

Urcaela

Tempus Sempervivi

Sempervivum soculense sp. nov.

a long time unknown houseleek from
the south-western Garda Prealps

by Davide Donati and Gérard Dumont



Summary: Highlighting a new *Sempervivum* species, living as a post-glacial relict in the Garda Prealps: *Sempervivum soculense* D.Donati & G.Dumont sp. nov.; confirming its diploidy by new chromosome counts, and general discussion regarding its integration in the geobotany and phylogeny of the genus *Sempervivum*, and particularly its possible relationship with the tetraploid *Sempervivum tectorum*.

Keywords: *Crassulaceae*, *Sempervivum*, Garda Prealps, Monte Pizzocolo, allopolyploidization, phylogeny, relict.

THE flora of the Garda Prealps is characterized by a richness of endemic plants, as well as plants that could be defined as post-glacial relicts, that is, plants that have found refuge, during the Quaternary glaciations, in the southernmost part of the Central Alps. Amongst them, we can find a houseleek which although already known, has been neglected, or whose nature has been misinterpreted up to now. In this article we want to give this plant all the importance it deserves, since it can challenge some phylogenetic hypotheses regarding some species belonging to the genus *Sempervivum* (*Crassulaceae*), and thus represents a very important element in the understanding of this genus.

The Garda Prealps

In Northern Italy, the Garda Prealps are a long mountain range, oriented north-south and encompassing Lake Garda (*Lago di Garda*), from which they get their name. To the West they are delimited by the Giudicarie valleys, to the North by the river Sarca, to the East by the river Adige and to the South by the hills of Brescia and Verona. They can be considered as the southern continuation of the Brenta Dolomite Alps and are separated from the Rhaetian Alps by the Mount Sella di Bondone.

The Garda Prealps can be divided in three massifs (“groups” *sensu* Marazzi 2005):

- The Giudicarie Prealps.
- The south-western Garda Prealps.
- The eastern Garda Prealps.

From a geological point of view, the Garda Prealps consist entirely of sedimentary rocks: Jurassic grey limestone (Masetti et al., 1998), dolomite limestone, and dolostone (Castellini et al., 2006).

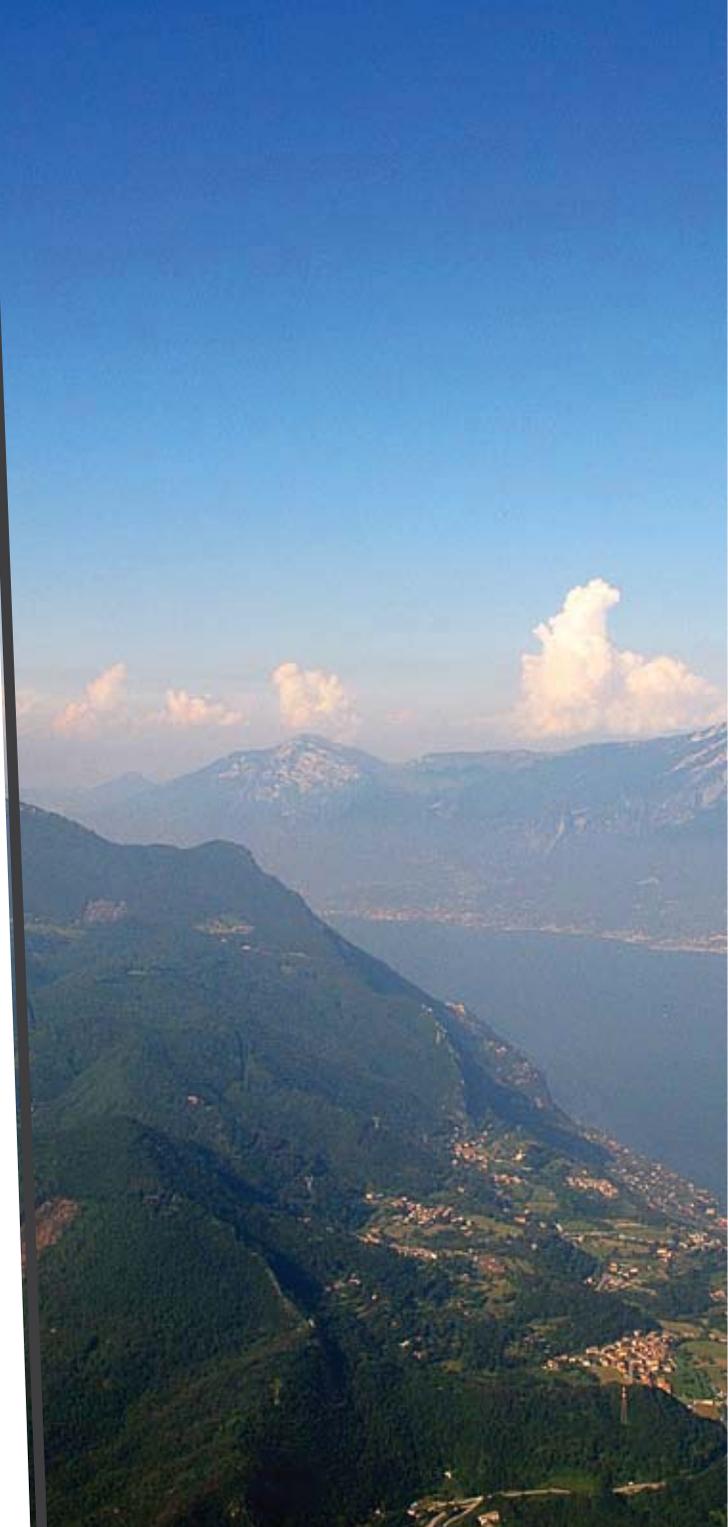
A refuge area for the alpine flora of the Tertiary period

The distinct southern position of the Garda Prealps compared to the rest of the Central Alps (they are basically a promontory wedged in the Padan Plain) has spared some parts of this mountain range from the Quaternary glaciations, and even in ice-covered areas, some cliffs remained ice-free, particularly those facing the lakes. Many plants coming from more northern and higher areas found refuge here during the glaciations. Some of them were then able to completely (or partially) re-colonize their original habitat when the climate became milder, some leaving behind some populations in their refuge areas⁽¹⁾, while others remained confined here⁽²⁾, following the dynamics of each species.

We should point out that the richness and variety of biotopes in this area, and their faunistic and floristic richness, have led to the establishment of various protected areas.

1 Amongst the plants presenting relict populations in the Garda Prealps we have, for example, *Saxifraga tombeanensis* Boiss. ex Engl., *Saxifraga vandellii* Sternb., *Daphne petraea* Leybold, etc.

2 Some species have survived exclusively in these areas next to Lake Garda: i.e *Saxifraga arachnoidea* Sternb., a relict of the Tertiary period that grows only below rocky outcrops, on a dry substrate, but with high hygrometry (see Reisigl & Keller, 1990).



Monte Pizzocolo

Monte Pizzocolo is one of the peaks of the Garda Prealps. It's a very imposing mountain that dominates the south-western part of Lake Garda, inland to the small town of Toscolano Maderno situated on the lake bank.

Monte Pizzocolo can be defined as a massif of limestone rock, which is particularly compact and sometimes dolomitized and is ivory or beige in colour, arranged in metric layers or even with indistinct stratification, on the eastern flank and near its top. This is the so-called "*Corna calcarea*" (Zecchini, 2009), a lower-Jurassic limestone, which often shows a pronounced morphology, as the local dialect term "*corna*", horn, implies. Even if the nearby Monte Spino (1486 m) is mainly formed by "*Corna*" limestone, the group Spino-Pizzocolo is almost entirely surrounded to the west and to the north by outcrops of Triassic dolostone (Camerini, 2004 ; Carlini et al., 2010).

The south-western flank and even more so, the lower northern flank of Monte Pizzocolo are densely covered by forests, with rocky meadows (more or less inclined) beginning at 1450 m and reaching right up to the top at 1581 m.

On the contrary, the eastern flank consists of almost vertical cliffs descending directly to the lake. These cliffs are very exposed and mainly consist of naked limestone, well eroded by weather, whilst the vegetation is sparse, particularly in the most exposed areas. On its north-eastern and northern portions, the slopes are very steep, almost vertical in some places and particularly on the northern flank, but this favours higher water availability, so the vegetation is a little denser and it is possible to see plant communities with *Potentilla caulescens* L.



Monte Pizzocolo
[south slope]

The position and configuration of the eastern flank of Monte Pizzocolo allow us to hypothesize that these cliffs weren't ice-covered during the Quaternary glaciations, or at least part of them weren't⁽³⁾; the part of the cliffs above the glacier which shaped the valley now occupied by the lake.

Monte Pizzocolo is currently located inside a Natural Preserve, the *Parco dell'Alto Garda Bresciano*.

³ According Corrà et al. (2000), the vertical cliffs to the north and north-east were formed by the erosion of huge glaciers which, according to their theories, could even have split Monte Pizzocolo from Monte Castello di Gaino, which hypothetically formed a single mountain range before the glaciations.

Karyology and variations within the genus *Sempervivum*

The genus *Sempervivum* is characterized by an evident general morphologic homogeneity, coupled with a great variability within each species, which can be considered next to each other with relatively undefined boundaries. This genus is probably still undergoing a very active speciation phase and quite often two specimens of the same species look more different than two specimens of two different species. As a consequence, the identification of *Sempervivum* is often difficult, and the current nomenclature poorly and inadequately describes this genus.

Faced with such a situation, one would expect the *Sempervivum* genus to be genetically very homogeneous, and that karyological studies would be of no help in understanding it. Completely wrong. On the contrary, the genus *Sempervivum* is a very variable and complex group from a karyological point of view: the somatic chromosome counts are very variable, starting from $2n = 16$ to $2n = 108$, with base numbers starting from $x = 16$ to $x = 21$ in a continuous succession⁽⁴⁾.

This karyological diversity shows that the genus *Sempervivum* is much less homogeneous than it would seem, and that many taxa are much more separated than their morphology would indicate. For this reason, an exclusively morphological approach to their taxonomy could prove unsatisfactory. Any attempts to solve the above-mentioned complexity of the genus by moving everything into a few large taxonomic frames is as unsatisfactory as exploding the genus into a myriad of mainly useless micro-taxa, since they would represent a simple local ecotype at most, but more often, one of the many levels inside the natural variation range of a single taxon (if not simple phenotypic variations). Unfortunately finding and maintaining an intermediate position is difficult.

The relatively high chromosome numbers and their marked diversity leads one to think that the speciation of the genus *Sempervivum* occurred largely by allopolyploidization⁽⁵⁾ rather than by cladogenesis or anagenesis⁽⁶⁾. For this reason, the study of chromosome numbers is particularly important for this genus, since it allows us sometimes to track or to guess the likely phylogeny of some species or groups of species.



Mitosis of somatic cell
(metaphase)
Sempervivum tectorum
[Monte Maddalena]

4 The base number of *Crassulaceae* is considered to be $x = 9$.

5 Allopolyploidization is an additive crossing: two not reduced gametes join to form a polyploid individual that is immediately stable and fertile and, if able to sustain itself, will make a new species. $AA + BB \rightarrow AABB$, whereas the result of a simple crossing is $AA \times BB \rightarrow AB$.

6 Speciation is called *cladogenesis* when the original branch splits into two or more branches; it's called *anagenesis* when a species replaces the one from which it derives. In both cases, the chromosome numbers are very often identical in the whole group, hence karyological studies of this group is of little interest.

The *Sempervivum tectorum* case

One of the most appealing hypotheses for this genus about speciation by allopolyploidization involves *Sempervivum tectorum*⁽⁷⁾, a widely distributed species in western and central Europe.

Sempervivum tectorum is a tetraploid species with a chromosome number of $2n^{(8)} = 72$, confirmed by numerous different counts. This high number has obviously led to the hypothesis that it could be the result of allopolyploidization between the ancestors of the current *Sempervivum marmoratum* Griseb. ($2n = 34$), a Balkan and Carpathian diploid species, and the current *Sempervivum calcareum* Jord. ($2n = 38$), a diploid species from the south-western Alps. In fact, $34 + 38 = 72$.

Many factors lead one to consider *Sempervivum tectorum* as a rather recent species within the *Sempervivum* genus, certainly more recent than its supposed parents, *S. calcareum* and *S. marmoratum*:

- unlike them, *S. tectorum* hasn't any evident relict populations that would suggest a Tertiary pre-glacial origin, contrarily to other houseleeks with which it often cohabits (*S. arachnoideum*, *S. wulfenii*, *S. calcareum*, etc.). The Monte Pizzocolo population is amongst the few (or the only one?) that could be considered a relict population, but the inclusion of the local houseleek in *S. tectorum* can be questioned, as we'll explain later.
- the distribution range of *S. tectorum* seems to be still expanding westwards, not having reached its potential limit yet; something which is particularly evident in the French Massif Central. This can be deduced by the uneven distribution of *S. tectorum* compared to other, often sympatric species, a difference that can be explained by the later arrival of *S. tectorum* only.

7 In this article, we consider *Sempervivum tectorum* L. in its widest sense, that is putting together all the numerous taxa that have been created by splitting this complex and very variable species. The only definition of these taxa is often their geographical location, and they could be sometimes considered at infraspecific level at most, being totally integrated in *S. tectorum*. In the area treated by this article, we can mention as an example *Sempervivum acuminatum* Schott non Decne, *Sempervivum schottii* Baker non C.B.Lehm. & Schnittsp., *Sempervivum alpinum* Griseb. & Schenk.

8 The cited articles report on the gametic chromosome count "n" or the somatic chromosome count "2n". We have converted the gametic counts into somatic counts, since in this paper we are using the somatic counts "2n" only. We understand that writing in this way is a relative linguistic abuse for polyploid genomes, but it makes the text easier to read and more understandable when we make comparisons.



Sempervivum tectorum
[Passo di Croce Domini]

A recent alpine origin (Quaternary, post-glacial) of *Sempervivum tectorum* is thus, if not certain, very likely at least.

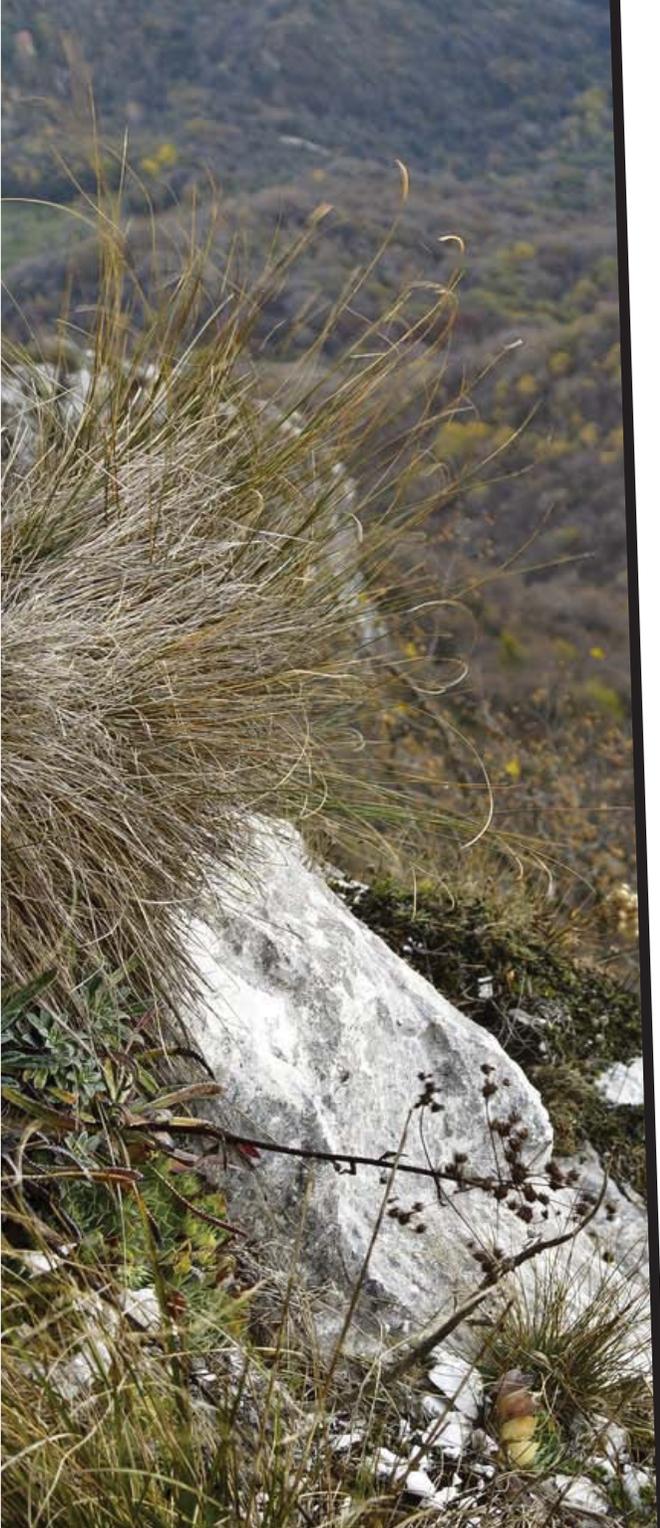
About the hypothesis of its appearance due to an allopolyploidization mechanism, many factors make it credible:

- The apparent youth of this species, compared to the two diploid species from which it supposedly derives.
- Its high chromosome number, which exactly matches the sum (additive crossing) of these two diploid species.
- Its morphology, that is somehow intermediate between the two putative parents.
- Its strength and ecological plasticity, markedly higher than that of its putative parents, as it happens with the majority of the allotetraploid species, whatever the plant group concerned.

The distribution ranges of the putative parents are presently very distant, but it's possible that they were once very much closer or even overlapped in some places, and were subsequently reduced and separated, disappearing from the central Alps and replaced perhaps by the tetraploid *S. tectorum* in the case of *S. marmoreum* (their current distribution ranges are in contact, but don't overlap). It must also be pointed out that the current distribution range of *S. tectorum* actually links the current distribution ranges of *S. marmoreum* and *S. calcareum*.

This hypothesis is certainly appealing, but it isn't the only one, or at least it could be improved.





Analysis of available data

Monte Pizzocolo hosts a population of a single houseleek species. Its presence has been known for a long time and it's been considered until now, as belonging to the common *Sempervivum tectorum*, omnipresent in the Alps. Some previous studies, which were essentially karyological, outlined its peculiarity, as reported below, but in our opinion, failed to draw the necessary conclusions.

Available data on the houseleek of Monte Pizzocolo

The status of this plant became less clear in 1961, when Zésiger, in a general study on the chromosome numbers of *Sempervivum*, discovered a $2n = 36$ number in a *Sempervivum* "tectorum" originating from the "pied sud des Alpes" (southern foot of the Alps), without giving further details about the locality. As all his other chromosome counts of *Sempervivum tectorum*, from various localities (about fifteen), gave a result of $2n = 72$, the author apparently neglected this seemingly diploid plant, nor made any hypothesis about it. Zésiger probably considered this odd count was due to an abnormal specimen, with little significance.

After that, Favarger in 1973 again reports a count of $2n = 40$ on the same clone of *Sempervivum* "tectorum" (M 552) previously counted by Zésiger as $2n = 36$, a result that was confirmed as $2n = 40$ by the same Favarger on two more plants (61/926 et 61/927) "récoltées plus tard au même endroit" (collected later at the same locality), again without giving any locality details. This time it was suggested that *S. tectorum* could be sometimes be diploid⁹, adding that a relationship with this plant and *Sempervivum wulfenii* Hoppe ex Mertens & W.D.J.Koch could also be possible.

In 1998 Zonneveld mentions the previous counts and reports a new count of $2n = 38$ he made for this population of *Sempervivum* "tectorum", but doesn't say whether it was made on a newly collected clone, or on a previously used clone (probably the latter, since no locality data is given). However, very importantly, he gives its locality at last (undoubtedly after having contacted the previous authors): Monte Pizzocolo. Following the previous authors, Zonneveld doesn't consider this *Sempervivum tectorum* anything special though, except for its diploidy, hence the possibility that the tetraploid populations of *Sempervivum tectorum* (the vast majority) wouldn't be allotetraploid, but autotetraploid, which would challenge the phylogenetic hypothesis of its appearance by allopolyploidization *S. calcareum* + *marmoreum*.

⁹ For many plants, the ploidy level is multiple and variable and it would be a mistake to assign any taxonomic value to it. I.e. *Sempervivum arachnoideum* can be both diploid ($2n = 16$) and tetraploid ($2n = 32$), without showing any differences, so much that even its infraspecific taxa aren't linked by ploidy levels at all. For what concerns the geographical subdivision of the diploid and polyploid lineages, this is very intricate in most of the distribution range. cf. Welter 1977.

About the range of the results

The small difference between the various chromosome numbers (36, 38, 40) should be of no surprise, since counting is technically difficult for the genus *Sempervivum*: it's difficult to obtain good metaphase plates, and their reading isn't very accurate, since the chromosomes of houseleeks are extremely small, punctiform, and so numerous that they often overlap, partially hiding each other. Counting the chromosomes of a houseleek could be compared to counting the marbles which fill a dirty jar from a distance. For this reason, all the chromosome counts published so far should be interpreted as approximate numbers with a variable error margin, directly proportional to the number of chromosomes. Only by repeating the counts over and over, we can arrive at sufficient precision for an individual or a taxon.

What can we gather from these karyological data?

1. – *Sempervivum tectorum* is really a tetraploid

Due to its vast distribution range, *Sempervivum tectorum* is the houseleek on which the highest number of chromosome counts have been made; practically on plants coming from all the European mountains where it lives. All the counts made on well documented plants have confirmed its polyploidy, except those relating to the population of Monte Pizzocolo.

2. – The *Sempervivum* population of Monte Pizzocolo definitely seems to be diploid

Subsequent counts, made by different biologists, on different clones, seem to prove that there's indeed a diploid population on Monte Pizzocolo, and it isn't simply an abnormal individual (which could always happen with a single count or even several counts on the same clone in culture).

3. – This population represents the only known case of diploid *Sempervivum tectorum*

Up to the present, no other chromosome count has ever found a diploid *Sempervivum* "tectorum", except in this isolated population of Monte Pizzocolo.

4. – There is some doubt about the real nature of this population

In all the cited studies there is no reported field data nor any information about the variability, so that even if it were highly unlikely, we can't exclude beforehand that this natural population could be a single abnormal sterile clone reproducing vegetatively at this locality.

5. – Further data are needed

The case of the diploid *Sempervivum* "tectorum" of Monte Pizzocolo is very intriguing, and the scarcity of field collected data led us to carry out further studies on this plant, *in situ*, in cultivation and in the laboratory.



Below, the young rosettes of the four individuals from which we made the chromosome counts.



Materials and methods

Studying the *Sempervivum* populations of Monte Pizzocolo *in situ* has been of fundamental importance, in order to verify its ecology, variability, the actual distribution, as well as the plants that share its habitat, including other houseleeks locally or in the neighbourhood.

We have also collected a few samples of this plant *in situ*⁽¹⁰⁾ (small lateral rosettes) and cultivated them to check their biological cycle and refine its morphological study. The radical tips needed for the chromosome study have been removed from these cultivated clones.

In the lab, the tips of some young and active roots have been cut 3 mm long, and then secured and coloured according to a protocol derived from that of Zonneveld (in litt.) :

1. immersion in a solution of hydroxyquinoline 0,002M;
2. immersion in a solution of pure ethanol (3 parts) and glacial acetic acid (1 part);
3. hydrolysis in 5N HCl;
4. immersion in Schiff reagent;
5. immersion in a solution of $K_2S_2O_5$ and distilled water;
6. squeezing between microscope slide and cover slip and searching by microscope of the best meta-phase plates amongst the mitotic cells, in order to count the chromosomes.

¹⁰ Authorization according to art. 8 of Regional Law n. 10/2008 (Collection of protected spontaneous flora for scientific purposes), by Regione Lombardia - Direzione generale ambiente, energia e sviluppo sostenibile - Parchi, tutela della biodiversità e paesaggio - Valorizzazione delle aree protette e biodiversità.

New data

Field data

Monte Pizzocolo:

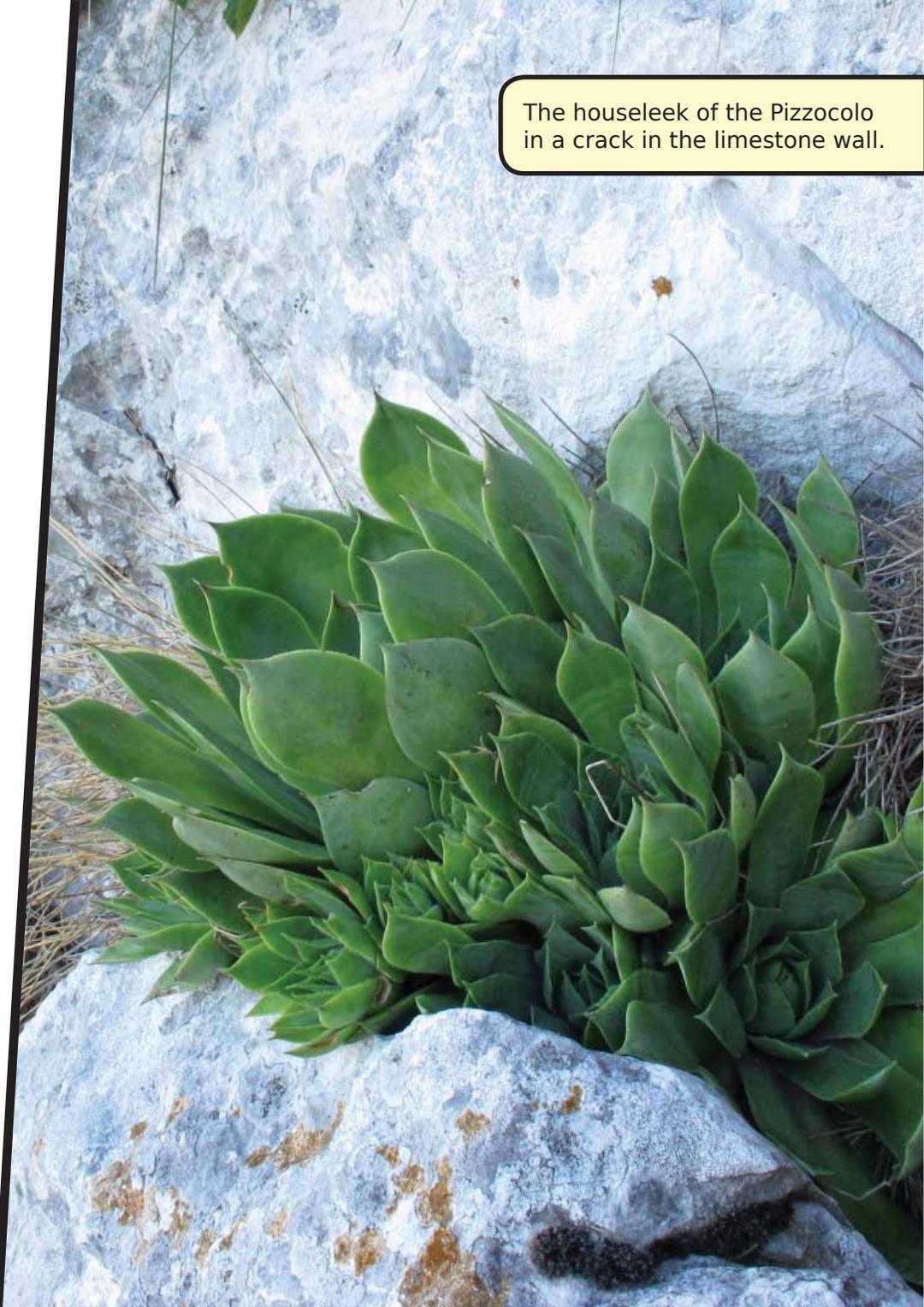
The main population of *Sempervivum* growing on Monte Pizzocolo is located on the limestone of the eastern flank, primarily on the almost vertical cliffs facing east-northeast, and extending up to the north-facing cliff, that could be defined as a vertical rock wall. A few dozen clumps are also present on the steepest points of the south-western flank.

The total population is composed of a single species, the famous “*tectorum*”. No other houseleek has been found.

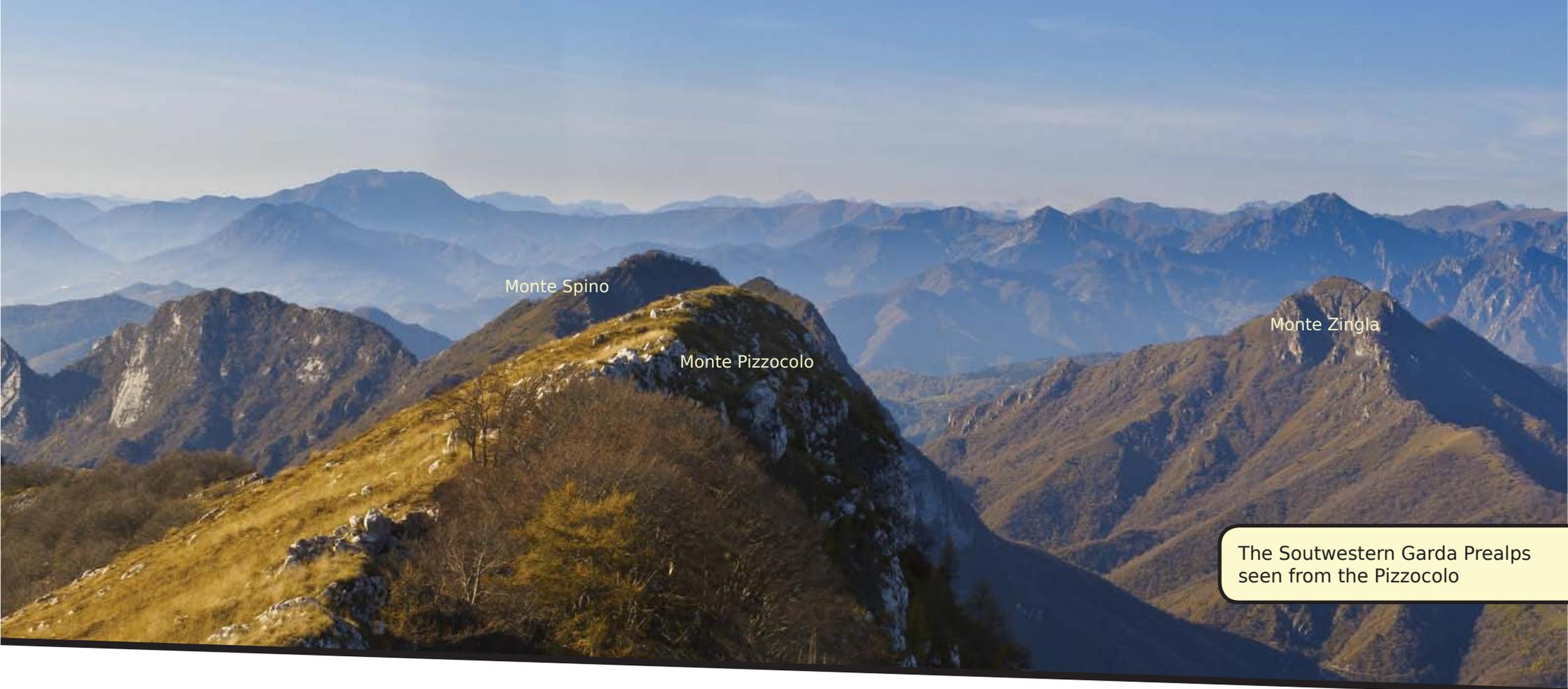
The majority of the plants are concentrated starting from about 1450 m a.s.l. up to the mountain top, although some clumps can be found starting from about 1350 m. The scarcity of plants on the rocky meadows near the top is maybe linked to the plant's ecology (preference for habitats in rupicolous situation), as the current concentration on steep or almost vertical rocky cliffs would suggest. It's also possible that the presence of a World War I fort on the top first, and the trampling by many tourists later (Monte Pizzocolo is a favourite destination for excursionists due to the fantastic panorama that can be enjoyed from the top) have reduced its presence on the summit meadows to a few sparse clumps, leaving most of the plants concentrated in less accessible areas (the almost vertical cliffs).

The plants grow directly in rock cracks or on rocky protrusions where a little humus and clay have accumulated and allowed the establishment of various types of rupicolous vegetation.

We verified that the population of Pizzocolo shows an evident morphological variability between the individuals, which can eliminate the above-mentioned hypothesis that it could be a single clone reproducing locally and vegetatively.



The houseleek of the Pizzocolo in a crack in the limestone wall.



The Southwestern Garda Prealps seen from the Pizzocolo

Surroundings of Monte Pizzocolo:

All the peaks and passes near Monte Pizzocolo (Monte Spino, Monte Zingla, Monte Carzen, Passo di Tremalzo) have been visited to check the presence of other houseleeks. This search was unsuccessful; we did not find the plant or any other houseleek.

The *Sempervivum* populations nearest to Pizzocolo can be found on Monte Maddalena, near Brescia, on one side, about 16 km distant as the crow flies, and on Monte Baldo, on the other shore of Lake Garda, on the other side, again about 16 km distant as the crow flies. These two populations consist exclusively of *Sempervivum tectorum*, and lack any morphological peculiarities. Another houseleek that can

be found nearby, eastwards from Monte Baldo is *Sempervivum globiferum* subsp. *hirtum* (L.) 't Hart & Bleij⁽¹¹⁾, but that belongs to the subgenus *Jovibarba*, a distinct group whose members don't hybridize with true *Sempervivum* (subgenus *Sempervivum*).

¹¹ *Sempervivum globiferum* subsp. *hirtum* represents a very wide taxonomic group, which includes morphologically very variable populations, very difficult to separate, unless an infinity of micro-taxa with little importance is created. The local form near of Monte Baldo has been recently differentiated as *Sempervivum globiferum* subsp. *lagarinianum* (Gallo) Stephenson, its definition mainly relying on a chorological criterion (isolated population on the southern boundary of the distribution range) rather than on ecological and morphological criteria: the debate about the taxonomical value of this taxon is still in progress and it's outside the scope of this paper, so we prefer here to keep the classical combination as above.

Description

Clump: not copious, with few daughter-rosettes, isolated rosettes flowering without having produced any daughter-rosettes aren't rare. Stolons are short, barely exceeding the diameter of the mother-rosette. As generally happens with *Sempervivum*, no stolons are produced during the year in which the rosette flowers, which occurs after a vegetative phase of several years (monocarpic rosettes).

Adult rosette: rather large, diam. 6-10 cm, exceptionally up to 20 cm; number of leaves variable depending on the individual (25-50+); symmetrically arranged leaves without any evident anisophylly¹². During the vegetative phase, the central leaves are often arranged like a cone, particularly at the end of the growing season (an inconsistent and variable character).

Leaves: wide lamina, with rather marked mucron; glabrous blades; non-glandular cartilaginous marginal cilia, densely and regularly arranged. A nice, often strongly glaucous colour, particularly at the beginning of the growing season and before flowering; many specimens show, especially on younger leaves, an alternation of paler, glaucous transversal bands and darker, greenish ones; basal blotch from cherry red to purple, more or less pronounced and clear, but relatively constant with good exposure; the blotch has indistinct margins gradually blending with the leaf's glaucous colour. A clear apical blotch is missing, but some specimens with a good exposure can show a light beige apical blotch, small and scarcely evident, mainly at the beginning of spring. Whenever present, the apical blotch is always less evident than the basal one.

Inflorescence: flower stalk tall, sometimes taller than 60 cm, hairy-glandular, with a "minaret"-like structure, that is, a tall stalk bearing many but short subequal lateral branches, distributed along the stalk. Such a stalk's morphology is rather rare in *Sempervivum*, occurring with good frequency and evidently only in *Sempervivum calcareum*. The stalk shape of this houseleek clearly differs from the common shape of the stalks of *Sempervivum tectorum*, including the populations of *S. tectorum* nearest to Monte Pizzocolo (Monte Baldo). The typical flower stalk of *S. tectorum* is markedly three-branched at the apex, whilst further down, there are a few lateral branches of decreasing importance (acrotonous branching), whose number and importance varies depending on the stalk's vigour.

Flower: large and markedly polymerous (often more than 12 subdivisions); greenish petals, pale and with a slight pinkish basal blotch (actually consisting of thin and short pink stripes on a whitish background), with a hairy-glandular external blade; reddish staminal filaments contrasting with the greenish petals.

Possible identifications: although this plant has been linked with *Sempervivum tectorum* up to now, in our opinion it more resembles *Sempervivum wulfenii*, both in situ and in cultivation, and it's not easy to determine at first glance whether it is one or the other, especially during the vegetative phase.

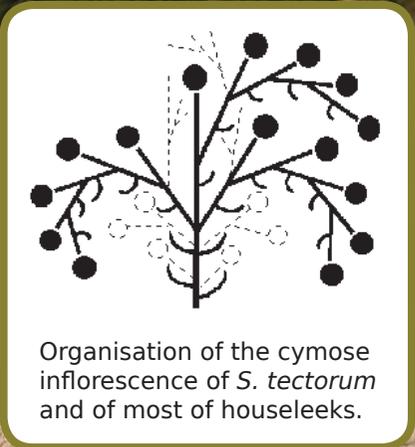
¹² Anisophylly is the difference in shape and size between leaves located nearly at the same level on an axis, hence with comparable age and function. Anisophylly frequently occurs in houseleeks in the vegetative phase during the growing season, *Sempervivum tectorum* being one of the species where this occurs more frequently and evidently.



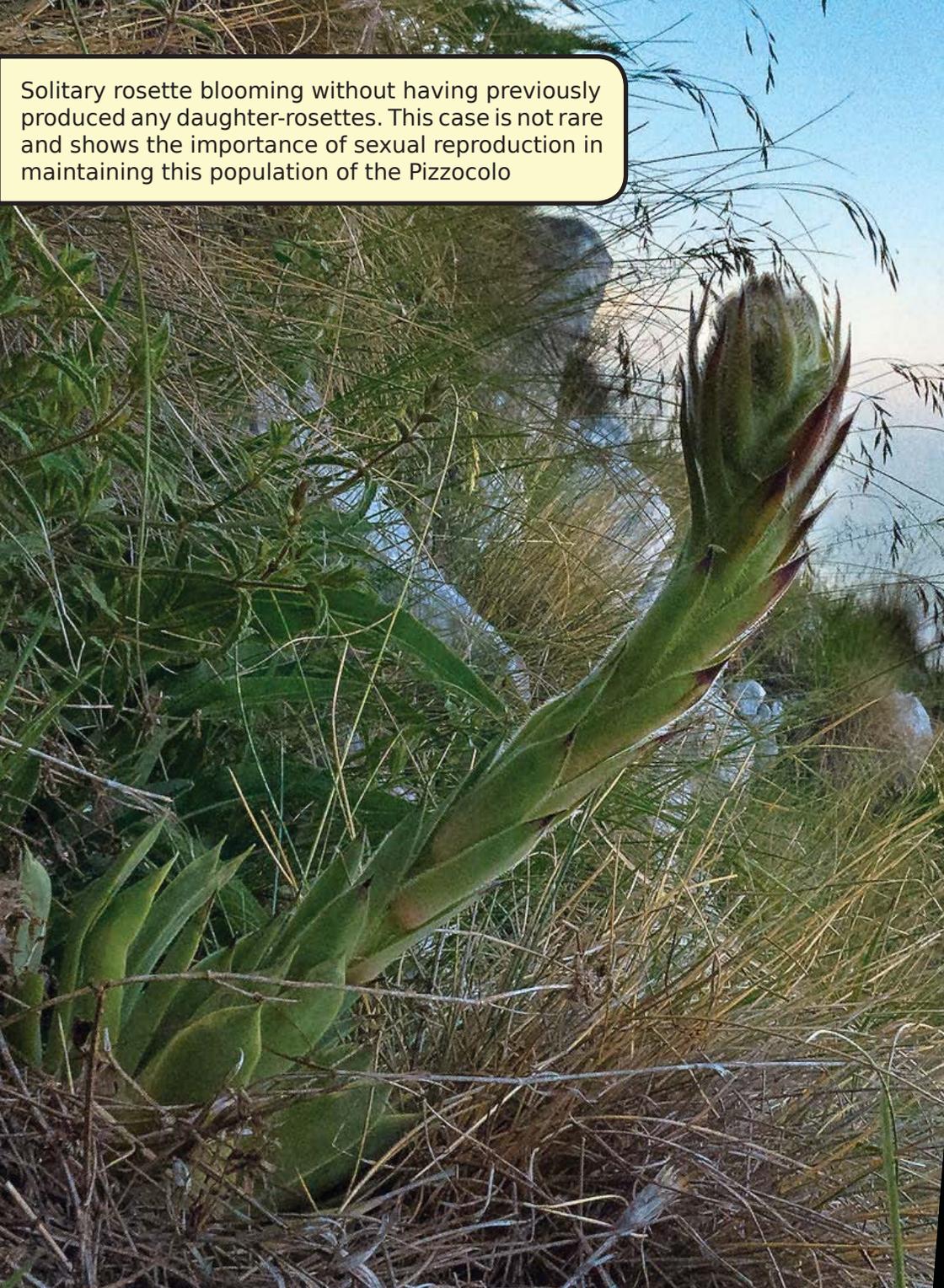
The characteristic aspect of the inflorescence.



Houseleek inflorescences show their maximum expansion at dry state, after fruiting. Even at this state, the inflorescences of the houseleek of the Pizzocolo (right side) keep their typical columnar aspect, very different from the trifurcated and acrotonous aspect of *S. tectorum* (left side).



Organisation of the cymose inflorescence of *S. tectorum* and of most of houseleeks.



Solitary rosette blooming without having previously produced any daughter-rosettes. This case is not rare and shows the importance of sexual reproduction in maintaining this population of the Pizzocolo





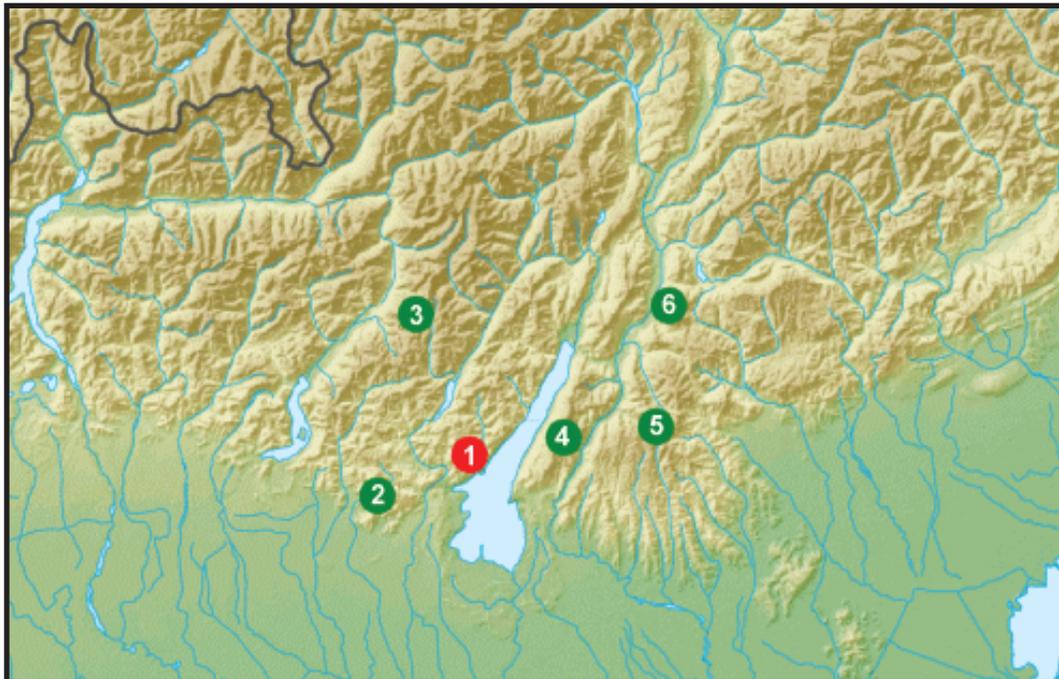
Note the alternating light and dark transverse bands on the leaf blades, a very common but not constant character.

Karyological data

We have performed new chromosome counts on this plant by using new clones we collected *in situ*, hence of known origin, and almost certainly different clones than the ones used in previous counts (see above).

Our results confirm the previously published data: this plant actually has a **diploid genome** and the somatic number we count is $2n = 38$. Since the quality of the meta-phase plates was rather good, the error margin should be low.

Our data agree with previously published counts ($2n = 36, 38, 40$), so we can consider the *Sempervivum* population of Monte Pizzocolo as truly diploid, with somatic chromosome number $2n = 38$.



We have also checked the chromosome numbers of the populations of *Sempervivum tectorum* nearest to Monte Pizzocolo, following an almost elliptical orbit around it. Again, we have used material collected by us in habitat, and followed the same counting method we used for the Pizzocolo plant. **1**

- 2** Monte Maddalena (Brescia): $2n = 72$
- 3** Proximity of Passo di Croce Domini (Brescia): $2n = 72$
- 4** Rifugio Telegrafo, monte Baldo (Verona): $2n = 72$
- 5** Campobrun, Lessinia (Verona): $2n = 72$
- 6** Forte Dosso del Sommo (Folgaria, Trento): $2n = 72$

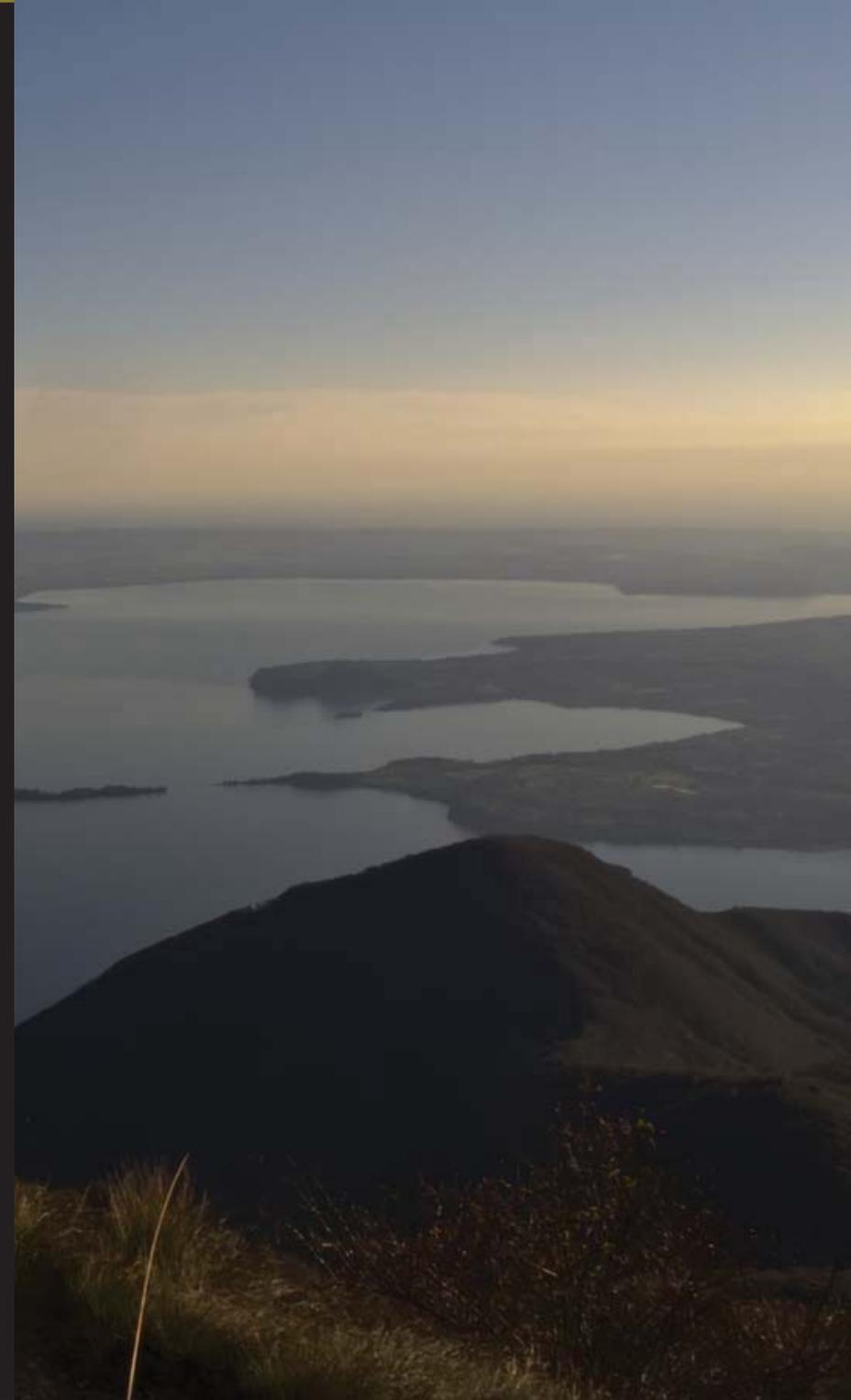
As the data indicate, we have found no diploid individuals and all the specimens were tetraploid.



DDGD13C
[Monte Pizzocolo]

Our chromosome counts can be checked through video files which we made at the same time. In viewing frame by frame, it is possible to change the focus as if you have your eye on the microscope. To perform an accurate count, you will need a graphics program managing layers and stack into it some snapshots of the video or, more simply, use transparent plastic sheets placed on the screen.

These video files are freely available under Creative Commons BY-NC-ND 3.0 license on the website of the journal (<http://acta-succulenta.eu>) or directly from the authors.



Discussion

At present, no tetraploid individual has been found amongst the Monte Pizzocolo population, after various counts on several specimens (clones). We are thus really in front of a **true diploid population**.

In addition, no diploid *Sempervivum tectorum* has ever been found in the proximity of Pizzocolo or in any other locality. This is definitely a **unique and isolated diploid population**, located in a **place recognized as a shelter-area** for some Tertiary species during the Quaternary glaciations. A tetraploid can easily stem from a diploid, but not vice-versa: we can then suspect that this is a relict population, a residual testimony of an ancestral population, that was once perhaps more widespread in the central Alps.

The inflorescence morphology compares to that of *Sempervivum calcareum* from the southwestern Alps, and its chromosome number is identical too ($2n = 38$). This latter species has been, until now, the only known true houseleek (subgenus *Sempervivum*) with this chromosome number, all the other houseleeks belong to the subgenus *Jovibarba*, and are very different plants⁽¹³⁾, with no direct parentage link with *S. calcareum*, so their matching chromosome number is certainly a coincidence.

All these karyological, morphological and chorological data make us doubt that the population of Pizzocolo represents a typical *Sempervivum tectorum*.

Starting from that premise, we can then examine five different hypotheses to understand this plant:

Hypothesis 1: it could be a *Sempervivum tectorum*, if not a typical plant, at least an intra-specific taxon

Hypothesis 2: it could be a very ectopic relict population of *Sempervivum calcareum*.

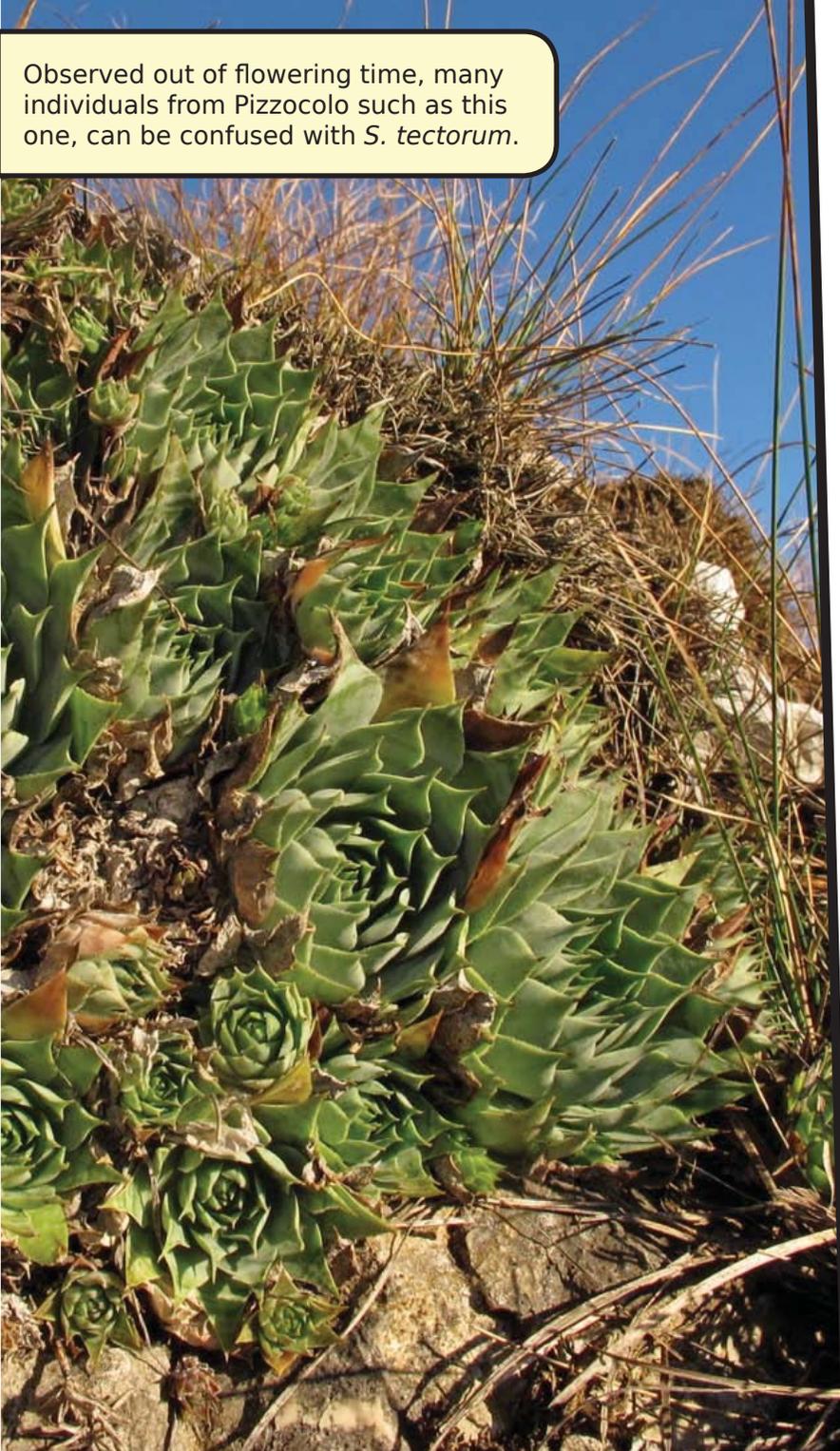
Hypothesis 3: it could be a relict population of *Sempervivum wulfenii*.

Hypothesis 4: it could be an ancient (more or less stable and fixed?) hybrid between *Sempervivum tectorum* and a now extinct species from Monte Pizzocolo.

Hypothesis 5: it could be a new species, overlooked and mis-interpreted up to now.

¹³ The separation between subgenus *Sempervivum* and the subgenus *Jovibarba* is clear and there are no species with intermediate characters between the two. Actually, many authors consider *Jovibarba* as a separate genus, but we prefer to treat it as a subgenus for reasons to which we shall return because they go beyond this article.





Observed out of flowering time, many individuals from Pizzocolo such as this one, can be confused with *S. tectorum*.

Hypothesis 1 : *Sempervivum tectorum*

This *Sempervivum* population, up to now considered belonging to *Sempervivum tectorum*, is rather isolated and, as we mentioned above, morphologically well distinguished from the nearest *Sempervivum tectorum* populations, since the latter don't show any difference compared to the typical *S. tectorum* morphotype of the central Alps. Furthermore, the chromosome number of this population (diploid $2n = 38$) differs from that of the nearest *Sempervivum tectorum* populations, as well as from those that have been counted so far (tetraploid, $2n = 72$).

Could this isolated population be the testimony of an ancestral diploid status of *Sempervivum tectorum*, that would then be an **autotetraploid** rather than an **allotetraploid**, and could this autotetraploid have replaced in the entire distribution range its diploid ancestor, now present only on the shelter-station of Pizzocolo? We can't rule that out completely, but it's rather unlikely: first of all because the autotetraploid would have lost two pairs of chromosomes, a significant loss, but this loss would also have happened homogeneously throughout the entire vast distribution range of *S. tectorum*; something difficult to imagine, since the appearance of autopolyploidy is very likely polytopic and diluted in time. We shouldn't also forget that although an allotetraploid is generally stronger and ecologically more adaptable compared to the diploid parents (due to its double genome), this strength is very rare in autopolyploids. An autopolyploid is often a victim of the expression of recessive defects and its fertility is usually lower⁽¹⁴⁾ than the diploid from which it derives⁽¹⁵⁾. It can be observed that an allopolyploid can easily replace one of the diploids from which it descends from, but an autopolyploid generally tends to cohabit with the original diploid as a simple "chromosomic race" here and there, without any tendency to replace it, and never completely. Hence, the hypothesis according to which the Pizzocolo's houseleek would be an ancestral diploid form of *Sempervivum tectorum* and that the latter is autotetraploid (with chromosome loss) seems highly unlikely. Even more so, if one considers the morphological peculiarity of the Pizzocolo population compared to *Sempervivum tectorum* as it is in the rest of the region and throughout its distribution range.

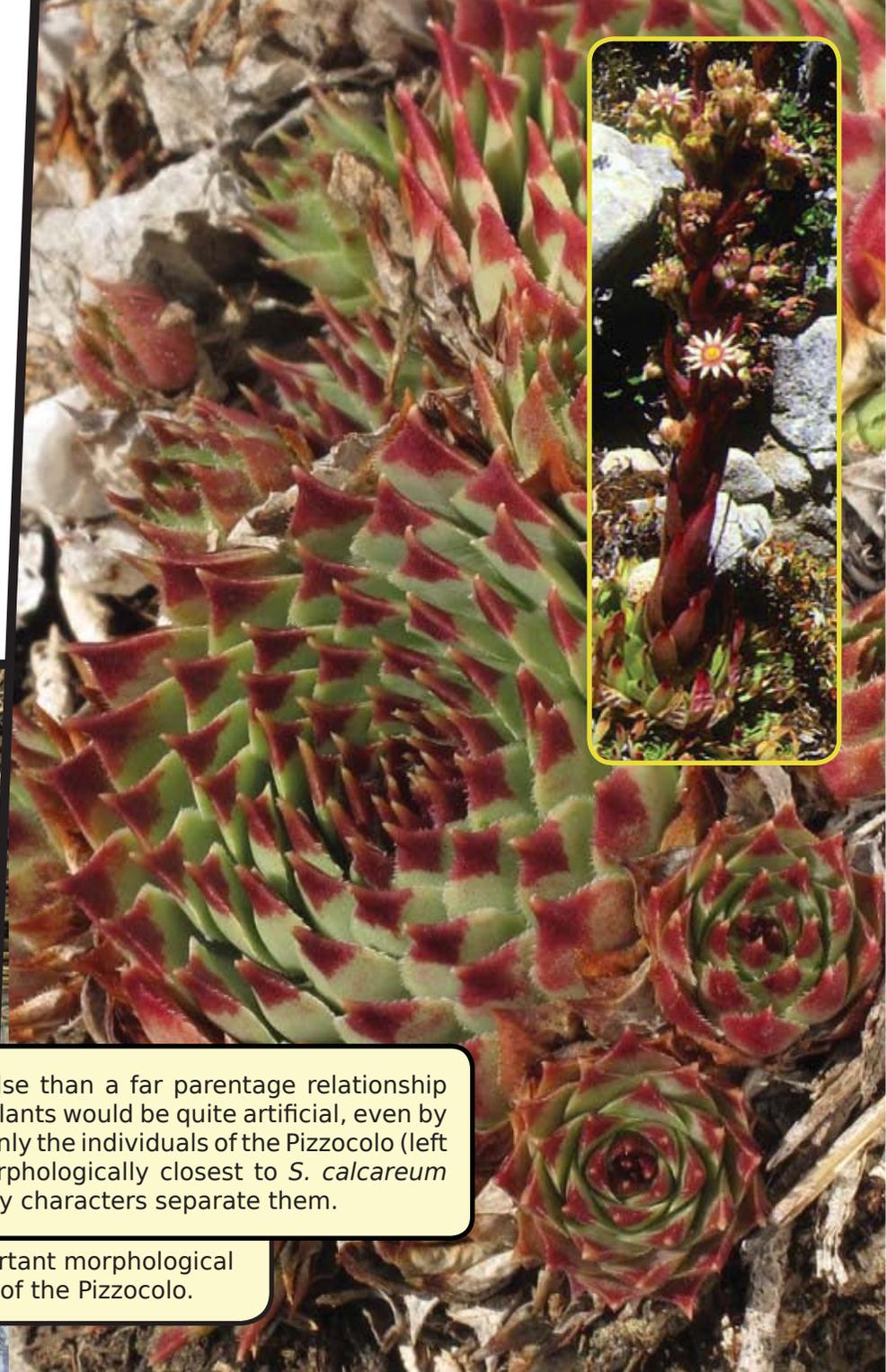
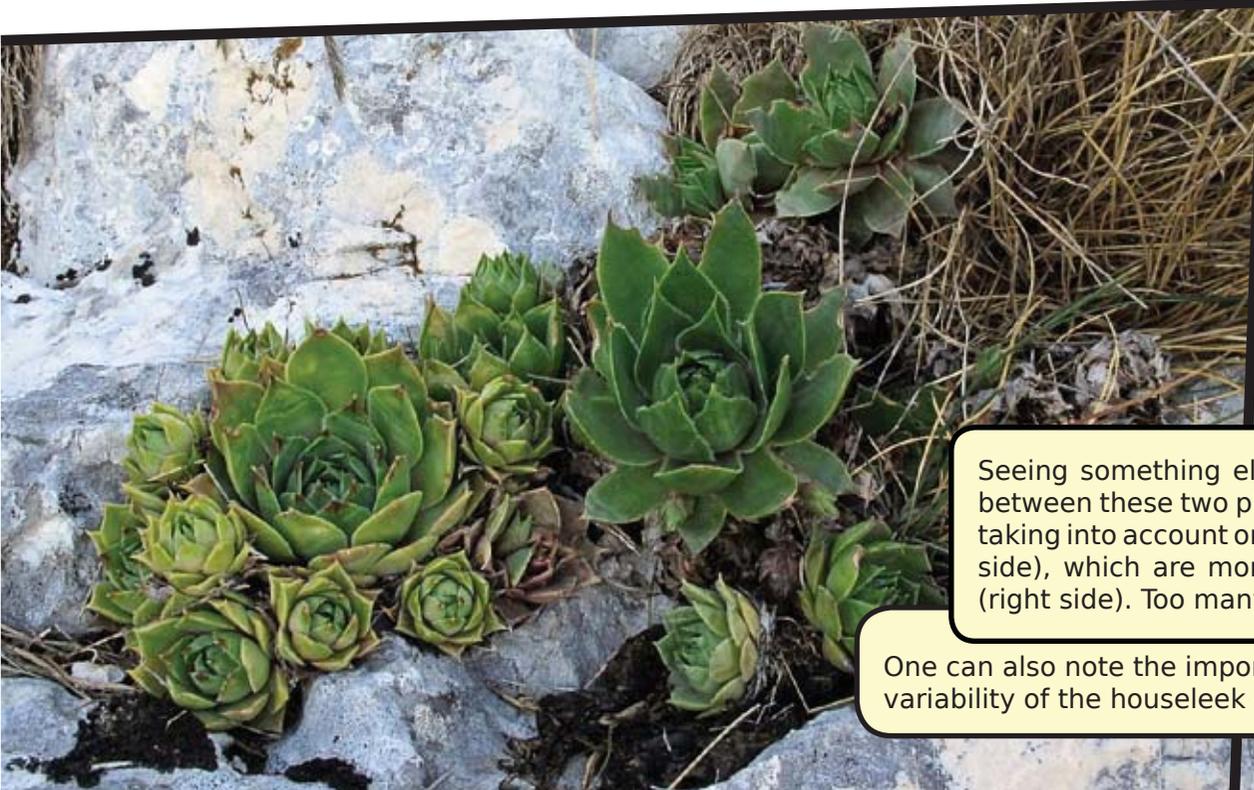
For this reason, it's not possible, in our opinion, to continue considering the Pizzocolo plant as *Sempervivum tectorum*.

14 Quadriavalents formation instead of bivalents in chromosomal pairing, during meiosis.
15 Mind you: all these statements refer to the result of polyploidy, not its mechanism: the spontaneous **autopolyploidy** of a diploid hybrid produces an **allopolyploid** actually, but this mechanism is rare compared to the creation of an allopolyploid by non-reduced gamete crossing.

Hypothesis 2: *Sempervivum calcareum*

As already reported, the Pizzocolo houseleek shares some characters with *Sempervivum calcareum*, that is, its chromosome number and the very peculiar morphology of its flower stalk. However, the distribution range of *S. calcareum* is very far (the limestone south-western Prealps) on the other side of the Alps, and never crosses the ridge line. Moreover, the Pizzocolo houseleek lacks many distinct and constant characters of *S. calcareum* (very numerous, rigid leaves, prickly apex, "raisin" looking old leaves, flowers shape, etc.).

Although a certain affinity between these two houseleeks can't be excluded (common ancestor?), their morphological and chorological differences are such that we don't think it would be possible to link the *Sempervivum* of Pizzocolo with *Sempervivum calcareum*, both as a subspecies or any other intra-specific rank.



Seeing something else than a far parentage relationship between these two plants would be quite artificial, even by taking into account only the individuals of the Pizzocolo (left side), which are morphologically closest to *S. calcareum* (right side). Too many characters separate them.

One can also note the important morphological variability of the houseleek of the Pizzocolo.

Hypothesis 3: *Sempervivum wulfenii*

The wide, glaucous leaves with a red basal blotch, but lacking the apical one, and the erratic presence of a leaf cone, can remind one of *Sempervivum wulfenii*, a species located a little more to the north and north-east, in the Rhaetian Alps. Rather than with *Sempervivum tectorum*, it's the resemblance with this species that comes to mind when observing the plant of Pizzocolo *in situ*. However, the inflorescence of the latter is clearly different, as is the flowers' shape; in addition, the marginal cilia aren't glandular (a very important character for the identification of *S. wulfenii*, although not every evident). From an ecological point of view, the plant grows at a slightly lower altitude than *S. wulfenii* which is a high altitude plant, although, there still are some relict populations in the eastern Alps where *S. wulfenii* grows at low altitude. However, *S. wulfenii* is a markedly silicicolous plant and can be found on limestone only exceptionally, whilst the Pizzocolo plant grows exclusively on calcareous rock. Finally, the chromosome number of *S. wulfenii* is $2n = 36$, close, but not identical to that of the plant of Pizzocolo.

Although the two plants show some similarities, it isn't possible to link the Pizzocolo's plant with *Sempervivum wulfenii* in our opinion, although a certain degree of affinity can't be excluded beforehand.



Some individuals of the Pizzocolo are strongly reminiscent of *S. wulfenii* out of flowering. Their cartilaginous marginal cilia differentiate them immediately. At flowering time, the confusion is not possible.





Hypothesis 4: hybrid

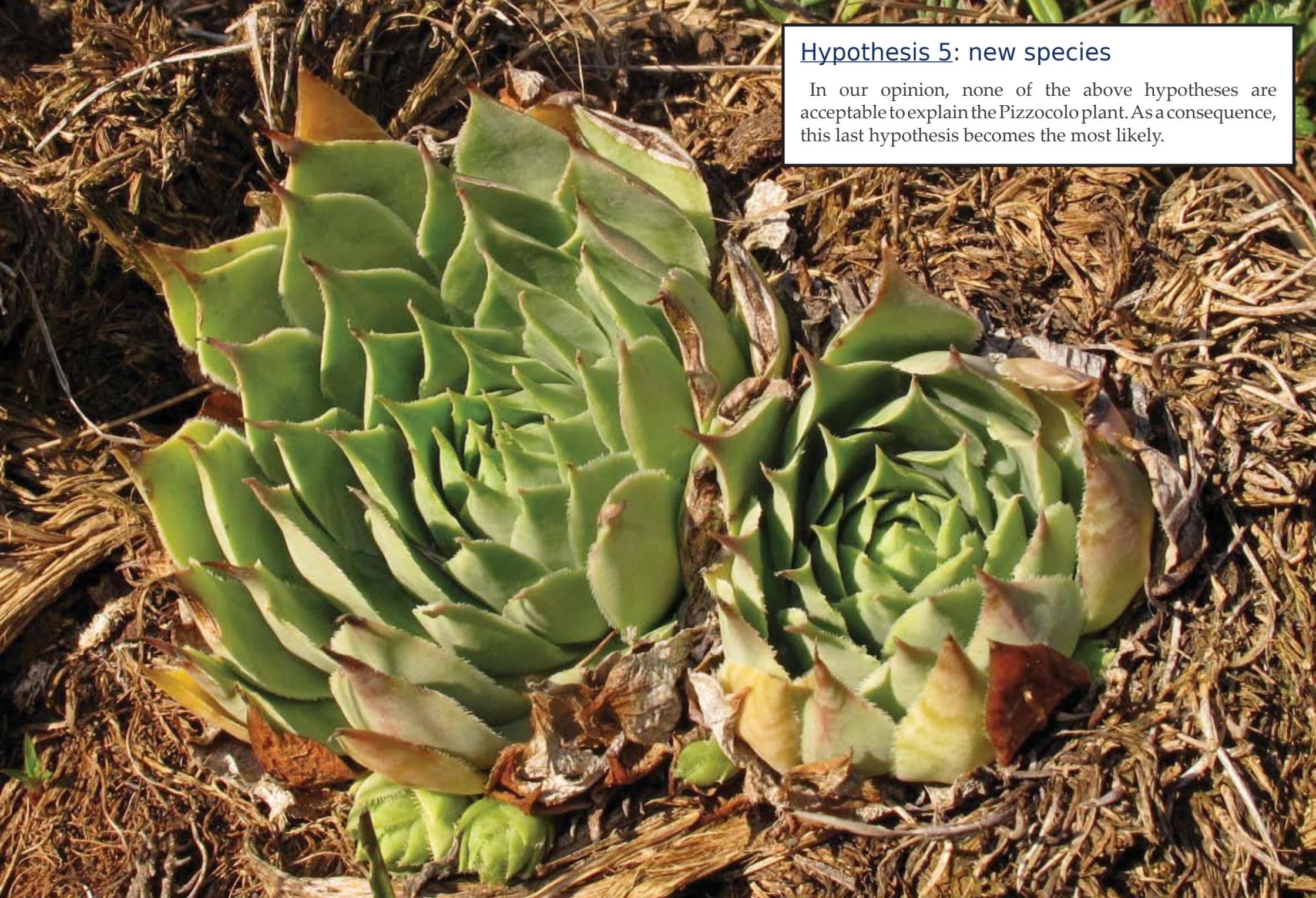
Hybrids are very frequent amongst houseleeks, and often dominant in certain natural populations, even replacing the parent species in some cases. The identification of a *Sempervivum* should always take into consideration that it might be a hybrid.

The pale flower with thin and numerous greenish-white petals, the glabrous rosettes with an inconspicuous or often missing apical blotch, the inconstant presence of an apical leaf cone; all this would be compatible with the aspect of a *Sempervivum tectorum* × *wulfenii* hybrid, a plant known for being difficult to distinguish from *S. tectorum*.

The somatic chromosome number of such a hybrid (*tectorum* $2n = 72$) × (*wulfenii* $2n = 36$) is $36 + 18 = 54$, would be quite different from 38, but, by backcrossing with *S. wulfenii* we would get $27 + 18 = 45$, a number closer to 38. However, to explain a somatic chromosome number of 38 starting from 45, we would have to introduce some meiotic anomalies in the hybrid, enough to force the loss of three pairs of chromosomes, which would be far, far too much. We would also have to expect a very dis-homogeneous hybrid population, with a high variability of chromosome numbers (not the case here), in which the triploid component having a chromosome number close to 40 would be one amongst others, very likely showing marked sterility (not the case here) being a triploid. Hence, we can exclude the hypothesis of the hybrid population between *S. tectorum* and *S. wulfenii*.

From a morphological point of view, we could also hypothesize a possible ancient hybridization of *S. tectorum* with a now defunct member⁽¹⁶⁾ of the complex group *Sempervivum zeleborei* ($2n = 64$), but it should be rapidly discarded, since the theoretical somatic chromosome number of such a hybrid would be $32 + 36 = 68$ and its backcrossing would still maintain a very high chromosome number.

¹⁶ This now eastern group of yellow flowered houseleeks, was once certainly distributed over the Alps, since a vestigial population (*Sempervivum pittonii* Schott) is still present in the Austrian Prealps.



Hypothesis 5: new species

In our opinion, none of the above hypotheses are acceptable to explain the Pizzocolo plant. As a consequence, this last hypothesis becomes the most likely.

Nomenclatural consequences

All the data reported above lead us to consider that the identification of the houseleek of Monte Pizzocolo as *Sempervivum tectorum* is an error, and that this plant is really a new, unknown species, which we describe as follows:



Sempervivum soculense D.Donati & G.Dumont sp. nov.

Sempervivum, **rosula** magna, symetrica, 6-10(-20) cm diam. ; **foliis** valde mucronatis, glauco-viridibus, basi obscure rubra, apice rare leviter colorato, glabris, margine ciliis brevibus eglandulosisque, externis patulis, centralibus nonnumquam conice condensatis ; **stolonibus** rosulae contiguus, saepe paucis, nonnumquam absentibus ; **caule florifero** alto usque 60 cm vel ultra, glanduloso-piloso, non acrotone successive breviter ramoso ; **floribus** breviter pedicellatis, plus minusve 12-partitis saepe ultra, petalis virido-albidis basi roseole leviter lineolatis, filamentis rubentibus.

A *Sempervivo tectorum* atque *S. wulfenii*, praesertim caulis habito et chromosomatico statu, a *S. wulfenii* insuper ciliis, differt.

Habitat in Italia septentrionali, in Alpibus benacensibus, in calcareis saxosis cacuminis clivorumque montis Soculi (Pizzocolo).

Holotypus a nobis designatus : leg. D.Donati & G.Dumont, n° DDGD13A, 2013-11-28 ; « Versante NE del monte Pizzocolo, Toscolano Maderno, Brescia », 1580 m s.m. ; in herbario bononiense (BOLO 507977) depositur. Isotypus in herbario florentino (FI).

NB: the protologue of this taxon is represented by the Italian edition of *Acta Succulenta*.



Cartilaginous not glandulous marginal cilia.



Some solitary or almost rosettes can reach a large diameter.

Relationship with *Sempervivum tectorum*

The fact that we consider *Sempervivum soculense* as a distinct species from *Sempervivum tectorum* doesn't mean that, in our opinion, it doesn't have any relationship with it. The only peculiarity of our point of view is that we don't see it as a progeny, but as an ancestor of the latter.

In our opinion, *Sempervivum soculense* could be considered as a direct descendant of the diploid plant having led to *S. tectorum* by allopolyploidization.

As reported above, the isolation of *S. soculense* on Monte Pizzocolo leads one to think of a post-glacial relict; that this plant or its direct ancestors were populating the central, and perhaps the eastern Alps at the end of the Tertiary period. Regarding *Sempervivum marmoreum*, this was probably populating, at it does now, the Balkans and the Carpathians, but maybe the eastern Alps too.

The most likely scenario is hence thus:

During the Quaternary glaciations, the distribution of *Sempervivum soculense* was squeezed southwards, reduced to some rare ice-free mountainous areas south of the Alps, with the wide Padan Plain blocking its further descent to the south and with a possible redistribution northwards between glaciations; one of these shelter stations was Monte Pizzocolo.

On the contrary, *Sempervivum marmoreum* had all the space it needed to regress towards the Balkans, where it's still abundant even today. No obstacle opposed its moving back, unlike *S. soculense*.

When the post-glacial climate became milder, the two plants gradually re-colonized the ice-free alpine areas and connected (or re-connected) themselves. Being inter-fertile⁽¹⁷⁾, hybrids were formed; by accident, some of them were allotetraploid additive hybrids, they are what we call today *Sempervivum tectorum*. This has in turn rapidly and totally replaced its two parents throughout its expanding distribution range, occupying the same ecological niche, but with a higher competitiveness and ecological amplitude in case of cohabitation⁽¹⁸⁾.

17 We haven't tested the interfertility between *S. soculense* and *S. marmoreum* in cultivation, but this can almost be taken for granted, since all the houseleeks belonging to the subgenus *Sempervivum* are more or less inter-fertile, so much that when a hybrid between two sympatric species is missing *in situ*, this is a symptom of the hybrid's scarce competitiveness, rather than the consequence of a sterile crossing. The proof of this is that it's very easy to obtain hybrids in cultivation which are unknown in the wild.

18 Some cases of allotetraploid plants that have rapidly replaced the parent plants can be observed even by using human life as a time scale. One of the most spectacular and well known cases is that of *Spartina anglica* C.E.Hubb, an allotetraploid, that in a few decades has almost entirely replaced its diploid parent *Spartina maritima* (Curtis) Fernald in every area where they cohabited. It's even likely that in a few decades the parent plant could become extinct, except in some rare residual stations where the tetraploid hasn't yet arrived, or where it's regularly cleared out to preserve *S. maritima* and its environment (see Lacambra 2004).



S. tectorum of the Monte Baldo, the nearest population from *S. soculense*. The inflorescence morphology is quite different.

R.Siniscalschi

This hypothesis seems chorologically more likely than the one that considers *S. calcareum* as one of the parents of the allotetraploid *S. tectorum*.

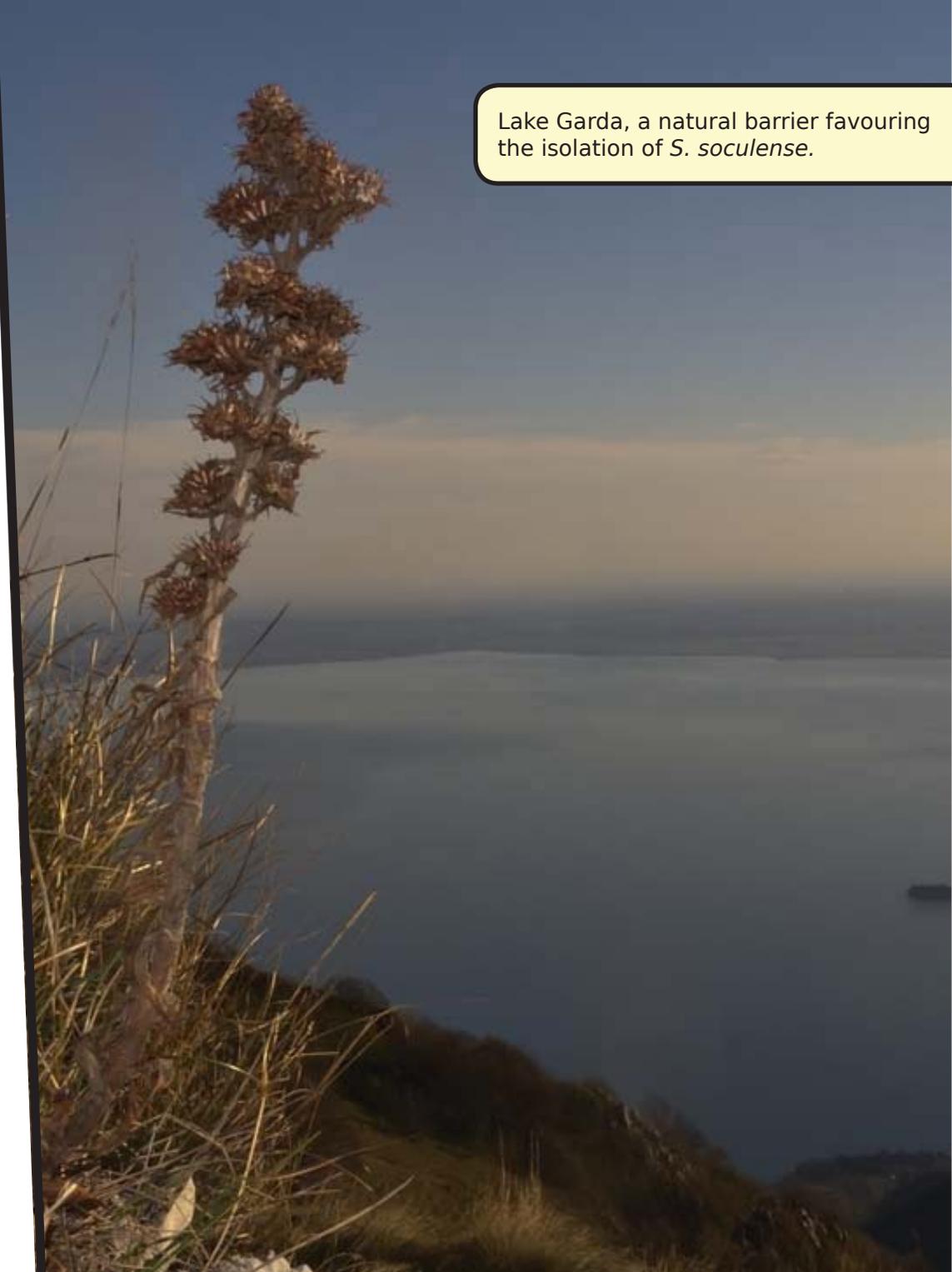
Therefore, *Sempervivum tectorum* has most likely replaced most of the ancient populations of *Sempervivum soculense* and the survival of this relict population on Monte Pizzocolo can only be explained by its isolation and the fact that *S. tectorum* hasn't colonized this mountain, at least not yet.

Amongst the possible explanations of the failed colonization of Monte Pizzocolo by *Sempervivum tectorum*, topography and geology have probably played a major role:

- As already mentioned, Monte Pizzocolo is surrounded by dolomitic rocks. Due to their richness in magnesium, dolostones are inhospitable substrates for many plants, including most houseleeks: *S. tectorum* is one of them, very common on the central Alps, on limestone and crystalline rocks, but absent or very rare on dolomitic rocks, which are quite frequent in this area of the southern central Alps. The only houseleek that grows on dolostone in this part of the Alps is a rare and localized species, *Sempervivum dolomiticum* Facchini, absent in the area near Pizzocolo.
- Lake Garda, a sort of small inland sea, represents an insurmountable barrier to the east and to the south of Pizzocolo for *S. tectorum*, despite the fact that it grows on the opposite side about fifteen km away only, as the crow flies. This impassability is enhanced by the fact that the enclosed and elongated shape of this big lake channels the winds in a north-south direction and vice-versa, blocking a possible transportation of the seeds by the wind from one shore to the other.

The theory that *Sempervivum tectorum* is a descendant of *Sempervivum soculense* (or more exactly its direct ancestors) by allopolyploidization, although it remains a hypothesis, is surely not pure botanical fiction because it seems to be backed-up with serious arguments.

Lake Garda, a natural barrier favouring the isolation of *S. soculense*.



The future of this plant

The unique, known population of *Sempervivum soculense* exists inside a Natural Preserve (Parco dell'Alto Garda Bresciano) where collecting or destruction is forbidden. Furthermore the collection, damage or the destruction of houseleeks is forbidden in the Lombardia Region by Art. 8 of the regional law n. 10/2008. So the plant is well protected by the local administration. This plant is also protected by its ecology, since it grows mainly in very dangerous places, barely accessible by trekkers, a fact which limits any trampling threat.

Nevertheless, the number of individuals on Monte Pizzocolo is quite limited⁽¹⁹⁾ and the species is presently unknown elsewhere. This strict endemism in a single massif, without any possibility of expansion, makes *S. soculense* potentially vulnerable: whilst it doesn't need a further protection, careful and regular monitoring will be very useful.

¹⁹ The total number of specimens is hard to define, due to the difficulty in exploring the northern vertical slope, but we can estimate them to be several hundred, though surely not several thousand.



The deteriorated aspect of this high trail shows the extent of the popularity of the Pizzocolo and thus the potential risk to the unique known population of *S. soculense*, from which we see an individual on the rocks at the left hand side.

In conclusion

In botany it is rather rare that chromosome counts can have a true taxonomical consequence, but for the genus *Sempervivum* they are very important and new karyological studies will help certainly in understanding this “difficult” genus.

The conclusion of this article is a question, which we cannot answer with confidence at the moment: does *Sempervivum soculense* grow elsewhere other than on Monte Pizzocolo; in the Alps or elsewhere? So far we cannot confirm this, but at the same time we cannot also exclude it completely. ■



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