

The genus *Pulmonaria* (Boraginaceae) in the province of Bologna

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ABSTRACT

Populations of *Pulmonaria* on Apennine highlands in the province of Bologna were investigated. Except for a few isolated sites with *P. officinalis*, most populations are referable to *P. apennina* on the basis of leaf shape, indumentum and chromosome number ($2n = 22$). *Pulmonaria apennina* occurs in two morphs: i) a green morph with few or no leaf spots, common in mountain sites and similar to its Alpine vicariant, *P. vallarsae*; ii) a variegated morph with diffuse, clear leaf spots occurring in cool locations at low altitude, often not distant from cities. Hilly areas along the Reno valley, however, are colonized by populations of *P. hirta* with $2n = 28$ chromosomes, characterized by lanceolate leaf shape and extensive maculation. Certain similarities between *P. hirta* and the variegated morph of *P. apennina* have long caused confusion and misidentifications, but the two species in the area of investigation are distinguishable on the basis of leaf morphology and indumentum.

Key words: basal leaves, foliar spots, karyology, lungwort, *Pulmonaria*, trichomes

RIASSUNTO

Il genere Pulmonaria (Boraginaceae) nella Provincia di Bologna

Sono state studiate le popolazioni di *Pulmonaria* L. sulle montagne della provincia di Bologna. Tranne che per pochi siti isolati con *P. officinalis*, la massima parte delle popolazioni sono riferibili a *P. apennina* in base alla forma fogliare, all'indumento e al numero cromosomico ($2n = 22$). *Pulmonaria apennina* si presenta in due morfe: i) una morfa verde con poche o nessuna macchia fogliare, comune in località montane e simile alla sua vicariante delle Alpi, *P. vallarsae*; ii) una morfa variegata con macchie fogliari diffuse, chiare, distribuita in siti freddi a bassa altitudine, spesso non distanti dalle città. Tuttavia, le aree collinari lungo la valle del Reno sono colonizzate da popolazioni di *P. hirta* con $2n = 28$, caratterizzate dalla forma lanceolata delle foglie e da macchiatura abbondante. Certe somiglianze fra *P. hirta* e la morfa variegata di *P. apennina* hanno a lungo causato confusioni e determinazioni erranee, ma le due specie nell'area in esame sono distinguibili sulla base della morfologia fogliare e dell'indumento.

Parole chiave: Foglie basali, macchie fogliari, cariologia, *Pulmonaria*, tricomi

INTRODUCTION

Several species and morphs of the genus *Pulmonaria* L. (lungworts) occur in the Bologna province, and their systematics is still debated. The situation is clear for the European species *P. officinalis* L. (Fig. 1A), which extends its discontinuous range in Italy as far south as the Gran Sasso massif in Abruzzo (Bartolucci *et al.* 2012). All other Apennine lungworts (Figs. 1B-1E) are often thought to fall within one large species complex (Lacaita 1927; Merxmüller & Grau 1969; Merxmüller & Sauer 1972; Bolliger 1982; Tison & de Foucault 2014). Anton Kerner von Marilaun in his *Monographia Pulmonariarum* (1878), the first modern approach to lungwort systematics, recognized two related Apennine species: *P. vallarsae* A.Kern. and *P. saccharata* Mill. The former was originally described based on plants from south-eastern Alps but later extended to the whole of Italian peninsula as the most common Italian *Pulmonaria*, the latter shows strongly speckled leaves and

occurs in south-western Alps and north-central Apennines. Although Kerner's treatment was criticized by Lacaita (1927) who reputed Kerner's *P. saccharata* to be, in fact, a variegated form of *P. vallarsae*, it was essentially accepted by all authors of Italian Floras (Fiori 1923; Zangheri 1976; Pignatti 1982). Puppi & Cristofolini (1991), however, argued that the name *P. saccharata* is not applicable to any Italian population and plants named *P. saccharata* sensu Kerner are to be referred to *P. picta* Rouy. More recently, the name *P. picta* has been rejected in favour of *P. hirta* L., the earlier name holding priority (Conti *et al.* 2007). Puppi & Cristofolini (1996) provided evidence that Alpine and Apennine populations attributed to *P. vallarsae* are distinct enough to be separated at species level, so that the peninsular populations were described as *Pulmonaria apennina* Cristof. & Puppi, sharing with *P. vallarsae* the same chromosome number $2n = 22$ ($2n = 22 + 2B$ for specimens from Alpi Apuane: Merxmüller & Grau 1969; Capineri 1986), although $2n = 26$ has now been reported for a population of *P. apennina*

(Astuti *et al.* 2019). On the other hand, most chromosome counts for *P. hirta* gave $2n = 28$, but some reports had $2n = 22$ as well as intermediate numbers such as $2n = 26$ and 27 , thus partially overlapping *P. apennina* and *P. vallarsae* (Merxmüller & Grau 1969; Sauer 1975; Capineri 1986; Puppi & Cristofolini 1996; Astuti *et al.*, 2019).

In a survey of some two hundred populations of *Pulmonaria* in northern Apennines, Vosa & Pistolesi (2004) found three major karyotypes: $2n = 16$ (*P. officinalis*), $2n = 22$ and $2n = 28$ (*P. apennina* and/or *P. hirta*), with cytological hybrids between these two species found in two sites. These authors were unable to correlate karyology and morphology of the plants examined and considered any distinction between *P. apennina* and *P. hirta* as unfeasible. Consequently, some authors treated this group of parapatric lungworts at the rank of subspecies, i.e. *P. hirta* subsp. *hirta* and *P. hirta* subsp. *apennina* (Cristofolini & Puppi) Peruzzi (Bernardo *et al.* 2010). In their recent monograph of Italian Boragineae, on the other hand, Cecchi & Selvi (2015) split *P. vallarsae* s.l. into two subspecies: *P. vallarsae* subsp. *apennina* (Cristof. & Puppi) L. Cecchi & Selvi, and the nominal subspecies, while no subspecies was recognized within

the separated *P. hirta* (with $2n = 28$; Cecchi & Selvi 2015). The latter treatment has been adopted in the updated checklist of Italian flora (Bartolucci *et al.* 2018).

In the attempt to clarify the situation, we have conducted a study of *Pulmonaria* populations of mountain areas of the Bologna province, which also includes the *locus classicus* of *P. apennina* near Bologna (Peruzzi *et al.* 2015). Our results confirm the presence of three different chromosome numbers, probably related to the three taxa described as *P. officinalis*, *P. hirta* and *P. vallarsae* subsp. *apennina*. For the sake of simplicity, we shall refer to this latter taxon throughout this paper as *P. apennina*, which is primarily applied to populations morphologically similar to *P. vallarsae* and mostly featuring full-green, uniform leaves (green morph), fairly common on mountains. In addition, we also recognize a variegated morph related to *P. apennina*, but showing some distinctive traits often including an extensive leaf maculation, either pale-green or white. *Pulmonaria officinalis* occurs in some isolated, cool hill sites. *Pulmonaria hirta* is mainly distributed along the Reno valley and at the border of Tuscany.



Figure 1. Plants and basal rosette leaves of: A) *Pulmonaria officinalis* subsp. *officinalis*, Val Rio Maggiore (Sasso Marconi) June 24, 2012; B) *P. vallarsae*, Passo Borcola (Recoaro, Vicenza) Aug. 11, 2014; C) green morph of *P. apennina*, M.te Bastione (S. Benedetto Val di Sambro) May 10, 2009; D) variegated morph of *P. apennina*, Dolina Spipola (S. Lazzaro di Savena) Nov. 7, 2010; E) *P. hirta*, Poggio di Carviano (Grizzana Morandi) Nov. 14, 2013. All from Bologna province except *P. vallarsae*.

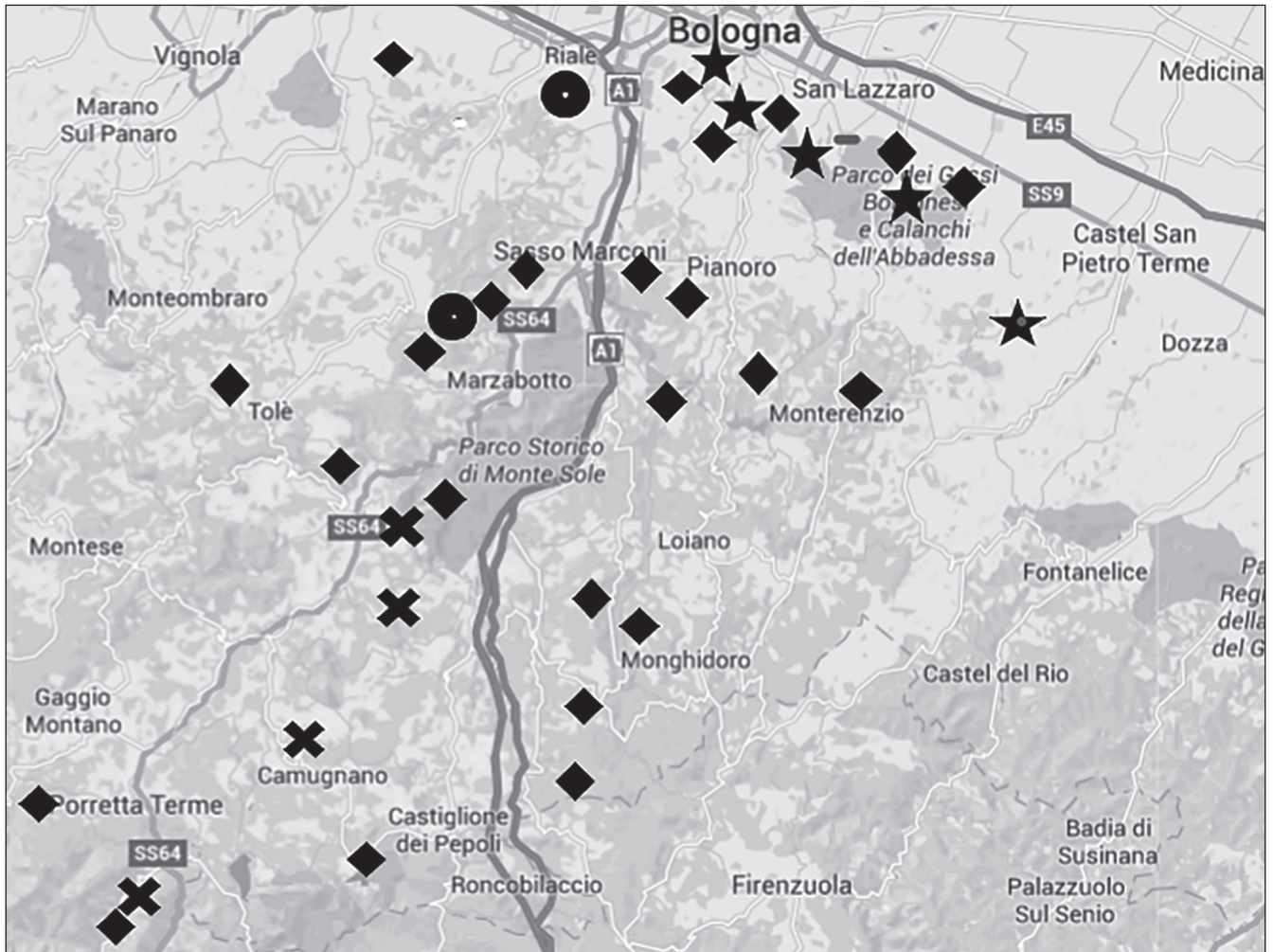


Figure 2. Distribution map of species and morphs of *Pulmonaria* in the province of Bologna: ● *P. officinalis* subsp. *officinalis*, ◆ *P. apennina* green morph, ★ *P. apennina* variegated morph, ✕ and *P. hirta*.

MATERIALS AND METHODS

Locations: Populations of *Pulmonaria* were sampled in the municipalities of Bologna (Colle d. Guardia 44.4791194,11.2958754; Roncizio 44.4680868,11.3348583; Villa Ghigi 44.4767947,11.3244979), Camugnano (Rigna 44.1706205,11.0838834), Casalecchio (Parco Talon 44.469414,11.2859311; Tizzano 44.4676736,11.252442), Castel San Pietro (Monte Calderaro 44.3607437,11.485439), Castiglione de' Pepoli (Brasimone 44.119942,11.1158439), Granaglione (Tre Croci 44.1136138,10.9259836; Molino del Pallone 44.0997872,10.9603472), Grizzana Morandi (Montovolo 44.2171075,11.0846472; Poggio di Carviano 44.2877256,11.1119003), Lizzano in Belvedere (Vidiciatico 44.1699822,10.8692671), Marzabotto (Medelana 44.3774143,11.1704765; Mt. Sole 44.3155265,11.2067627), Monterezeno (road to Monter. Vecchio 44.3231773,11.4209194), Val Samoggia (Oliveto 44.4730288,11.1209472), Monzuno (M.te Adone 44.3402166,11.2854829; Villa Cedrecca 44.278493,11.261639), Porretta (Castelluccio 44.1515204,10.9251357), San Benedetto Val di Sambro

(Madonna dei Fornelli 44.2031283,11.25641; M.te Bastione 44.1594668,11.2557502), San Lazzaro di Savena (Acquafredda-Spìpola 44.4452735,11.3777701; Farneto-Goibola 44.4375954,11.4041804), Sasso Marconi (M.te Mario 44.380054,11.2569622; Rio Sasso 44.3949208,11.2521483; Val Rio Maggiore 44.3798475,11.2031001). A *Pulmonaria vallarsae* population in beechwoods of Passo Borcola at the border between the provinces of Trento and Vicenza (municip. Recoaro, 1420-1600 m height) was also sampled for comparison.

Cultivation: Three to five specimens for each population were collected in years 2013, 2014, and 2015 mostly in September and grown in pots in the Orto Botanico of the University of Bologna until used.

Morphological analysis: *Pulmonaria* species show variation in leaf morphology such as overall shape, spot colour and outline, and hair type which are often difficult to quantify and are subject to seasonal changes (Kerner 1878; Bolliger 1982; Astuti *et al.* 2014). We have defined the major features of the basal leaves using 4 easily recognizable traits for leaf shape and spot

Spot type				
Apparently no spots	Very small	Roundish, regularly scattered	Shape and distribution irregular (mortar-like)	Spots widely confluent
ns	sm	ro	mo	cf
Spot colour				
Ground green	Inconspicuous (but visible)	Gaudy green	Light green to yellowish	Bold white
iv	ic	gr	ye	bw
Leaf base				
Cordate (indented) with peduncle	Truncate with distinct peduncle	Arcuate (crescent-shaped) with peduncle	Shortly decurrent on peduncle	Tapering into winged peduncle
cd	tr	ar	dr	wg
Leaf shape				
Roundish (plump)	Heart-shaped	Oval to spear-shaped	Elongated*	Very long & thin
pl	he	sp	el	th

Table 1. Major traits (character-states) of *Pulmonaria* basal leaves used as combined population markers. Abbreviations in bold. Elongated*: about 2× longer than wide at max leaf width.

type, with 5 states each (Astuti *et al.* 2014). The character-state frequency in a population is calculated as $\frac{N_s}{N_p} * 100$, where N_s is the number of individuals showing the state and N_p is the total number of individuals investigated for that population (see Table 2). Observations were made on three young basal rosette leaves of each individual. The abbreviations for the character states are reported in Table 1. Hair types were examined in fresh or desiccated material using a binocular microscope and classified according to the hair typology and terminology proposed by Kerner (1878) (Table 3).

Karyological analysis: Metaphase chromosome plates were obtained by squashing root tips cells according to the following protocol: pretreatment in 0.4% colchicine solution for 3 hr; Carnoy fixing for one hr; hydrolysis in HCl 1N for 9 min at 60°C; staining with leucobasic fucine for at least 2 hr.

RESULTS

Pulmonaria officinalis subsp. *officinalis*.

A few, isolated populations of typical *P. officinalis* are found in very fresh broadleaf woods and chilly gorges at 300-1000 m of elevation (Fig. 1A). *Pulmonaria officinalis* of Bologna province is easily identified by plants with a diagnostic leaf morphology and hair types, and vivid green spots (never white) frequently confluent (Table 2A), generally similar to their Alpine relatives. The distribution of this and other *Pulmonaria* species and morphs as known to us is illustrated in the map of Fig. 2.

Characterization of *P. apennina*.

In early stages of the present study, attention was mainly given to *Pulmonaria* populations consisting of faintly spotted or unspotted plants (“dark”) with oval or triangular basal leaves (Fig.

1C). Such plants are fairly common in faraway mountain localities, but also in fresh woods and slopes near Bologna. A statistical profile of the morphological traits of a “dark” lungwort population, reported in Table 2C, supports the assignment to *P. apennina* and remarks an overall resemblance to *P. vallarsae* (Fig. 1B and Table 2B). The diagnostic soft hair patterns with abundant short hairs (*puberes*) and some bristles (*setae*) fit the protologue of *P. apennina* (Table 3). We refer to these widespread *P. apennina* plants (Fig. 2) as the green morph. Several populations of *Pulmonaria* also referable to *P. apennina*, but showing partially divergent morphological traits, are found in cool, shady sites often not distant from human settlements (Fig. 2). An interesting example is the lungwort belt on hills overlooking Bologna, in part on gypsum rocks (Parco dei Gessi Bolognesi). These plants are clearly related to *P. apennina* on the basis of hair types and karyology (see below), but they often display large, irregular leaf spots or even spot confluence particularly in autumn (Fig. 1D, Table 2D), which can result in a certain resemblance to *P. hirta*. We refer to this type as the variegated morph of *P. apennina*.

Characterization of *P. hirta*.

Typical features of this plant group are elongated summer leaves showing *setae* and few *puberes* (contrary to *P. apennina*), and a gaudy leaf speckling ranging from pale green to bold white, which often changes to a confluent, “glazed” leaf surface in autumn (Fig. 1E). However, some specimens with limited maculation also occur. A statistical analysis of *P. hirta* morphology reveals significant differences with respect to the variegated morph of *P. apennina*, in particular longer and slenderer leaves (lanceolate) in all seasons (Table 2E, Table 3). Plants showing these characters are infrequent around Bologna, where the variegated morph of *P. apennina* is dominant, but they are widely distributed on hills on the right hand of river Reno

in chestnut groves and along roads and ditches at 400-700 m elevation, mainly in the municipalities of Grizzana and Camugnano (Fig. 2).

Karyology.

Several *Pulmonaria* populations mentioned in this paper were sampled and metaphase plates of one or more plants were inspected. All plants labelled as the green morph of *P. apennina* had a chromosome number $2n = 22$ (Fig. 3A). A limited sample of the variegated morph also showed $2n = 22$ (Fig. 3B). All plants identified as *P. hirta* on the basis of general morphology and hair types had a chromosome number $2n = 28$ (Fig. 3C). No plants with intermediate chromosome numbers were found. In our area of investigation, therefore, leaf and hair morphology appear to be related to chromosome set.

Table 2A - *Pulmonaria officinalis*
Val Rio Maggiore (Sasso Marconi), 94 plants - Oct. 12, 2011

Spot color		Spot type		Leaf base		Leaf shape	
ns 2	2%	iv 1	1%	cd 18	19%	pl 4	4%-
ic 24	26%	sm 1	1%	tr 46	49%	he 52	55%
gr 67	72%	ro 28	30%	ar 28	30%	sp 32	34%
ye 4	4%	mo 56	60%	dr 1	1%	el 6	6%
bw -	-	cf 8	8%	wg -	-	th -	-
TYPE: gr 72% - mo 60% - tr 49% - he 55%							

Table 2B - *Pulmonaria vallisae*
Passo Borcola, 126 plants - Aug. 11, 2014

Spot color		Spot type		Leaf base		Leaf shape	
ns 35	28%	iv 28	22%	cd 11	9%	pl 7	6%-
ic 66	52%	sm 41	33%	tr 78	63%	he 84	67%
gr 25	20%	ro 54	43%	ar 33	27%	sp 32	25%
ye -	-	mo 3	2%	dr 1	1%	el 3	2%
bw -	-	cf -	-	wg -	-	th -	-
TYPE: ic 52% - ro 43% - tr 63% - he 67%							

Table 2D - *Pulmonaria apennina* variegated morph
Valle dell'Acquafredda, 442 plants - July 12, 2013

Spot color		Spot type		Leaf base		Leaf shape	
ns 5	1%	iv 4	1%	cd 16	4%	pl 5	1%
ic 16	4%	sm 6	1%	tr 146	33%	he 182	41%
gr 101	23%	ro 37	8%	ar 238	55%	sp 140	32%
ye 161	36%	mo 314	71%	dr 37	8%	el 109	25%
bw 159	36%	cf 81	18%	wg -	-	th 6	1%
TYPE: ye/bw 72% - mo 71% - ar 55% - he 41%							

DISCUSSION

According to our findings, lungworts in the Bologna province can be assigned to three groups. A first group is represented by scattered populations of *P. officinalis* subsp. *officinalis* occurring in cool, shady mountain woods and brook gorges, probably representing a relict of colder climates of the past. An instructive example is given by a healthy *P. officinalis* population in the inner, sheltered part of Val Rio Maggiore near Sasso Marconi, where *P. apennina* (green morph) occupies the lower, open part of the small valley. These plants have a distinctive morphology typical of *P. officinalis* subsp. *officinalis*, in contrast to the southern Alps where, in addition to the diffuse nominal subspecies, high-mountain *P. officinalis* subsp. *marzolae* and other morphs also occur (Astuti *et al.* 2014). *Pulmonaria officinalis* is unequivocally characterized by $2n = 16$ chromosomes (Astuti *et al.* 2014, and literature therein).

A second group of lungworts consists of populations of *P. hirta*, mainly distributed along the valley of river Reno, where it seems to largely replace *P. apennina*. The map of Fig. 2 shows the different, major areas of occurrence of this and other *Pulmonaria* species and morphs in Bologna province as known to us, although other lungwort populations undoubtedly exist. The Reno valley was used for thousands of years as a major route to Tuscany and central Italy and it cannot be excluded that the present distribution of *P. hirta* in the area was influenced by human activities; in addition the Montovolo-Vigese

Table 2C - *Pulmonaria apennina* green morph
Poggio dell'Oca, 42 plants - June 2, 2011

Spot color		Spot type		Leaf base		Leaf shape	
ns 22	52%	iv 22	52%	cd 5	12%	pl 1	2%-
ic 10	24%	sm 13	31%	tr 24	57%	he 21	50%
gr 5	12%	ro 7	17%	ar 12	29%	sp 14	33%
ye 5	12%	mo -	-	dr 1	2%	el 6	14%
bw -	-	cf -	-	wg -	-	th -	-
TYPE: ns 52% - iv 52% - tr 57% - he 50%							

Table 2E - *Pulmonaria hirta*
Poggio di Carviano, 51 plants - July 12, 2008

Spot color		Spot type		Leaf base		Leaf shape	
ns 1	2%	iv 1	2%	cd -	-	pl -	-
ic 4	8%	sm 4	8%	tr -	-	he -	-
gr 32	63%	ro 13	25%	ar 24	47%	sp 19	37%
ye 11	22%	mo 28	55%	dr 25	49%	el 8	16%
bw 3	6%-	cf 5	10%	wg 2	4%-	th 24	47%
TYPE: gr 63% - mo 55% - dr 49% - th 47%							

Table 2. A comparison of character-states of basal leaves of five lungwort populations belonging to: A) *Pulmonaria officinalis* subsp. *officinalis*; B) *P. vallisae*; C) green morph of *P. apennina*; D) variegated morph of *P. apennina*; E) *P. hirta*. Except for *P. vallisae* of Passo Borcola (Vicenza), all populations examined grow within the Bologna province as indicated.

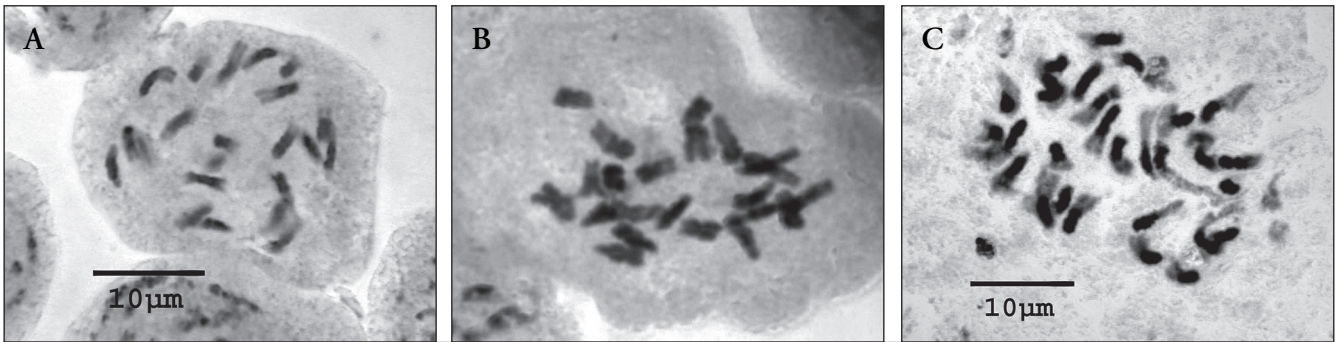


Figure 3. Examples of mitotic metaphase plates: A) of *P. apennina* green morph from M.te Adone (Monzuno), B) of *P. apennina* variegated morph from Valle dell'Acquafredda (S. Lazzaro di Savena), C) plate of *P. hirta* from Poggio di Carviano (Grizzana).

range, dominating the high course of river Reno, has been an important religious area since Etruscan times. Most of these plants show elongated summer leaves with conspicuous (“mortar-like”) to confluent spots, and a strigose indumentum formed by bristles (*setae*) and a few short hairs (*puberes*) (Puppi & Cristofolini 1996). All *P. hirta* plants sampled had $2n = 28$ chromosomes. This species has a mainly western distribution in Italy in continuity with southern France, and a southern limit at the latitude of Rome as stated by Vosa & Pistolesi (2004) for their $2n = 28$ cytotype. Puppi & Cristofolini (1996) reported on the presence of *P. hirta* (under the name *P. picta* Rouy) in high woods up to the tree line (e.g., at Lago Baccio, Modena, with $2n = 22$ chromosomes). Again, this point needs confirmation because of possible confusion with the variegated morph of *P. apennina*.

The third and more diverse group is represented by the widespread and dominant *P. apennina*, which is found in many localities of the area considered (Fig. 2). Most mountain populations of *P. apennina* are faintly spotted or unspotted (green morph) and resemble the south-east-Alpine *P. vallisarsae* in many details, notably the chromosome number $2n = 22$. Plants corresponding to the description of *P. apennina* are common in secluded localities e.g., along northern slopes of the Contrafforte Pliocenico Reserve, but also in fresh hillsides around Bologna as already known to Bertoloni (1855) and Kerner (1878, p. 19: “Jola, Ronc(o)rio, alle Grotte”). At least two-thirds of the *P. apennina* locations known to us in the Bologna province (and in adjoining Romagna mountains to the south-east, down to Marche: D. Ubaldi, pers. comm.) pertain

to the green morph, and the original circumscription of this species as found in central and southern Apennines also refers to weakly spotted or unspotted plants (Puppi & Cristofolini 1996). The close relationship between *P. apennina* and *P. vallisarsae* and the wide highland distribution of the green morph suggest that the latter may be the original, dominant form of Apennines. It can be supposed that there was once a continuity of this species from southern Alps to Apennines across the Po plain in postglacial times, although only a few traces of it, if any, remain by now in the Pianura Padana. On the other hand, the variegated morph may be the product of occasional gene flow between *P. hirta* and the green morph of *P. apennina*. The two Apennine taxa *P. apennina* and *P. hirta* are reported to be interfertile (Puppi & Cristofolini 1996), as also witnessed by the occurrence of plants with chromosome numbers intermediate between $2n = 22$ and $2n = 28$ (Puppi & Cristofolini 1996; Vosa & Pistolesi 2004; Astuti *et al.* 2019). In addition, recent phylogenetic and DNA fingerprint analyses (Liu *et al.*, unpublished) have confirmed that some interspecific gene flow does occur between *P. apennina* (including that from the *locus classicus* in the vicinity of Bologna) and *P. hirta*.

While the green morph of *P. apennina* dominates the mountains, other populations of *P. apennina* are from moderately to heavily spotted and can even show confluent speckling (variegated morph), to the point that some individuals may resemble *P. hirta* at a first glance (Fig. 1D-E). This deceiving resemblance may be at the origin of reports of $2n = 22$ chromosome numbers for *P. hirta* (as *P. saccharata* or *P. picta*: Merxmüller & Grau 1969; Sauer 1975; Puppi & Cristofolini 1996), con-

Summer basal leaves, upper face	<i>P. apennina</i> green morph	<i>P. apennina</i> variegated morph	<i>P. hirta</i>
Bristles (“Setae”)	sparse	sparse	sparse
Short hairs (“Puberes”)	numerous	numerous	scanty
Small pins (“Aculeoli”)	absent	absent	absent
Glandular hairs (“Colleteres”)	present	present	present
Microglands (sessile glands)	sparse	sparse	sparse

Table 3. *Pulmonaria* trichomes. Comparing the hair types of the green and variegated morphs of *P. apennina* with those of *P. hirta*. Kerner’s (1878) hair types in brackets.

tributing to a rather confusing situation until today. Kerner (1878) himself considered plants from Bologna as *P. saccharata*, although no *P. hirta* is present in the outskirts of Bologna, where the variegated morph of *P. apennina* is still abundant in some shady, cool places (karst valleys in gypsum of the Acquafredda-Spipola area, in particular) and along hill roads. Despite their variable macromorphological characters, all *P. apennina* populations can be recognized by a set of specific traits: triangular or oval leaf form, soft indumentum with plentiful short hairs, and a diploid chromosome number $2n = 22$. The variable appearance of variegated plants undoubtedly contributed to the fame of morphological instability surrounding the genus *Pulmonaria*, at least in Italy (Lacaita 1927; Pignatti 1982; Vosa & Pistolesi 2004).

Our findings, therefore, agree with Vosa & Pistolesi (2004) that all properly examined *Pulmonaria* of northern and central Apennines fall within three sharply distinct karyotype classes, with $2n = 16$ (*P. officinalis*), $2n = 22$ and $2n = 28$ (*P. hirta* complex). However, we observe that the $2n = 22$ karyotype is mostly correlated with microcharacters typical of *P. apennina*, both in its green and variegated morphs, whereas $2n = 28$ is related to microcharacters typical of *P. hirta*. Cecchi & Selvi (2015) also refer to *P. hirta* as having $2n = 28$ chromosomes. In general, we find that the distinctive characters of *P. hirta* with respect to the variegated morph of *P. apennina* mainly rely on the relative abundance of short hairs (more *puberes* in the latter) (Table 3), in accordance with Puppi & Cristofolini (1996), and on the differences in leaf shape (Table 2D-E).

Current evidence supports a simple scheme with *P. apennina* and the closely related *P. vallarsae* having $2n = 22$, and *P. hirta* having $2n = 28$ chromosomes. This could help clarifying the systematic position of this species complex. However, the variability of variegation patterns in *P. apennina*, and in *P. hirta* to some extent, is not easy to understand. That it originated by a mechanism of hybridization was early proposed by Lacaita (1927). Vosa & Pistolesi (2004) reported that only a few hybrids were found in their extensive investigations, but Astuti *et al.* (2019) found that all individuals sampled from a population referable to *P. apennina* in Abruzzo had invariably $2n = 26$ (see also Capineri 1986). It may be hypothesized that *Pulmonaria* hybrids have a lower fitness, which would account for their rarity in nature as already pointed out by Meeus *et al.* (2016). The relationships of the two morphs of *P. apennina*, green and variegated one, therefore deserve to be investigated in terms of possible genetic introgression between the interfertile (Puppi & Cristofolini 1996) and partially sympatric *P. apennina* and *P. hirta*, although a more complex scenario of the origins of *Pulmonaria* populations in northern Apennines cannot be ruled out at this stage of knowledge.

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