutionary implications [6], polyploidy is also of taxonomic relevance, since populations of different ploidy levels are at least partly reproductively isolated, allowing for divergent evolutionary trajectories that can be treated at species level [7].

The genus *Polylepis* (Rosaceae) is a taxonomically complex genus that has variously been treated as comprising 33 species [8], 15 species [9], 26 species [10], or 45 species [11]. This variability already hints at the difficulty of delimiting species in this genus, which is due to overall morphological similarity between species coupled with high intraspecific variability and plasticity, which themselves are linked to hybridization and polyploidization [12]. The family Rosaceae, to which *Polylepis* belongs, is renowned for the occurrence of polyploid complexes, e.g., in *Crataegus* [13] and *Sorbus* [14,15].

In *Polylepis*, polyploidization has long been hypothesized to occur [9,12,16], but direct chromosome counts in *Polylepis* have been hampered by their small size and the difficulty of preparation [9,17–19]. Different ploidy level in *Polylepis* were first documented by Schmidt-Lebuhn et al. [19] who made flow cytometry measurements of 11 species, finding that the majority of species have nucleus masses of around 1.5–1.65 pg, which they interpreted as diploids, whereas some species had values around 2.9–3.1 (tetraploids), with a single species having a mean value of 5.7 (octoploid). Because flow cytometry in *Polylepis* requires live material (all efforts to obtain measures from dried material have failed so far, even when working in collaboration with experts such as J. Suda) and not all species were available in cultivation, they additionally used guard cell measurements of herbarium specimens to estimate ploidy levels of additional species. Guard cell size is well known to be correlated to ploidy level in angiosperms in general [5,20,21] and

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also Rosaceae in [22], although in some genera such as *Crataegus* guard cell sizes overlap between ploidy levels, so that ploidy inference is challenging [23]. In *Polylepis*, by sampling the same individuals as used for flow cytometry, Schmidt-Lebuhn et al. [19] confirmed the applicability of guard cell length for separating at diploid and polyploid individuals, even though the differentiation between tetra- and octoploids was not possible with their limited sample size. Based on their combined flow cytometry and guard cell size data, they arrived at a phylogenetic hypothesis for the development of ploidy level in *Polylepis*, stating that the ancestral diploid condition is found in the phylogenetically older sections *Sericea* and *Reticulata* (as defined by [11]), whereas the more derived section *Incana* is largely polyploid. The latter section on average occurs at higher elevations and in more arid environments, which corresponds well with the polyploid condition since polyploids are well known to be overrepresented at high latitudes and elevations [5,24], possibly because the different paralogs offer higher adaptive potential [25]. A major limitation of this study was that each species was only sampled with 2–4 individuals, so that intraspecific variability may have gone undetected. The only species for which several ploidy levels were detected were *P. australis* and *P. pauta*.

In a later study, Kessler et al. [26] conducted more detailed flow cytometry measurements of three Argentinian species of *Polylepis*, finding constant ploidy levels in *P. hieronymi* (diploid, N = 52 individuals from three sites) and *P. tomentella* (tetraploid, N = 43, three sites) but marked variation in *P. australis*. In this species, among 361 individuals from 27 populations, 75 (21%) were interpreted as diploids, 24 (7%) as triploids, 261 (72%) as tetraploids, and one (0.003%) as a hexaploid. These ploidy levels showed a clear geographical pattern, with populations from the northern Argentinean Andes being purely diploid and those from the central Andes tetraploid, whereas in the isolated Sierra de Córdoba, all four ploidy levels co-occurred in mixed populations. This suggests that the triploid plants may be hybrids between the di- and tetraploid ones, but whether they are sterile primary hybrids or can reproduce by themselves is unresolved. Also, the degree of reproductive isolation and hence evolutionary independence between the diploid and tetraploid populations remains unknown.

The next studies examining ploidy levels in *Polylepis* were conducted by Segovia-Salgado and colleagues on Ecuadorean species [27–33]. Using flow cytometric measures of nucleus mass, chromosome numbers, and guard cell lengths, of dozens of individuals of each species, they documented that many species have variable chromosome numbers and nucleus mass, suggesting reductions in chromosome numbers. These reductions in chromosome numbers and DNA content could be the result of aneuploidy (loss of DNA and reduction of chromosome size) and dysploidy (chromosome fusion) [34–36].

Currently, a new taxonomic treatment of *Polylepis* is being prepared by TB and MK which applies a narrower species concept than previous treatments, resulting in 45 species recognized [11,37–39]. This species concept combined information on morphology, distribution, and ecology to infer evolutionarily independent units that are treated as species. For example, applying this concept to the previously very widespread and morphologically variable *P. sericea* as defined by Simpson [9], has resulted in the recognition of seven species, which partly occur in sympatry without hybridization, clearly supporting their treatment as independent species [38, 40. Because ploidy level can also yield important taxonomic information, in the present study we set out to estimate ploidy levels of all species based on guard cell measurements. We used this methodological approach because it can be applied to herbarium material and because accessing live plants of so many species would have been impossible. Also, guard cell measurement is the only possible approach to assess ploidy level in type specimens.

Our study also aims to provide a framework to understand the implications of polyploidy of *Polylepis*, and in particular regarding intraspecific ploidy variation, in strategies of conservation and restoration for the genus. This is particularly important in species reintroduction or ecological restoration, because there are potentially negative consequences when ploidy variants are unintentionally mixed within populations, such as the formation of sterile triploid individuals that may reduce overall population fitness.

Methods

Sampling

To measure guard cell length, we selected 1–24 herbarium specimens of 33 species from the herbaria F, GOET, MO, NY, QCA, and Z/ZH, depending on the availability of previous measurements (previously well-studied species were not sampled). Species level taxonomy follows Boza & Kessler [11].

Guard cell measurements

Guard cell size measurements followed the approach of Kessler et al. [26], with some modifications. Three leaflets per specimen were used for measurements. On each leaflet, we selected a central part halfway between the midvein and the margin to measure the guard cells. In this area, we first carefully removed the hair and wax cover with a scalpel or brush. In species with a wax layer on the leaflet surface, we soaked the leaflets for 1–4 h in acetone or isopropanol, then rinsed

them in clear water, and finally dried samples between tissue paper. Once all covering substances had been eliminated from the leaves, we applied clear nail varnish that was allowed to dry for 4–12 h before removal. Application of the varnish was complicated by the fact that the guard cells of many *Polylepis* species are small and deeply sunken in stomatal pits, so that we used different dilution levels of the varnish with acetone. Often, several layers of varnish were applied to obtain a cover that would not change shape when pulling it off. In species in which the nail varnish level did not work, we used a second method: we mixed ethanol (80%) and hydrogen peroxide (30%) in a relation 1 to 2. Leaflets were left in this solution for up to 3 days until bleached and bloated, with the epidermis separating from the parenchyma. The samples were then rinsed with clear water and stained with safranin. Once the samples were considered adequate, we measured the guard cell length on 10–30 stomata from each sample at 400X magnification under a light microscope and averaged these measurements. For some species, obtaining measurements was difficult, especially when the leaves were thick, covered by a dense hair layer, and when the stomata were deeply sunken in pits. In such cases, repeated attempts with different methods were necessary. Nevertheless, by combining different preparation methods, we obtained at least one measurement for each species.

Ploidy level assignment

Inferring ploidy levels based on guard cell measurements faces several challenges. First there is the issue of assigning the correct base chromosome number. Segovia-Salcedo [31] used the base haploid chromosome number of n = 7 in the family Rosaceae [40] as reference, thus interpreting a chromosome count of 42 as hexaploid (x = 6). Schmidt-Lebuhn *et al*. [19] and Kessler *et al*. [26] instead used the lowest number in the genus (2 n = 42) as baseline, interpreting this as a diploid (x = 2). *Polylepis* has been hypothesized to have originated from the homoploid hybridization between two species of *Acaena* with n = 21 each [31,41,42], which might be taken as indication of a chromosome base number of n = 21 in the genus. However, we consider that ultimately, within a plant group, the crucial factor is the meiotic pairing behavior of the chromosomes, i.e., whether they behave as bivalents so that during chromosome pairing each chromosome has a single counterpart, or as polyvalents where they can pair with several other chromosomes. This behavior is unknown for *Polylepis* or related genera. For simplicity, we here use a chromosome number of 2 n = 42 as baseline for defining diploids, while acknowledging that a baseline of x = 7 may ultimately prove to be more appropriate.

The second issue in inferring ploidy levels concerns variation in measurements and conflicts between different data sources. For inferring ploidy from flow cytometry measurements of nucleus mass, we considered that the diploid condition is related to values around 1.4–1.7 pg, triploidy to 2.0–2.3, tetraploidy to 2.6–3.4 pg, hexaploidy with 4.6–4.9 pg, and octoploidy to 5.7–5.8 pg [19,26]. Following the assignement of a base chromosome number of x = 21, chromosome counts of around 42 correspond to diploids, around 84 to tetraploids, and around 126 to hexaploids. However, numerous published counts differ notably from these values. For example, Caiza et al. [33] and Segovia-Salcedo & Quijia [32] reported chromosome counts of 59–77 for nine individuals of *P. ochreata* (as *P. sericea*) from Yanacocha, Ecuador. In this situation, it is unclear if these numbers reflect the difficulty of fully counting the tiny chromosomes, or whether they correspond to real values with would suggest triploidy and other intermediate chromosome levels as a result of aneuploidy and dysploidy.

Regarding guard cell measurements, there is notable variation within species, even when only a single ploidy level is believed to occur in the species [19]. This variation may be due to anatomical plasticity of a species depending on growth conditions as also found in *Crataegus* [23], differences in measurement methods, or aneuploidy. Thus, to assess the suitability of guard cell measurements in inferring ploidy levels, we plotted the frequencies of measurements in size classes of 1 µm (Figure 1), and indicated those measurements that come from species for which available flow cytometry or chromosome counts have so far only indicated a single ploidy level. Overall, we found that the guard cell measurements showed a bimodal distribution, with one peak at around 13 µm, and the other at around 17 µm, with perhaps a third minor peak at 22 µm. All measurements that can be linked with known diploid species show guard cell measurements below 15 µm, whereas the majority of measurements that can be linked with known tetraploid species correspond to guard cell measurements of 15–20 µm. However, three measurements associated with tetraploid species had measurements of 13–14 µm. Two of these measurements come from *P. triacontandra*, which has a single chromosome count of around 80 (tetraploid) [19] and three guard cell measurements of 18.0–20.4, which is consistent with tetraploidy, but where we also have two measurements of 13.5 µm and 14.1 µm. *Polylepis triacontandra* is morphologically variable and occurs in a region with a long history of human impact, where species of *Polylepis* have been transplanted for long times [10], and where hybridization with other species cannot be ruled out, so that we cannot say with any certainty whether these low measurements truly correspond to tetraploid individuals or whether they might not

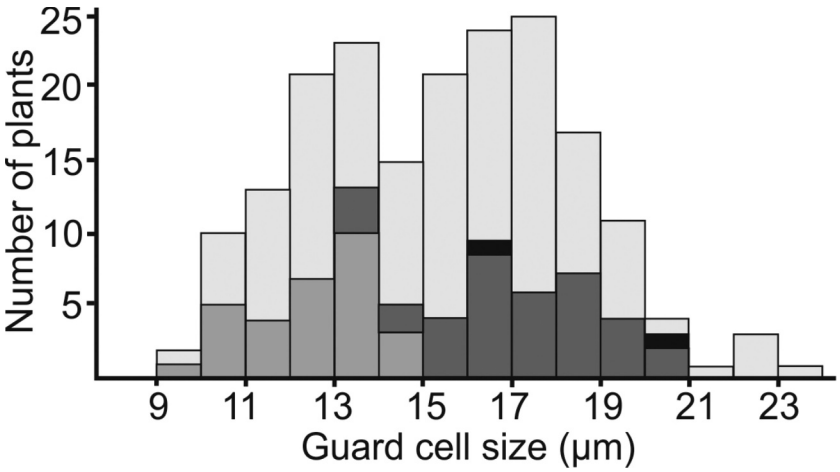

Figure 1. Number of *Polylepis* guard cell measurements in size classes (µm, with 9 indicating measurements from 9.0 to 9.9 µm), including all previously published and newly generated measurements. Total height of each bar shows the total number of measurements in this size class, with white indicating measurements that cannot be directly linked with chromosome counts or flow cytometry measurements, light gray the number of samples from species with only diploid chromosome counts or flow cytometry measurements between 1.4 and1.7 pg, dark gray species with tetraploid chromosome counts or flow cytometry measurements between 2.6 and 3.4 pg, and black species with octoploid flow cytometry measurements.

actually be diploid or triploid plants. The other tetraploid species with a low guard cell measurement is *P. nana*, with two flow cytometry measurements of 2.93–2.96 pg (tetraploid) and five corresponding guard cells measurements between 18.6 µm and 20.3 µm (tetraploid), but also two measurements of 13.1 µm and 15.4 µm. Here, again, it is clear that the species has tetraploid individuals, but it is unclear whether the low measurements are from individuals that are tetraploid or diploid. In addition, we have two measurements of a species known to be octoploid based on flow cytometry (undescribed species *P. sacra*), where the same individuals used for flow cytometry had guard cells sizes of 16.7 µm and 20.2 µm, respectively, thus overlapping with measurements of known tetraploid plants of other species.

From these data we can conclude several things. First, the bimodal distribution of guard cell measurements and the strong association of the two peaks with known diploid and tetraploid ploidy levels confirmed by flow cytometry or chromosome counts support the notion that guard cell measurements reflect ploidy levels in *Polylepis*. Second, however, the overlap between the two peaks and the overlap of the individuals that can be associated with known ploidy levels shows that unambiguous assignment of ploidy levels is not possible for each single guard cell measurement. We conclude that guard cell measurements below 13 µm are very likely to correspond to diploid plants and that measurements above 15 µm are equally likely to be tetraploids or higher ploidy level plants. Values between 13.0 µm and 15.0 µm may correspond to both diploid or tetraploid individuals. Finally, plants with guard cell measurements above 21 may well correspond to hexa- or octoploids, but our data is insufficient to confirm this.

Based on these considerations and in combining all available data sources, our inferences of ploidy levels in species of *Polylepis* were based on the majority of the data available. For instance, in the case of *P. nana*, 5 of 6 guard cell measurements are above 15 µm, indicating tetraploidy, and flow cytometry also points to tetraploidy, so that we interpret this species as being tetraploid. The single guard cell measurement of 13.1 is intriguing in that it might indicate that lower ploidy levels also occur in this species, but not given much weight until further data are available. In Table 1, we indicate our assessment of the confidence we have in the ploidy inferences based on the amount and quality of data, and concordance between different data sources, in three steps, ranging from "low confidence" (*) to "high confidence" (***).

Results and discussion

We obtained guard cell measurements from 114 individuals of 33 species of *Polylepis*, including all species for which no previous measurements were available (Table 1).

For those species for which previous data were available, our new measurements were largely congruent. For example, for *P. argentea* Kessler *et al*. [26] reported sizes from two living plants as 12.4 µm and 13.0 µm, which is in the diploid range, and our measurements from herbarium specimens amounted to 13.4–13.9 µm which is within the mixed diploid/tetraploid range. In this case, we interpreted the species to be diploid, with an intermediate level of confidence. In the case of *P. tarapacana*, we measured the same collection *Kessler 3599* that was also measured by Schmidt-Lebuhn *et al*. [19]. Although the measurements were not identical, the standard deviations overlapped broadly (17.4 ± 2.2 and 19.7 ± 2.0),

Table 1. Overview of the available data on DNA content, chromosome numbers, and guard cell sizes in species of the genus *Polylepis*. Species taxonomy follows Boza *et al*. [14] and Boza & Kessler [11]. Where possible, literature records were assigned to this taxonomy, but a few data points (especially from the hybrid zone at Mojanda, Ecuador) had to be excluded because they could not be unambiguously assigned to a species. Species arrangement in sections and subsections follows Boza & Kessler [11]. Data from the literature are in italics, newly generated data in bold. Depending on data source, we report the mean ± standard deviation, only the mean, or a range. Our confidence in the inferences of ploidy levels, based on the amount and quality of data, and concordance of data from different sources, is indicated by asterisks ranging from low (*) to high (***). Data sources: 1: Schmidt-Lebuhn *et al* [19].; 2: Quijia *et al*. [26]; 3: Montalvo [28]; 4: Zurita *et al*. [29]; 5: Segovia-Salcedo & Quijia [31]; 6: Segovia-Salcedo [30]; 7: Kessler *et al*. [25]; 8: Caiza *et al*. [32]; 9: this study. Abbreviations: Ar = Argentina, Ec = Ecuador, cult. = cultivated (in botanical garden), ind. = individuals, pl = planted.

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
	Voucher/locality	**Size (pg)**	**Voucher/locality**	**N**	**Voucher/locality**	**Length (µm)**	
Section ***Sericea***							
Subsection ***Lanuginosa***							
P. lanuginosa Kunth	3 ind. Sangay, Ec [6]	*1.42 ± 0.13*					diploid***
			6 ind. Zhud, Ec [2]	*38–42*			
					Laegaard 55,036 [1]	*10.8 ± 1.4*	
					Laegaard 102,637 [1]	*11.8 ± 1.6*	
P. multijuga Pilger					***Boza 3070*** [9]	**11.0 ± 2.0**	diploid**
					Boza 3074 [9]	**11.2 ± 1.7**	
					Boza 3076 [9]	**12.8 ± 2.3**	
Subsection ***Pauta***							
P. longipilosa T.Boza *et al*. ined.					***Jaramillo 10,862*** [9]	**10.3 ± 1.6**	diploid**
P. pauta Hieron.	2 ind. Oyacachi, Ec [6]	*3.21 ± 0.04*					tetraploid plus aneuploids; perhaps also diploid*
	2 ind. Oyacachi, Ec [6]	*3.37 ± 0.18*					
			25 ind. Papallacta, Ec [2, 5]	*67–83*			
			16 ind. Papallacta, Ec [6]	*72*			
			15 ind. Cayambe-Coca, Ec. [3]	*68–77*			
					Oyacachi, Ec [8]	*14.4 ± 2.5*	
					Papallacta, Ec [8]	*12.3 ± 1.9*	
					Kessler 2749 [1]	*12.5 ± 1.8*	
					Laegaard 102,327 [1]	*16.5 ± 2.8*	
					Papallacta, Ec [9]	**12.7 ± 1.9**	
					Papallacta, Ec [9]	**12.7 ± 2.1**	
					Papallacta, Ec [9]	**16.6 ± 1.6**	
P. serrata Pilg.	Cult. Göttingen [1]	*1.57 ± 0.11*			Cult. Göttingen [1]	*10.6 ± 0.9*	diploid***
	Cult. Göttingen [1]	*1.61 ± 0.11*			Cult. Göttingen [1]	*12.7 ± 1.8*	
Subsection ***Sericea***							
P. albicans Pilg.					***Boza 3014*** [9]	**10.8 ± 1.3**	diploid**
					Frimer 44 [9]	**13.5 ± 1.4**	
					Renvoize 5074 [9]	**12.2 ± 1.2**	
P. argentea T.Boza & H.R.Quispe	Cult. Göttingen [1]	*1.63 ± 0.15*			Cult. Göttingen [1]	*12.4 ± 2.4*	diploid***
	Cult. Göttingen [1]	*1.67 ± 0.15*			Cult. Göttingen [1]	*13.0 ± 1.7*	
					Cult. Zurich [9]	**13.9 ± 1.7**	
					Chevarria 1035 [9]	**13.4 ± 1.2**	
					Hanold 85 [9]	**13.4 ± 1.2**	
P. canoi W.Mend.					*Kessler 2880* [1]	*14.3 ± 2.6*	diploid*
P. frontinensis T.Boza & M.Kessler ined.					***Kessler 2772*** [9]	**11.5 ± 1.3**	diploid**
					Kessler 2776 [9]	**13.0 ± 1.3**	
P. humboldtii T.Boza *et al*. ined.					***Carate 185*** [9]	**12.4 ± 1.4**	diploid*
P. loxensis T.Boza *et al*. ined.			25 ind. Fierro Urco, Ec [2, 5]	*39–42*			
					Laegaard 19,109 [9]	**12.0 ± 1.5**	

(*Continued*)

Table 1. (Continued).

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
					Lewis 3804 [9]	**11.4 ± 1.1**	
P. ochreata (Wedd.) Bitter	2 ind. Yanacocha, Ec [6]	*3.41 ± 0.09*					diploid, tetraploid, and hexaploid; perhaps plus aneuploids or hybrids*
	2 ind. El Ángel, Ec [6]	*4.66 ± 0.57*					
			8 ind. El Ángel, Ec [2, 5]	*37–40*			
			9 ind. Yanacocha, Ec [2, 5]	*59–77*			
			16 ind. Yanacocha, Ec [6]	*82*			
			15 ind. Yanacocha, Ec [3]	*73–88*			
					Molau 2536 [9]	**10.7 ± 1.3**	
					Laegaard 54,474 [9]	**11.9 ± 1.1**	
					Romoleroux 1060 [9]	**11.7 ± 1.0**	
					Yanacocha, Ec [8]	*14.1 ± 2.3*	
P. sericea Wedd.					***Dorr 5220*** [9]	**13.3 ± 1.8**	diploid*
Subsection *Pepei*							
P. pepei B.B.Simpson					*Kessler 2795* [1]	*10.9 ± 1.6*	diploid**
					Kessler 3386 [1]	*11.8 ± 1.6*	
P. rodolfo-vasquezii L.Valenz. & Villalba	Cult. Göttingen [1]	*1.60 ± 0.07*			Cult. Göttingen [1]	*10.2 ± 1.5*	diploid***
	Cult. Göttingen [1]	*1.70 ± 0.05*			Cult. Göttingen [1]	*10.8 ± 1.3*	
Section *Reticulata*							
P. hieronymi Pilg.	Cult. Göttingen [1]	*1.52 ± 0.02*			Cult. Göttingen [1]	*12.6 ± 1.8*	diploid***
	Cult. Göttingen [1]	*1.49 ± 0.04*			Cult. Göttingen [1]	*11.9 ± 2.0*	
	52 ind. Ar [7]	*1.45–1.57*					
					Beck 9345 [1]	*13.2 ± 1.8*	
					Kessler 3123 [1]	*11.2 ± 1.0*	
P. microphylla (Wedd.) Bitter	Cult. Göttingen [1]	*1.53 ± 0.06*			Cult. Göttingen [1]	*13.9 ± 1.1*	diploid and tetraploid plus aneuploids**
	Cult. Göttingen [1]	*1.53 ± 0.07*			Cult. Göttingen [1]	*14.3 ± 2.2*	
	2 ind. Ozongoche, Ec [6]	*2.03 ± 0.22*					
			8 ind. Achupallas, Ec [2, 6]	*70–82*			
					Galiano 1999 [1]	*14.2 ± 1.8*	
					Achupallas, Ec [8]	*10.7 ± 1.9*	
P. occidentalis T.Boza & M.Kessler ined.					***Diaz 2879*** [9]	**11.3 ± 1.2**	diploid**
					Diaz 4012 [9]	**10.8 ± 1.0**	
					Sánchez 10,285 [9]	**12.7 ± 1.6**	
P. quadrijuga Bitter					*Gradstein s.n.* [1]	*12.2 ± 1.7*	diploid**
					Gradstein s.n. [1]	*12.3 ± 2.0*	
					Olivares 570 [9]	**14.3 ± 1.3**	
P. reticulata Hieron.			11 ind. Soldados, Ec [2]	*36–42*			diploid plus higher ploidy (hexaploidy?; in cultivated plants only?)*
			3 ind. Oyacachi, Ec (pl) [6]	*~118*			
					Kessler 2746a [1]	*12.2 ± 1.4*	
					Laegaard 102,691 [1]	*10.0 ± 0.9*	
					Cajas, Ec [9]	**10.5 ± 1.8**	
					Cajas, Ec [9]	**12.3 ± 1.6**	
					Cajas, Ec [9]	**11.4 ± 1.4**	
P. simpsonii T.Boza & M.Kessler ined.	3 ind. Sangay, Ec [6]	*1.40 ± 0.08*					diploid***
			25 ind. Zhud, Ec [2]	*37–42*			
			2 ind. Sangay, Ec [6]	*38*			
					Laegaard 102,677 [1]	*12.2 ± 1.0*	
					Cajas, Ec [9]	**9.1 ± 1.1**	
P. weberbaueri Pilg.					*Acleto 364* [1]	*12.3 ± 0.9*	diploid**

(*Continued*)

Table 1. (Continued).

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
					Boza 3018 [9]	**14.1 ± 1.1**	
					Boza 3148 [9]	**14.3 ± 1.5**	
					Smith 9568 [9]	**14.9 ± 1.1**	
Section ***Australis***							
P. australis Bitter	Cult. Göttingen [1]	*2.98 ± 0.06*			Cult. Göttingen [1]	*16.7 ± 2.6*	tetraploid and diploid plus triploid hybrids and hexaploid autopolyploid derivate***
	Cult. Göttingen [1]	*3.03 ± 0.03*			Cult. Göttingen [1]	*17.4 ± 1.6*	
	261 indiv. [7]	*2.84–2.97*					
	75 indiv. [7]	*1.44–1.54*					
	24 indiv. [7]	*2.09–2.24*					
	1 indiv. [7]	*4.15*					
					Kessler 3350 [1]	*18.9 ± 1.9*	
					Lorentz 760 [1]	*12.8 ± 1.4*	
					Cult. Zurich [9]	**22.7 ± 2.7**	
					Lazaro 6695 [9]	**17.6 ± 2.4**	
					Venturi 3010 [9]	**12.2 ± 1.8**	
					w/colector 2330 [9]	**15.5 ± 2.5**	
P. neglecta M.Kessler	Cult. Göttingen [1]	*1.54 ± 0.06*	Cult. Göttingen [1]	*~80*	Cult. Göttingen [1]	*13.9 ± 1.1*	diploid; perhaps also tetraploid*
	Cult. Göttingen [1]	*1.55 ± 0.09*			Cult. Göttingen [1]	*14.3 ± 2.2*	
					Kessler 3531 [1]	*13.6 ± 2.3*	
					Kessler 3633 [1]	*13.2 ± 2.0*	
Subsection ***Subsericans***							
P. flavipila (Bitter) M.Kessler & Schmidt-Leb.					***Boza 3163*** [9]	**17.9 ± 1.8**	tetraploid**
					Boza 3167 [9]	**16.0 ± 1.5**	
					Boza 3168 [9]	**15.3 ± 1.6**	
P. pilosissima T.Boza & M.Kessler ined.					*Kessler 3426* [1]	*18.0 ± 2.0*	tetraploid**
					Kessler 3591 [1]	*17.2 ± 2.2*	
					Boza 3023 [9]	**17.3 ± 1.6**	
					Cerrate 1265 [9]	**15.6 ± 1.4**	
					Gentry 638 [9]	**16.2 ± 1.3**	
					Kessler 3428 [9]	**15.7 ± 1.1**	
P. subsericans Macbr.	Cult. Göttingen [1]	*3.12 ± 0.18*			Cult. Göttingen [1]	*16.6 ± 2.2*	tetraploid**
	Cult. Göttingen [1]	*3.21 ± 0.11*			Cult. Göttingen [1]	*17.3 ± 2.2*	
					Toivonen s.n. [1]	*18.3 ± 1.5*	
					Toivonen s.n. [1]	*18.5 ± 2.4*	
					Sylvester 428 [9]	**13.9 ± 1.2**	
					Sylvester 868 [9]	**16.2 ± 1.7**	
					Sylvester 1287 [9]	**15.9 ± 1.8**	
Section ***Incana***							
Subsection ***Racemosa***							
P. acomayensis T.Boza & M.Kessler ined.					***Boza 3135*** [9]	**16.2 ± 1.8**	tetraploid**
					Boza 3141 [9]	**15.1 ± 1.4**	
P. incarum (Bitter) M.Kessler & Schmidt-Leb.					*Jimenez 2716* [1]	*18.3 ± 0.8*	tetraploid**
					Kessler 3465 [1]	*17.2 ± 1.9*	
					Jimenez 2716 [9]	**17.6 ± 1.6**	
					Kessler 13,515 [9]	**16.9 ± 2.1**	
					Shepard 150 [9]	**17.8 ± 2.2**	
P. lanata (Bitter) M.Kessler & Schmidt-Leb.					*Kessler 2851* [1]	*19.6 ± 2.1*	tetraploid**
					Kessler 2962 [1]	*18.8 ± 1.7*	

(*Continued*)

Table 1. (Continued).

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
P. pacensis M.Kessler & Schmidt-Leb.					*Kessler 3028* [1]	*15.9 ± 2.7*	tetraploid**
					Mendez & Arcienaga 14 [1]	*17.7 ± 1.3*	
					Kessler 14,528 [9]	**19.2 ± 1.7**	
					Lopez & Bermejo 4 [9]	**19.1 ± 1.9**	
					Lopez & Bermejo 10 [9]	**19.7 ± 2.0**	
P. racemosa Ruiz & Pav. (all pl)	2 ind. Cotopaxi, Ec. [6]	*4.48 ± 0.19*					tetraploid to octoploid, with many intermediate and aneuploid ploidy levels*
	2 ind. Cotopaxi, Ec. [6]	*2.63 ± 0.20*					
	3 ind. Oyacachi, Ec. [6]	*4.57 ± 0.11*					
			12 ind. Oyacachi, Ec [2, 5]	*80–82*			
			2 ind. Oyacachi, Ec [2, 5]	*72–77*			
			10 ind. Oyacaci, Ec. [6]	*62–80*			
					Ferreyra 12,418 [1]	*18.0 ± 1.6*	
					Papallacta, Ec [8]	*21.7 ± 3.8*	
					Oyacachi, Ec [8]	*17.6 ± 2.6*	
					Arce 161 [9]	**17.2 ± 1.5**	
					Arce 167 [9]	**15.1 ± 1.3**	
					Arce 207 [9]	**13.8 ± 1.4**	
					Bird 1384 [9]	**16.1 ± 1.3**	
					Boza 3020 [9]	**16.7 ± 1.6**	
					Boza 3030 [9]	**15.1 ± 1.0**	
					Boza 3031 [9]	**14.2 ± 1.4**	
					Boza 3119 [9]	**18.0 ± 1.5**	
					Ferreyra 3792 [9]	**15.3 ± 1.6**	
					Kenehira 5 [9]	**15.9 ± 1.1**	
					Kessler 14,608 [9]	**17.1 ± 1.1**	
					Laegaard 20,465 [9]	**19.8 ± 2.0**	
					Laegaard 22,351 [9]	**17.0 ± 1.9**	
					Leiva 741 [9]	**14.9 ± 1.2**	
					Leiva 1090 [9]	**16.4 ± 1.8**	
					Nuñez 8117 [9]	**16.3 ± 1.3**	
					Renvoize 4847 [9]	**17.7 ± 1.4**	
					Sánchez Vega 5322 [9]	**13.5 ± 1.1**	
					Smith 11,076 [9]	**12.6 ± 1.0**	
					Soukup 3498 [9]	**16.7 ± 1.5**	
					Stork 9972 [9]	**16.6 ± 1.8**	
					Tovar 2371 [9]	**15.3 ± 1.4**	
					Velásquez 12 [9]	**16.3 ± 1.1**	
					West 3787 [9]	**13.2 ± 2.2**	
P. sacra T.Boza & M.Kessler ined.	Cult. Göttingen [1]	*5.76 ± 0.26*			Cult. Göttingen [1]	*20.2 ± 3.3*	octoploid; perhaps also tetraploid or intermediates*
	Cult. Göttingen [1]	*5.72 ± 0.15*			Cult. Göttingen [1]	*16.7 ± 3.3*	
					Rosales 04 [1]	*19.5 ± 0.8*	
					Sylvester 644 [9]	**15.6 ± 1.4**	
					Sylvester 1262 [9]	**22.1 ± 1.2**	
					Sylvester 1270 [9]	**15.8 ± 1.3**	

(*Continued*)

Table 1. (Continued).

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
P. triacontandra Bitter			Cult. Göttingen [1]	*~80*			tetraploid; perhaps also lower ploidy levels*
					Beck 4976 [1]	*18.9 ± 1.9*	
					Kessler 3420 [1]	*20.4 ± 1.1*	
					Steudel 427 [9]	**13.5 ± 1.9**	
					Steudel 431 [9]	**14.1 ± 2.6**	
					Steudel 433 [9]	**18.0 ± 2.1**	
Subsection ***Besseri***							
P. besseri Hieron.					*Kessler 2989* [1]	*20.4 ± 2.8*	tetraploid or higher ploidy level*
					Kessler 2985 [1]	*19.2 ± 1.9*	
P. crista-galli Bitter					*Beck 9343* [1]	*16.6 ± 1.4*	tetraploid**
					Kessler 3155 [1]	*17.8 ± 2.1*	
P. pallidistigma Bitter					***Boza 3005*** [9]	**17.2 ± 1.9**	tetraploid**
					Boza 3006 [9]	**17.1 ± 2.2**	
					Boza 3007 [9]	**18.5 ± 1.9**	
					Sylvester 1807 [9]	**16.4 ± 1.4**	
					Sylvester 1816 [9]	**17.3 ± 1.9**	
					Sylvester 1825 [9]	**18.5 ± 1.9**	
P. rugulosa Bitter					*Ferreyra 2594* [1]	*16.8 ± 1.9*	tetraploid*
P. subtusalbida (Bitter) M.Kessler & Schmidt-Leb.					***Beck 7395*** [9]	**22.1 ± 1.5**	tetraploid and higher ploidy level**
					Kessler 216 [9]	**23.4 ± 2.4**	
					Ritter 1196 [9]	**15.9 ± 1.4**	
Subsection ***Incana***							
P. fjeldsaai T.Boza & M.Kessler ined.					***Mendoza 1019*** [9]	**11.9 ± 2.6**	diploid**
					Mendoza 1032 [9]	**13.3 ± 1.4**	
					Mendoza 1057 [9]	**15.2 ± 1.7**	
P. incana Kunth	3 ind. Sincholagua, Ec [6]	*1.99 ± 0.34*					mainly diploid but also tetra- and hexaploid (in cultivated plants only?)**
	3 ind. Illinizas, Ec [6]	*1.60 ± 0.14*					
	3 ind. Inga-Raya, Ec [6]	*1.67 ± 0.30*					
	3 ind. Cayambe-Coca, Ec (pl) [6]	*1.42 ± 0.10*					
	3 ind. Antisana, Ec (pl) [6]	*4.67 ± 018*					
			16 ind. El Ángel, Ec [2, 5, 6]	*(38 –) 42*			
			6 ind. Illinizas, Ec [2, 5, 6]	*38*			
			30 ind. Cayambe-Coca, Ec [2, 5, 6]	*(39 –) 42*			
			15 ind. Inga-Raya, Ec [6]	*42*			
			15 ind. El Inga, Ec [4]	*40–42*			
			15 ind. Papallacta, Ec [4]	*41–42*			
			15 ind. El Ángel, Ec. [4]	*40–42*			
					Laegaard 102,647 [1]	*17.6 ± 2.0*	
					Schmidt-Lebuhn 521 [1]	*17.0 ± 2.3*	
					Illinizas, Ec [8]	*9.7 ± 0.5*	
					Boza 3066 [9]	**15.4 ± 1.8**	
					Boza 3095 [9]	**13.1 ± 1.5**	
					Laegaard 102,282 [9]	**18.6 ± 2.2**	
P. incanoides (M.Kessler) T.Boza & M.Kessler ined.					*Kessler 3288* [1]	*16.4 ± 1.6*	tetraploid; also lower ploidy levels?*
					Kessler 3293 [1]	*18.3 ± 2.4*	

(*Continued*)

Table 1. (Continued).

Species	DNA Content		Chromosome number		Guard cell length		Inferred ploidy levels (2 n)
					Beck 34,512 [9]	**15.3 ± 1.9**	
					Kessler 2954 [9]	**13.2 ± 0.7**	
P. nana (M.Kessler) T.Boza & M.Kessler ined.	Cult. Göttingen [1]	*2.93 ± 0.05*			Cult. Göttingen [1]	*18.7 ± 1.7*	tetraploid; also lower ploidy levels?**
	Cult. Göttingen [1]	*2.96 ± 0.05*			Cult. Göttingen [1]	*19.6 ± 1.6*	
					Kessler 3514 [1]	*20.3 ± 2.6*	
					Kessler 3642 [1]	*19.5 ± 1.3*	
					Kessler 3501 [9]	**15.4 ± 1.8**	
					Kessler 3518 [9]	**13.1 ± 1.5**	
					Kessler 3519 [9]	**18.6 ± 2.2**	
P. tarapacana Phil.	Cult. Göttingen [1]	*3.02 ± 0.17*	Cult. Göttingen [1]	*~80*	Cult. Göttingen [1]	*17.4 ± 1.8*	tetraploid***
	Cult. Göttingen [1]	*3.00 ± 0.16*			Cult. Göttingen [1]	*16.9 ± 2.3*	
					Kessler 3599 [1]	*17.4 ± 2.2*	
					Kumar 6 [1]	*17.1 ± 1.1*	
					Beck 9008 [9]	**14.9 ± 2.8**	
					Beck 19,897 [9]	**16.1 ± 1.1**	
					Beck 32,470 [9]	**15.0 ± 1.2**	
					Boza 3009 [9]	**14.9 ± 2.3**	
					Kessler 3599 [9]	**19.7 ± 2.0**	
P. tomentella Wedd.	43 ind. Ar [7]	*2.90–3.01*			*Kessler 3188* [1]	*17.9 ± 1.8*	tetraploid***
					Kessler 3368 [1]	*18.7 ± 2.0*	
					Boza 3107 [9]	**16.6 ± 1.6**	
					Boza 3110 [9]	**13.9 ± 2.0**	
					Boza 3111 [9]	**15.3 ± 1.4**	
					Kessler 3200 [9]	**19.1 ± 2.5**	

confirming the replicability of the measurements while also showing some study-dependent variation which may be due to the preparation and measurement methods.

Where all three types of data are available for a species, they are often congruent and point to a single ploidy level, as in *P. lanuginosa*, undescribed species *P. simpsonii*, and *P. tarapacana* (Table 1). In other cases, the data are complementary, as in *P. ochreata*, where the two flow cytometry measurements point to tetra- and hexaploidy, the chromosome counts to di- and tetraploidy (and perhaps intermediate values as discussed under ploidy level assignment), and the guard cell measurements to diploidy. In this situation, we infer that the species includes di-, tetra-, and hexaploid individuals, possibly with aneuploids or hybrids. None of the three data sources would have by itself provided this picture, pointing to the importance of obtaining complementary data and highlighting the limitations of single types of data. At present, for the 45 species of *Polylepis*, we have combined data on guard cell length, chromosome number, and genome size for 9 (20%) species, on guard cell length and genome size for another 9 (20%) species, and on guard cell length and chromosome number for 3 (7%) species, whereas for 24 (53%) species only guard cell measurements are available.

Bearing in mind the potential limitations of incomplete data and some uncertainty in the interpretation of the data, we infer that at present knowledge, 19 (42%) species appear to be purely diploid, 15 (33%) purely tetraploid, and one (2%) purely octoploid. The remaining 8 (18%) species have mixed ploidy levels, with 3 (7%) being di- and tetraploid, 2 (4%) di- and hexaploid, 1 (2%) tetra- and hexaploid, 1 (2%) tetra- and octoploid, and 1 (2%) di-, tri-, tetra- and hexaploid. While it is likely that further studies will reveal more cases of mixed ploidy, at least some well-studied species appear to consistently show diploid (*P. hieronymi, P. lanuginosa, P. simpsonii*) or tetraploid (*P. tarapacana, P. tomentella*) conditions, suggesting that not all species have mixed ploidy levels.

From an evolutionary point of view, Schmidt-Lebuhn *et al*. [19] proposed that the a chromosome number of 2 n = 42 is ancestral in *Polylepis*, and that higher ploidy levels evolved once at the base of the *Incana* complex as defined by Simpson [9] and twice within species outside of this section (*P. australis, P. pauta*). The data that has since then been compiled in this and other studies points to a more complex picture (Figure 2). In section *Sericea*, which has been considered to include a grade of the phylogenetically basal members of the genus [9,12,16], most species are diploid (taking n = 21 as the base number), but polyploidy is present in *P. ochreata* and *P. pauta*. These two species overlap in northern Ecuador where they hybridize extensively [11,38]. It is conceivable that the polyploid condition in these two species stems from this hybridization, as polyploidy is often correlated with hybridization [3,4]. In the presumably monophyletic section *Reticulata*, again most species are diploid, but polyploidy occurs in two species that appear not to be closely related (*P. microphylla, P. reticulata*). Interestingly, at least in *P. reticulata*, polyploidy is so far only known from cultivated plants [31], suggesting that this condition may be related to domestication. Polyploidization as a result of domestication is a common phenomenon in plants [43]. In section *Australis, P. australis* has been well studied and includes di-, tri-, tetra- and hexaploids, most likely due to autopolyploidization [26]. Section *Subsericans* includes three purely tetraploid species. Finally, section *Incana* mainly includes tetraploid species, but diploidy occurs in *P. incana* and the closely related undescribed species *P. fjeldsaai* of subsection *Incana*. *Polylepis incana* has been considered to be one of the most derived species in this section [9,12,16], so that a diploid condition is surprising if one assumes that *P. incana* is nested within a tetraploid clade. This suggests that the assumption that *P. incana* is a derived member of this section is wrong, and that the evolution of this section is more complex than previously assumed. In any case, assuming that the species sections recognized here based on morphological and ecological similarity are evolutionary units, we now can deduce that polyploidy evolved at least eight times in the genus, possibly more often. However, considering that the evolution of *Polylepis* is probably reticulate and that the phylogeny of the genus remains poorly understood [12,42], inferring the origins of polyploidy in *Polylepis* is very difficult, and the conclusions drawn here should be viewed as hypotheses to be tested by future studies.

Placing *Polylepis* in the broader context of the family Rosaceae and particularly the tribe Sanguisorbae to which it belongs, shows that it higher chromosome numbers than many other genera of the tribe (e.g., 14–56 in *Sanguisorba*, 28 in *Bencomia*, and 28–56 in *Agrimonia* [40]). On the other hand, the values in *Polylepis* are comparable to those of the most closely related genus *Acaena*, which has reported chromosome counts between 42 and 126. This is consistent with the interpretation of *Polylepis* having evolved from the homoploid hybridization between two species of *Acaena* with n = 21 each [31,41]. Whether 2 n = 42 is a functionally polyploid or diploid conditions in these genera remains unknown.

Finally, focusing on the taxonomic implications of ploidy levels in the genus, we found that in some cases, closely related species have different ploidy levels, supporting their treatment as distinct species. For example, *P. fjeldsaai* has previously been identified as part of *P. tomentella* [44], but whereas the first species is diploid based on our data, the latter is consistently tetraploid. On the other hand, at least eight species include individuals of different ploidy levels. At least in *P. australis*, this is clearly a natural condition [27], which raises the question as to how to treat the different ploidy levels taxonomically. It has been suggested that different ploidy levels within a "species" should be treated at species level if

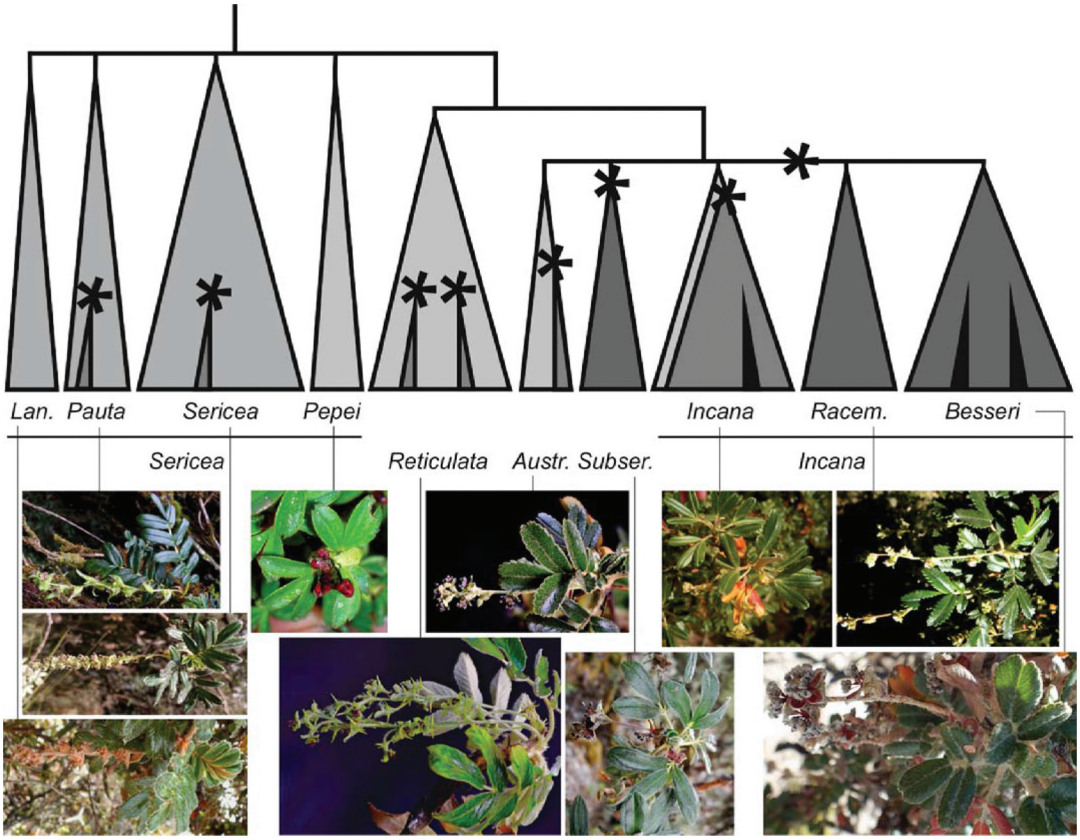

Figure 2. Simplified phylogenetic hypothesis of *Polylepis* based on Schmidt-Lebuhn et al. [19] indicating the ploidy levels as inferred in this study (diploid = light gray, tetraploid = dark gray, hexa- to octoploid = black) and the presumed origins of polyploidy (*). Triangles are proportional to species numbers in each section or subsection; see Table 1 for species. Names of subsections (left) and sections (right) are indicated next to the triangles; *Austr.* = *Australis, Lan.* = *Lanuginosa, Subser.* = *Subsericans*. Photos show representative species of the sections and subsections. From top to bottom: *Polylepis rugulosa* (E. Urquiaga), *P. racemosa* (T. Boza), *P. incana* (E. Urquiaga), *P. subsericans* (T. Boza), *P. australis* (A. Bernhard), *P. hieronymi* (A. Bernhard), *P. rodolfo-vasquezii* (T. Boza), *P. humboldtii* (T. Boza), *P. pauta* (T. Boza), and *P. lanuginosa* (T. Boza).

there is morphological, ecological, or biogeographical evidence that they are evolutionarily largely independent units [7]. This approach has been taken in polyploid-apomict species complexes of other genera of Rosaceae such as *Crataegus* [45] and *Sorbus* [14], but more information is needed before this approach can be applied to *Polylepis*. On the other hand, in several species polyploidization is apparently linked to cultivation, as in *P. incana, P. racemosa*, and *P. reticulata*. Polyploidization of cultivated plants is a common phenomenon either via auto- or allopolyploidization where higher ploidy levels are often associated with higher plant vigor and adaptive potential [46–48]. *Polylepis* has long been planted by Andean inhabitants as a source of building material, firewood, and as fences [17], and it is conceivable that natural or artificial hybrids have been favored.

Conclusions

The novel data on guard cell length presented in this study, in combination with previous data, provide a more comprehensive view of the distribution of ploidy levels within the genus *Polylepis*. This reveals a complex evolutionary history, with repeated polyploidization events partly linked to hybridization and cultivation. Understanding variation in ploidy has important practical implications for the management and conservation of *Polylepis*, many species of which are threatened with extinction [49]. For instance, if populations of a species have different ploidy levels, mixing these origins in reforestation schemes may lead to the formation of sterile hybrids, reducing the reproductive potential of the entire population. On the other hand, if different ploidy levels are reproductively isolated, then they represent different evolutionary units that deserve independent conservation attention. Clearly, ploidy level needs to be taken into account for the conservation of the genus. While the assessment of ploidy level using flow cytometry requires expensive technology, and direct chromosome counts are quite challenging [27], the measurement of guard cells is a low cost and simple technique that can be readily applied to both live and dried plant material. However, guard cell measurements are also the least precise of the three methods, and our study only represents a further step in our understanding of ploidy level distribution and evolution in *Polylepis*.

More detailed studies are clearly needed to clarify many important questions related to polyploidy and its consequences within *Polylepis*. Some that we consider to be particularly important include:

(1) What is the functional chromosome base number in *Polylepis*? In species with 42 chromosomes here considered as diploids, do they behave as 21 pairs of bivalents or as six groups of seven chromosomes that can pair in different combinations? Detailed studies of meiotic pairing behavior are needed, as done, e.g., for *Crataegus* [50].
(2) Are individuals of naturally occurring different ploidy levels with a "species" evolutionarily independent? This might be addressed in species of known multiple ploidy levels by combining ploidy measurements with population genetic approaches. Particularly intriguing target species for this kind of study include *P. australis, P. incana, P. racemosa*, and *P. reticulata*.
(3) Are triploid individuals, which are known to occur at least in *P. australis* and which may occur in other species having both diploid and tetraploid individuals, sterile or can they reproduce via apomixis? In *P. australis*, seed viability is very low in many individuals [51–53] but it is not known if this related to ploidy.
(4) More generally, does apomixis occur in *Polylepis*? This is commonly found in other genera of Rosaceae, where it is frequently linked with polyploidization [40]. This can be dome via pollination experiments and flow cytometry of embryonic tissue, as conducted, e.g., for *Lachemilla* [54].
(5) What is the prevalence of aneuploidy and dysploidy in the genus? Studies in Ecuador [27–33] suggest that these occur in the genus, but data are inconclusive.
(6) How does hybridization between ploidy levels induced by translocation and reforestation activities affect the population viability of species? This is a situation where guard cell measurements may be very helpful, since their variability is likely to be lower within individual species and among individuals growing under similar environmental conditions.

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Author contribution

This work is part of the Ph.D. thesis research of T. E. Boza E. and was developed under the supervision of M. Kessler. Both authors consolidated the ploidy database and contributed to any taxonomic decision adopted in this paper. The labwork was done by V. Popp. All authors provided comments on drafts of this manuscript.

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