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Development of unifacial leaves in *Senecio* L. s. lat. (Asteraceae)

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Summary: The unifacial leaves of the investigated *Senecio* species are terete (*S. citriformis*, *S. radicans*, *S. spiculosus*), ensiform (*S. ficoides*), and linear pseudo-bifacial (*S. serpens*). Each has a very short bifacial leaf base and an unifacial blade. All leaf primordia generated at the flat shoot-apex are transversely flattened. Therefore, adaxial and abaxial sides and the margin in between are clearly discernible in the primordium. We consider those margins as provisionary. The primordium tip remains throughout the leaf development to become the tip of the mature leaf. No secondary tip arises to cause an unifacial design of the mature leaf. Unifacial leaf design establishes after the primordium has differentiated into Unterblatt and Oberblatt. The former retains its provisionary sides and its margin to develop the leaf base. Thus, the provisionary sides and margin of the leaf primordium become definite in the bifacial leaf base. The Oberblatt transversely rounds in both, terete and ensiform leaves, to cause the provisionary margin to disappear. Thereupon, the unifacial part arises and gives rise to the unifacial definite leaf blade. The Oberblatt remains flattened in the pseudo-bifacial leaf, but a new margin develops on its provisionary adaxial side and becomes the definite leaf margin. It delimits definite adaxial and definite abaxial sides. The latter consists of the whole provisionary abaxial part of the Oberblatt and most of its provisionary adaxial side. Thus, the basal bifacial part of the Oberblatt and its distal unifacial one differentiate. Later, the unifacial part grows to a pseudo-bifacial, linear blade. Then, the facial design of the leaf changes during leaf development.

Keywords: unifacial leaf, terete leaf, ensiform leaf, pseudo-unifacial leaf, leaf development, *Senecio*, leaf morphology

We investigated selected *Senecio*-species of the section *Rowleyani* (Ozerova & Timonin 1993; Timonin & Ozerova 1993b). Their succulent leaves generally fit the criteria of the unifacial leaf as conceived by Troll (1939) in morphology as well as in anatomy. Only the short basalmost parts of such leaves show an adaxial side that narrows acropetally to become unrecognizable in the nearest vicinity of the leaf base. We have qualified these leaves as unifacial for distinguishing them from very similar leaves of other *Senecio* species, which have a narrow adaxial side nearly throughout the leaf (Ozerova & Timonin 1993; Timonin & Ozerova 1993b).

We have discerned 3 variants of the revealed, unifacial leaves according to their cross outlines (Timonin & Ozerova 1993b). The leaves of *S. citriformis* Rowl., *S. radicans* (L. fil.) Sch. Bip., and *S. spiculosus* (Seph.) Rowl. are transversely round. They are generally considered as terete (fig. 1 A–C) here in spite of the leaf diameter varying along the leaf in both, *S. radicans* and *S. citriformis*. Thereupon, the leaves are rather fusiform in the former species (fig. 1 B) and differentiated into an ellipsoidal to globular 'quasi blade', and a thinner 'quasi periole' in the latter one (fig. 1 C). Ensiform leaves are inherent in *S. ficoides* (L.) Sch. Bip. (fig. 3 A) (Timonin & Ozerova 1993b). *S. serpens* Rowl. has thickened linear to concave-convex pseudo-bifacial leaves (fig. 4 A) (Timonin & Ozerova 1993a).

The development of the Senecios' unifacial leaves is unlikely to be scrutinized. Meanwhile, the development of the unifacial leaves in Angiosperms in general is interpreted controversially.

According to Thielke (1948) and Roth (1949), every leaf is asserted to start as a bifacial primordium throughout. If there is an unifacial leaf, the secondary tip of the leaf appears on its abaxial side to supersede the arrested primary tip in time ('Dorsalauswuchs' sensu Roth l.c.). The bigger part of the leaf, arising from the secondary tip, has only one, viz. abaxial side. The unifacial part of a leaf is argued by Troll & Meyer (1955) to be *ab initio* unifacial, though it is initially flattened or even concave top down in some species. There is a characteristic rounding meristem ('Rundungsmeristem') in such species that differentiates under the adaxial epidermis of the flattened resp. concave part of leaf to cause often, but not always, this part to round in crossection. Every leaf primordium is considered by Hagemann (1970) and Eberwein (1995) as really bifacial. The adaxial side can neither disappear nor decrease in the developing leaf since it has originated in the primordium. Considerable thickening of the primordium accompanied by intensive growing of its abaxial side can result in apparent diminishing of the adaxial side, however. Such false diminishing excessively issues in a nearly unrecognizable adaxial side in mature leaf, especially when the marginal fold of the leaf surface has rounded during leaf thickening. Therefore, unifacial leaves don't appear. Only sub-unifacial leaves with highly suppressed adaxial sides can accordingly be acceptable.

In respect thereof, a developmental study of the unifacial leaves in *Senecio* species is necessary for proper realizing both, their structure and origin.

Materials and methods

Living plants of the species mentioned above growing in the greenhouses of Tsitsin Main Botanical Garden of Russian Academy of Science (RAS), of Komarov Botanical Institute of RAS, and of Lomonosov Moscow State University (MSU) were used for our investigations.

Tips of intensively growing shoots were picked up. All leaves exceeding 1 mm were removed from the tip with dissecting needles or pincers, respectively. The prepared tips were fixed in an excessive amount (30 or more : 1 v/v) of 70% ethanol for 3 days or longer to extract abundant slime, which otherwise prevented the complete dehydration needed for subsequent SEM studying.

Apical buds and apices of the fixed shoot tip kept in 70% ethanol medium were uncovered under a long-focused optical microscope with sharp pincers and hypodermic needles. The prepared apical buds were dehydrated in a series of 90% ethanol, 96% ethanol : 100% acetone (1 : 1 v/v), 100% acetone (3 times in each for 1.5 to 2 hrs), critical point-dried and covered with Pt, Pd, Au, and Ag variably combined, and examined by means of SEM Camscan 4DV or Hitachi S405–A.

Results

Apical bud

Apical buds are bare in all the species concerned. There are 2 to 3 developing leaves per bud in *S. spiculosus* and 3 to 5 in the other species (figs. 1 A; 3 A; 4 A). The developing leaves rarely vault over the apex (fig. 1 D), but they are regularly straight (figs. 1 E, F; 3 C, G; 4 B–F) . Therefore, the apical meristem is protected by the ventrally protruded thickened part of young leaves (figs. 1 E; 3 F–G; 4 E) as well as by a bunch of dense trichomes situated on the base of the larger leaves of the bud (figs. 2 C; 3 F–H; 4 F).

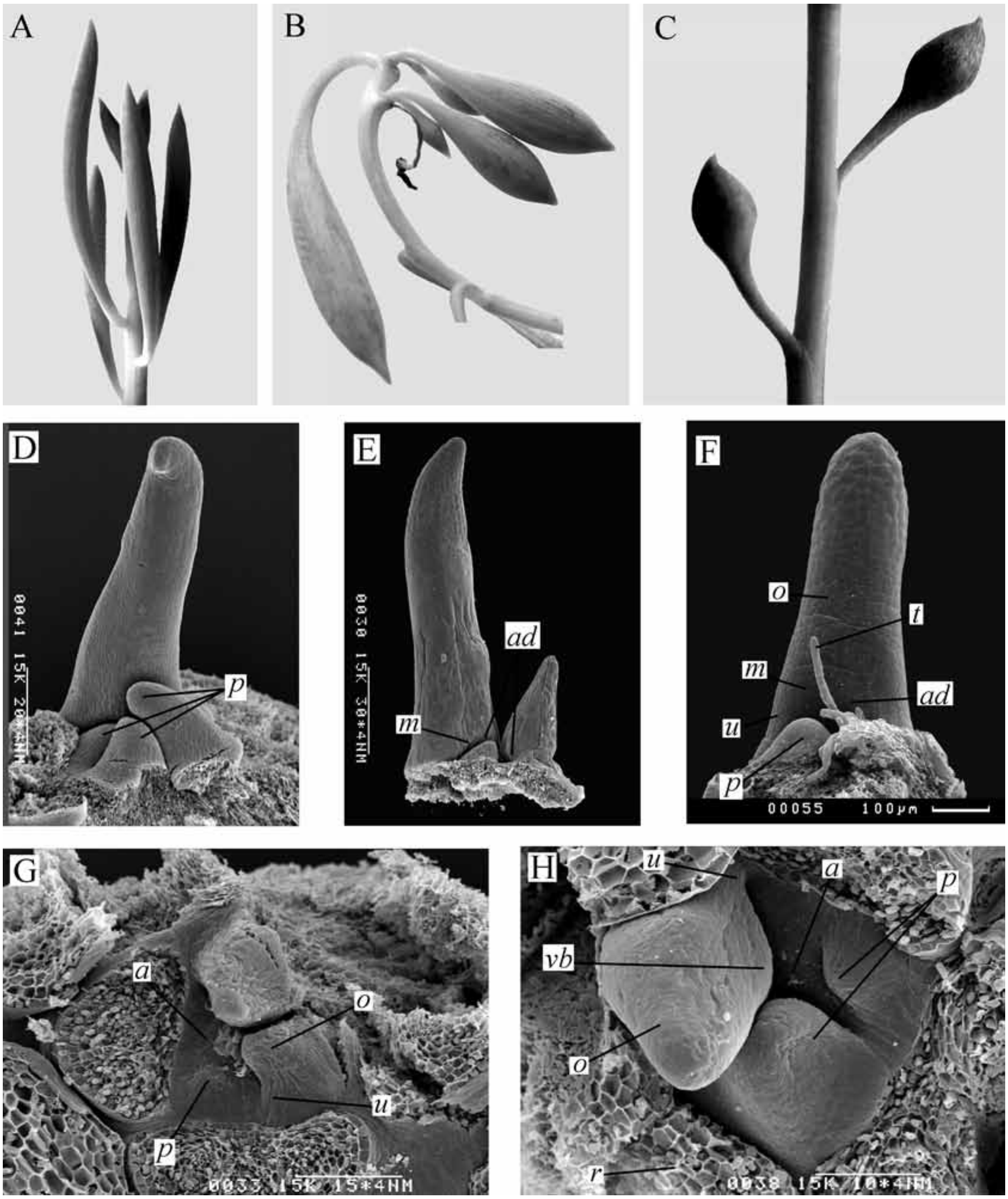

Figure 1: Mature and developing terete leaves. A) Shoot fragment, *S. spiculosus*. B) Shoot fragment, *S. radicans*. C) Shoot fragment, *S. citriformis*. D) Terminal bud partly uncovered, *S. spiculosus*. E) Terminal bud partly uncovered, *S. spiculosus*. F) Developing leaf, *S. radicans*. G) Initial leaf primordium, *S. spiculosus*. H) More advanced leaf primordium, *S. spiculosus*. – *a*, apex, *ad*, adaxial side, *m*, margin, *o*, Oberblatt, *p*, leaf primordium, *r*, rounding meristem, *t*, trichome, *u*, Unterblatt, *vb*, ventral bulge.

The apex hardly changes during the plastochrone. It is flattened to slightly depressed (figs. 1 G–H; 2 A–B; 3 D–C; 4 D). There are few initial cells of the outer tunic layer which are observable from the outside (fig. 2 A).

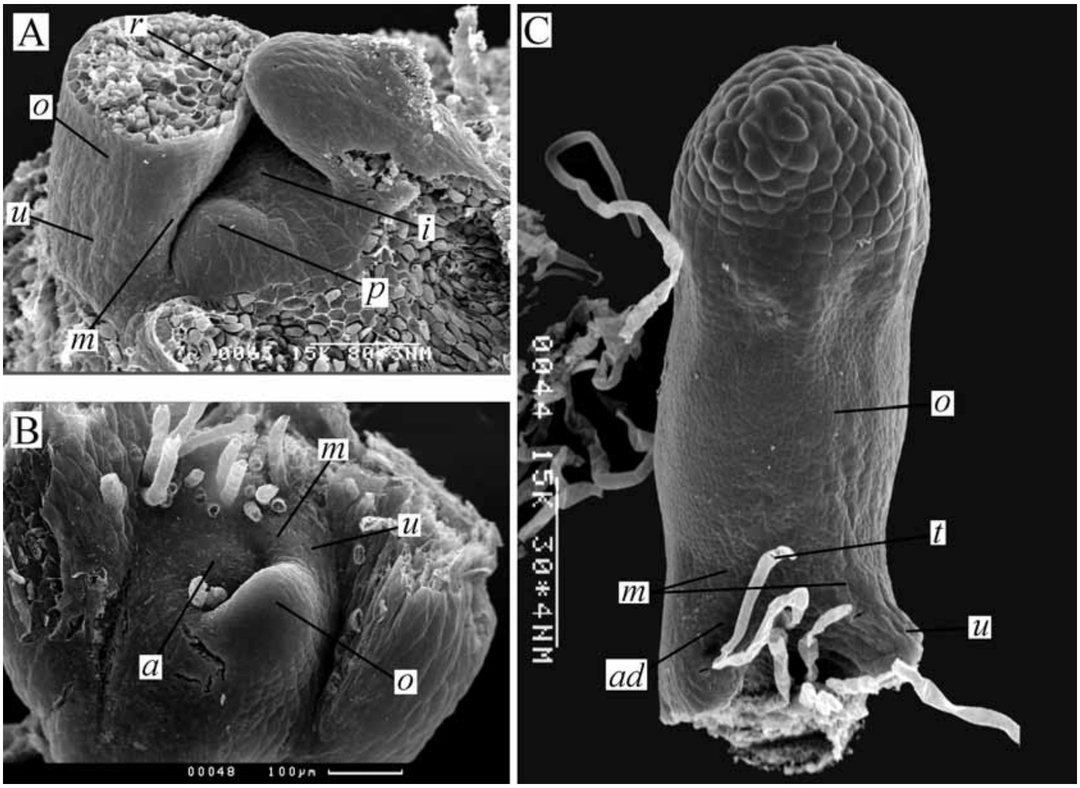

Figure 2: Developing terete leaves in *Senecio*. A) Shoot apex, *S. spiculosus*. B) Developing leaf differentiated into Unter- and Oberblatt, *S. citriformis*. C) Growing young leaf, *S. citriformis*. – *a*, apex, *ad*, adaxial side, *i*, tunic initial cells, *m*, margin, *o*, Oberblatt, *p*, leaf primordium, *r*, rounding meristem, *t*, trichome, *u*, Unterblatt.

Raising frequencies of cell divisions in the outer tunic layer on the periphery of the apex indicate externally the beginning of the development of the next leaf primordium (fig. 3B). The latter appears as a small transversely elliptic bulge in the median plane of the coming leaf (figs. 1G; 2A; 3C; 4B). Cell divisions divergently spread round the apex to wide the primordium up to 1/5–1/4 of the circumference of the apex. Primordium widening co-occurs with its lengthening and slight thickening. These three growing processes combined make the primordium triangular concave (figs. 1G–H; 2A; 3B–C; 4B–C). Both of its margins are rather roundish in the elevated median part of the primordium, but they become sharper further away from its median plane.

Subsequent developmental pathways of primordia diverge in the concerned species in accordance with their characteristic leaf variants.

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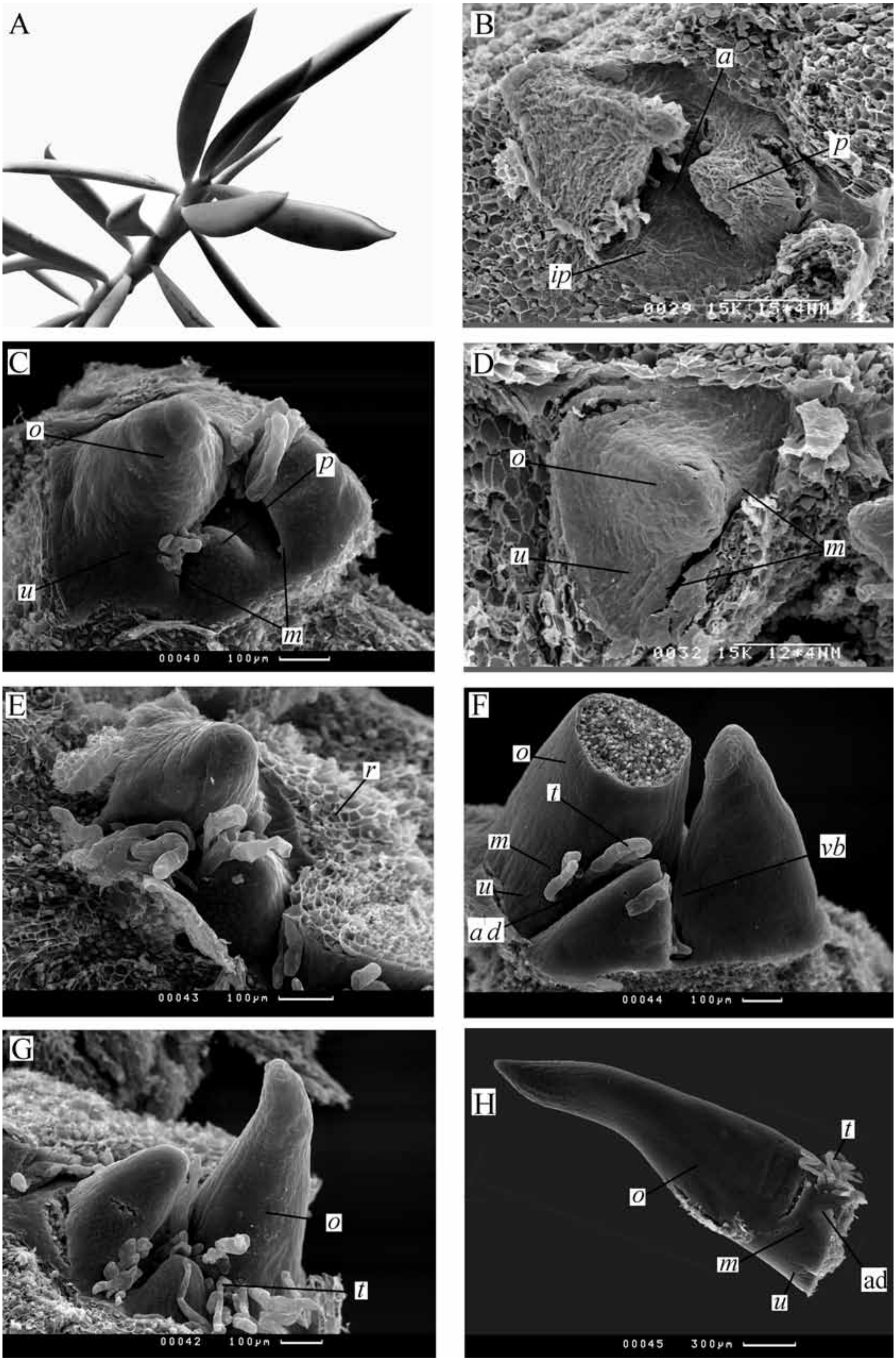
Figure 3: Ensiform leaves of *Senecio ficoides*. A) Shoot fragment. B) Initiation of leaf primordium. C) Leaf primordia. D) Developing leaf differentiated into Unter- and Oberblatt. E) Innermost part of terminal bud. F) Terminal bud partly uncovered. G) Terminal bud partly uncovered. H) Growing young leaf. – *a*, apex, *ad*, adaxial side, *ip*, initiating leaf primordium, *m*, margin, *o*, Oberblatt, *p*, leaf primordium, *r*, rounding meristem, *t*, trichome, *u*, Unterblatt, *vb*, ventral bulge.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Development of the terete leaves in *Senecio citriformis*, *S. radicans*, and *S. spiculosus*

The median part of the primordium highly exceeds in lengthening both of its laterals. Thereupon, the protrudent tip becomes a distinctive element of the primordium as opposed to its short base (figs. 1 D; 2 B). The primordium never becomes 3-lobed. However, 3-lobed primordia are considered as a *sine qua non*-stage of leaf development in Dicotyledons (e.g. Hagemann 1970; Gendels 1988; Eberwein 2005). The short base of the primordium and its protrudent tip correspond with the interpretation as 'Unterblatt' and 'Oberblatt' as established by German botanists (see Troll 1939 for more details).

The Unterblatt grows a bit subsequently. It retains its sharp margins and thus allows to distinguish easily its adaxial and abaxial sides (figs. 1 E–F; 2 A). Later, a bunch of dense trichomes develops basally on its adaxial side (see above). The Oberblatt soon becomes cylindrical due to repeated periclinal cell-divisions just below the adaxial epidermis (fig. 2 A). As soon as the original margins of the Oberblatt have rounded, the borderline between the adaxial and the abaxial side disappears (fig. 1 D–F, H). Therefore, the two sides can only be delimited conventionally. Originally the apical and the total growing of the Oberblatt concentrates on intercalary growing of the very base of the Oberblatt. Thus, two developmentally different parts of the Oberblatt appear. They are clearly recognizable because of differences in their epidermal cells (figs. 1 F; 2 C). Most of the mature leaf is a derivation from the basal intercalary growing part of the Oberblatt. Thickening of this part results in a ventral bulge of the leaf (fig. 1 H) which overhangs the shoot apex (see above). The basal edge of the bulge continues basipetally to the Unterblatt margins (figs. 1 E–F; 2 C). So, it is a distal edge of the Unterblatt.

Development of the ensiform leaves of *Senecio ficoides*

The developmental pathway of the ensiform leaves is similar to those of the terete leaves described above. The primordium of the ensiform leaf also differentiates into Unterblatt and Oberblatt (fig. 3 C). The former remains flattened while the latter becomes cylindrical. The Unterblatt is subsequently high suppressed. The apical or total lengthening of the Oberblatt changes to its intercalary growing. This leads to arising, developmentally different, distal and basal parts of the Oberblatt which are easily distinguishable in their epidermal cells (fig. 3 G). The distal part stops growing soon whereas the basal one gives rise to nearly the whole leaf blade. This part thickens considerably towards the shoot apex (fig. 3 F) due to the activity of the rounding meristem (fig. 3 E) and thus results in a medianly flattened ensiform blade (fig. 3 D, H).

Development of the pseudo-bifacial leaves of *Senecio serpens*

There is no distinctive Unterblatt and Oberblatt in the progressing primordium (fig. 4 C). The latter even tapers upwards and rounds up transversely to the cylindrical tip. There is a thinner basal part of the primordium and a thicker distal one which are, nevertheless, recognizable, and a zone of intercalary growing in between. The leaf part produced by intercalary growing remains flattened. Two buttresses originate on the adaxial side of this part at subsequent developmental stage of the leaf (fig. 4 D). Each is hardly raised above the surface but quite visible. Two buttresses run up closer acropetally and merge (fig. 4 E). They limit the basal part of the leaf ventral side. This part was identified as an adaxial side of the mature leaf elsewhere (Timonin & Ozerova 1993a). Intercalary growth of the leaf blade continues more distantly to the place where the buttresses have merged. Thus, the developing blade remains flattened or even becomes concave top down, though its lengthening co-occurs with its thickening. The leaf margins are much more roundish than those in a typical bifacial leaf, however (fig. 4 A).

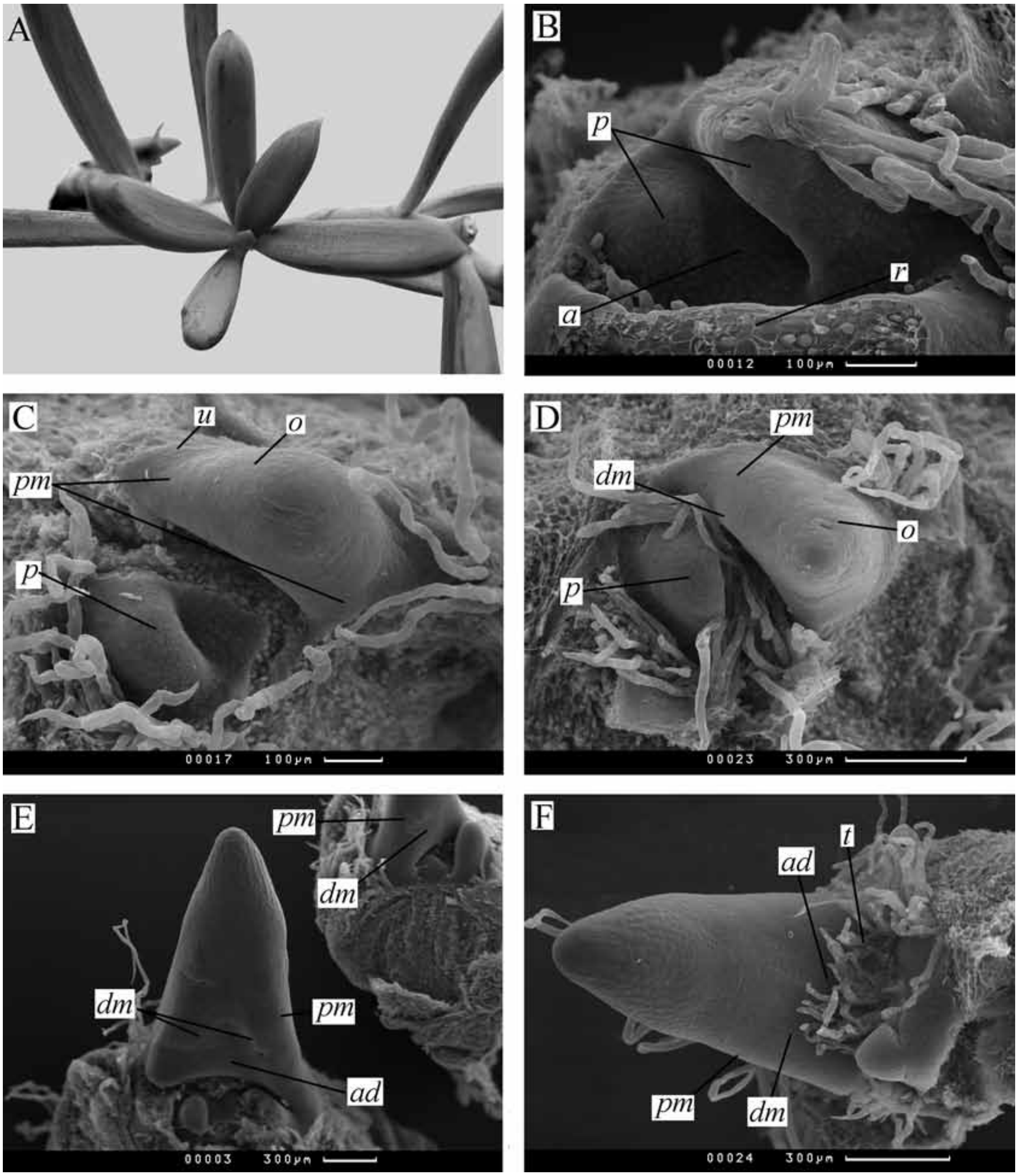

Figure 4: Pseudo-bifacial leaf of *S. serpens*. A) Shoot fragment. B) Leaf primordia. C) Developing leaf differentiated into Unter- and Oberblatt. D) Developing definite margins. E) Growing young leaf. F) More advanced growing leaf. – *a*, apex, *dm*, definite margin, *o*, Oberblatt, *p*, primordium, *pm*, provisional margin, *r*, rounding meristem, *t*, trichome, *u*, Unterblatt.

Discussion

There is a developmental stage in all three-typed leaves where the growing leaf vaults over the shoot apex to protect it (acrovergence). None of them is typically curved toward the apex, quite contrary to its counterparts in many other plants with unifacial leaves as shown by Thielke (1948) and Roth (1949). Instead, uneven thickening of the Oberblatt – predominantly towards the apex – results in shielding 'concha' over the latter. This thickening clearly takes place in the basalmost part of the Oberblatt. Therefore, the ventral outgrowth of the developing leaf, which partly covers the shoot apex, is by no means the primary tip of the mature leaf. That is why we have no reasons to consider the unifacial part of Senecios' leaves to be a derivation of a secondary leaf tip appearing on the abaxial side, suppressing and finally replacing the primary leaf tip. Thus, the mode of unifacial leaf development as suggested by Thielke (1948) and Roth (1949) is invalid in relation to the unifacial leaves of the *Senecio* species investigated.

The Oberblatt rounding at a certain developmental stage of both, terete and ensiform leaves is indeed caused by the activity of a characteristic rounding meristem ('Rundungsmeristem') according to Troll & Meyer (1955). This developmental process might hardly be the reason to be interpreted as rounding of the originally unifacial but flattened part of forming leaf. We are sure that adaxial/abaxial sides of the leaf primordium can be detected using some really observable traits of the primordium. Because the inner structure of the primordium is still underdeveloped at the developmental stage under consideration, the sharply folded surface along the primordium margins is the only reliable indicator of a bifacial structure of the primordium. As far as we know, this trait has never been discussed so far.

Very early primordia are in all species investigated transversely elliptic with rather roundish margins. This shape is characteristic for early primordia of dicotyledonous leaves (Esau 1965, 1977). Such primordia might generally be considered as flattened unifacial, but evidently nobody does so. Otherwise, if such primordia are considered as unifacial, every dicotyledonous leaf would have to be regarded as completely unifacial and no bifacial leaf could be accepted. That is why we qualify early primordia of leaves in the Senecios investigated as really bifacial.

The margins become sharper in more developed primordia. Such primordia are quite similar to those of undoubtfully bifacial leaves. Moreover, these margins remain in the flattened Unterblatt which develops in time other characters of its bifacial design in epidermis, mesophyll, and vascular system (see Timonin & Ozerova 1993b for more details). That is the reason, why the margins concerned are considered here as real margins of the developing primordium/leaf which delimit its genuine adaxial and abaxial sides. Thus, the primordia of *Senecio*-leaves at this developmental stage have no unifacial parts. They are still bifacial throughout.

The smallest hint of margins is missing in the Oberblatt after it has rounded. Besides, the epidermis is uniform throughout the cylindrical Oberblatt. Bifacial design of such an Oberblatt may only be postulated following Hagemann (1970) and Eberwein (2005). This Oberblatt really has no traits which indicate remaining its two original sides, however. Both authors would describe these leaves as 'aequifacial' (also see Troll 1939). If the interpretation of the leaf structure is based on actually recognizable characters instead of theoretical postulates, then the Oberblatt of the *Senecio* species investigated is worth being considered as unifacial, but not subunifacial. We believe that only the side of the cylindrical Oberblatt is homologized reasonably with the abaxial one because the abaxial side prevails over the adaxial side in the still bifacial primordium before Unter- and Oberblatt are appearing.

The facial structure of *S. serpens* leaves is very difficult to realize in the correct way. On the one hand, each primordium develops margins which remain in the Unterblatt and the bigger part of the Oberblatt up to leaf maturity, though they are rather roundish in the Oberblatt. This looks like a proof of *S. serpens* leaves being bifacial, but it sharply contrasts with anatomical and morphological data (Timonin & Ozerova 1993a). On the other hand, the primordium margins become too roundish in the more advanced Oberblatt. Additionally, a ∩-shaped bulge arises on the adaxial side of the primordium in the transitional zone from Unterblatt to Oberblatt. It is important that this bulge is congruent with the borderline which is identical in its property with those delimiting bifacial and unifacial leaf parts in other *Senecio* species investigated. We consider these data combined to be a sufficient reason for identifying the bulge with the leaf definite margins and for interpreting only the Unterblatt and basalmost Oberblatt up to the bulge as really bifacial.

We think, that this inconsistency of the data revealed is solvable by assuming Troll & Meyer's (1955) idea that both, definite margins of a leaf and its facial design, are not always congruent with the margins and facial design of the flattened primordium. This assumption does not mean that we consider the Oberblatt of Senecio's leaves to be primarily unifacial, however.

Leaf development in the six species investigated can be presented according to the already proclaimed criterion of leaf facial design as follows. The shoot apex generates leaf primordia which are really bifacial throughout their lengths. Each primordium misses a clearly 3-lobed stage, but differentiates into Unterblatt and Oberblatt. Unterblatt develops into a short basalmost part of the mature leaf remaining bifacial. Thus, the margins and both sides of this part directly derive from the margins and both sides of the primordium, respectively. Oberblatt, which gives rise to nearly the whole leaf blade is also initially bifacial but it rounds more or less highly to become partly (*S. serpens*) or completely (other species) unifacial. Though the Oberblatt in *S. serpens* remains flattened or slightly concave, its roundish edges are no longer leaf margins which have been lost during Oberblatt rounding. The genuine margins of the Oberblatt are hardly visible now, the ∩-shaped bulge, which arose on its ventral side, continues the margins of the Unterblatt.

Conclusion

After investigating the leaf development in 6 *Senecio* species we have realized that the facial design of the leaf is developmentally changeable in certain plants. Every leaf primordium is bifacial throughout. It has more or less obvious margins and adaxial/abaxial sides. All of them are transitory, however. Transitory margins of the primordium mostly become definitive leaf margins while its transitory adaxial/abaxial sides develop into definitive adaxial/abaxial sides of the mature leaf. In certain species, the primordium transitory margins disappear partly. Thereupon, a certain part of the developing leaf becomes unifacial and its transitory adaxial side becomes included in the definitive abaxial side of this part. The pseudo-bifacial leaf retains the primordium transitory margins as definitive pseudo-margins of the leaf blade. Its pseudo-adaxial side is congruent with the transitory adaxial side of the primordium, but really it is completely incorporated into the definitive abaxial side.

Some genes are revealed to express specifically either on the adaxial side of the developing leaf or on its abaxial side (Bowman 2000). Dynamics of differential gene expressions on the sides of developing leaves should be scrutinized in *Senecio* species and other plants with unifacial leaves to test if our narration, as just presented, is correct.

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References

Bowman J. L. (2000): Axial patterning in leaves and other lateral organs. – Current Opinions in Genetics & Development **10**: 399–404.

Gendels T. V. (1988): Leaf formation in woody dicotyledons. – Bot. Zhurn. **43**(4): 553–562. [In Russian]

Eberwein R. K. (1995): Bau und Ontogenese unkonventioneller Blätter des Typs 'unifaziale Phyllome' und deren Beitrag zur Theorie des Spermatophytenblattes. – Aachen: PhD Dissertation, Rheinisch-Westfälische Technische Hochschule Aachen.

Eberwein R. K. (2005): Blatt oder nicht Blatt – eine einfache Frage? – In: Harlan V. [Hrsg.]: Wert und Grenzen des Typus in der botanischen Morphologie: 149–162. – Nümbrecht: Martina Galunder-Verlag.

Esau K. (1965): Plant anatomy. – New York, London, Sydney: John Wiley and Sons.

Esau K. (1977): Anatomy of seed plants. [2nd ed.] – New York, Santa Barbara, London, Sydney, Toronto: John Wiley and Sons.

Hagemann W. (1970): Studien zur Entwicklungsgeschichte der Angiospermenblätter. – Bot. Jahrb. Syst. **90**: 297–413.

Ozerova L. V. & Timonin A. C. (1993): About the new type of leaf anatomical structure in Dicotyledons. – Bull. Main Bot. Gard. Russian Acad. Sci. **167**: 84–87. [In Russian]

Roth I. (1949): Zur Entwicklungsgeschichte des Blattes, mit besonderer Berücksichtigung von Stipular- und Ligularbildungen. – Planta **37**: 299–336.

Thielke C. (1948): Beiträge zur Entwicklungsgeschichte unifazialer Blätter. – Planta **36**: 154–177.

Timonin A. C. & Ozerova L. V. (1993a): Pseudo-bifacial leaves in *Senecio serpens*. – Bull. Main. Bot. Gard. Russian Acad. Sci. **168**: 72–78. [In Russian]

Timonin A. C. & Ozerova L. V. (1993b): Structure, origin and evolution of the terete leaves in section *Rowleyani* C. Jeffrey of genus *Senecio* L. (Asteraceae). – Proc. Russian Acad. Sci. Ser. Biol. **3**: 393–401. [In Russian]

Troll W. (1939): Vergleichende Morphologie der höheren Pflanzen. 1. Bd. Vegetationsorgane. 2. Teil. – Berlin: Gebrüder Borntraeger.

Troll W. & Meyer H.-J. (1955): Entwicklungsgeschichtliche Untersuchungen über das Zustandekommen unifazialer Blattstrukturen. – Planta **40**: 286–360.

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