

Molecular and phytochemical systematics of the subtribe Hypochaeridinae (Asteraceae, Cichorieae)

Neela Enke · Birgit Gemeinholzer · Christian Zidorn

Received: 22 November 2010 / Accepted: 28 November 2011 / Published online: 30 December 2011
© Gesellschaft für Biologische Systematik 2011

Abstract The systematics of the Hypochaeridinae subtribe was re-evaluated based on a combination of published and new molecular data. Newly found clades were additionally characterized using published and new phytochemical data. In addition to flavonoids and sesquiterpene lactones, which had been reviewed recently as chemosystematic markers in the Cichorieae, we analysed the reported occurrences of caffeic acid derivatives and their potential as chemosystematic markers. Our molecular results required further changes in the systematics of the genus *Leontodon*. Based on previous molecular data, *Leontodon* s.l.—i.e. including sections *Asterothrix*, *Leontodon*, *Thrinicia*, *Kalbfussia*, and *Oporinia* (Widder 1975)—had been split into the genera *Leontodon* s.str. (sections *Asterothrix*, *Leontodon*, and *Thrinicia*) and *Scorzoneroideis* (sections *Kalbfussia* and *Oporinia*). Instead of splitting *Leontodon* into even a higher number of segregate genera we propose to include *Hedypnois* into *Leontodon* s.str. and here into section *Leontodon*. Moreover, sections *Asterothrix* and *Leontodon* should be

merged into a single section *Leontodon*. The newly defined genus *Leontodon* is characterised by the unique occurrence of hydroxyhypocretenolides. The monophyly of the genus *Hypochaeris* is neither supported nor contradicted and potentially comprises two separate molecular clades. The clade *Hypochaeris* I comprises the majority of the European and Mediterranean as well as all South American taxa of *Hypochaeris* s.l. while the clade *Hypochaeris* II encompasses only *H. achyrophorus* L., *H. glabra* L., *H. laevigata* Benth. & Hook.f., and *H. radicata* L.

Keywords Asteraceae · Chemosystematics · Cichorieae · Hypochaeridinae · Molecular systematics

Introduction

The Hypochaeridinae are a subtribe of the Cichorieae, an Asteraceae tribe defined mainly by having only ligulate flowers and milky latex. According to the most recent treatment of the Cichorieae (Kilian et al. 2009), the Hypochaeridinae comprise seven genera: *Hedypnois*, *Helminthotheca*, *Hypochaeris*, *Leontodon*, *Picris*, *Scorzoneroideis*, and *Urospermum*. The monotypic genus *Prenanthes* s.str. has also been placed preliminarily within this subtribe but it is distinct morphologically and also shows affiliation to the subtribe *Lactucinae* in a chloroplast-marker-based phylogeny (Kilian et al. 2009). Using molecular methods, Samuel et al. (2006) found the genus *Leontodon*, in its traditional delimitation, to be diphyletic. Moreover, the same research group (Samuel et al., 2003) showed that all South American representatives from the genus *Hypochaeris* are related closely to, and are derived from, a European/North African ancestor that was putatively introduced via long distance dispersal in a single event.

N. Enke (✉)
Botanischer Garten und Botanisches Museum Berlin-Dahlem,
Freie Universität Berlin,
Königin-Luise-Str. 6-8,
14195 Berlin, Germany
e-mail: N.Enke@bgbm.org

B. Gemeinholzer
AG Spezielle Botanik, Justus-Liebig-Universität Giessen,
Heinrich-Buff-Ring 38,
35392 Giessen, Germany

C. Zidorn (✉)
Institut für Pharmazie and Center for Molecular Biosciences
Innsbruck, Universität Innsbruck,
Josef-Moeller-Haus, Innrain 52,
6020 Innsbruck, Austria
e-mail: Christian.H.Zidorn@uibk.ac.at

Recently, the flavonoids (Sareedenchai and Zidorn 2010) and sesquiterpenoids (Zidorn 2006, 2008b) known from taxa of the Cichorieae (Lactuceae) tribe were reviewed. Both classes of natural products proved to be suitable chemosystematic markers within the Cichorieae, though sesquiterpene lactones represent the more systematically informative class of compounds. In particular, hypocretenolides and isoetin derivatives are characteristic of the Hypochaeridinae (Zidorn 2008b; Sareedenchai and Zidorn 2010). While isoetin derivatives are also found in unrelated taxa such as *Isoetes*, hypocretenolides are virtually restricted to members of the Hypochaeridinae.

Although some genera of the Hypochaeridinae have already been analysed using molecular methods (Samuel et al. 2003, 2006), the present report constitutes the first comprehensive analysis combining data for all genera of the subtribe. In the current investigation we added additional ITS sequences to the published datasets, including sequences of representatives of the genus *Leontodon*. Moreover, we reviewed the literature on caffeic acid derivatives in the Hypochaeridinae to test their applicability as chemosystematic markers in the subtribe. Caffeic acid derivatives and chlorogenic acid in particular are ubiquitous in the plant kingdom. However, different sub-classes of caffeic acid derivatives like caffeoyl quinic and caffeoyl tartaric acids proved to be reliable chemosystematic markers within and for other genera of the Cichorieae tribe of the Asteraceae family (Zidorn et al. 2002, 2008). Moreover, caffeic acid derivatives are receiving growing attention due to their antioxidant and antiviral bioactivities (Bailly and Cotelle 2005).

The emerging systematic groupings are discussed in respect to evidence from molecular phylogenetics, phytochemical analysis, and morphology.

Material and methods

DNA analysis

Plant material

For ITS analysis, sequences of 99 taxa of the Hypochaeridinae, comprising the genera *Hedypnois*, *Helminthotheca*, *Hypochaeris*, *Leontodon*, *Picris*, *Prenanthes*, *Scorzoneroides*, and *Urospermum*, were generated or downloaded from GenBank (Table 1). All Hypochaeridinae sequences available in GenBank were included in the analysis, except very short fragments (200–300 bp). A reference list of valid Hypochaeridinae taxa (ICN International Cichorieae Network et al. 2009+) was used. Furthermore, *Hypochaeridinae* taxa of interest were added (GenBank

accession numbers JF801910–JF801918). As outgroups, taxa from the Hyoseridinae (*Hyoseris*, *Launaea*), Crepidinae (*Crepis*), and Lactucinae (*Lactuca*) were chosen (Table 1)—three subtribes related closely to the Hypochaeridinae subtribe (Kilian et al. 2009).

A list of taxa included in the DNA analysis is given in Table 1.

DNA extraction, amplification and sequencing

For the extraction of total genomic DNA, 20 mg dried leaf material was ground. DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol.

The ITS region (ITS1, 5.8 rRNA and ITS2) was amplified using the primer pairs ITS-P1 (White et al. 1990) and ITS-B (Blattner 1999). As external sequencing primers ITS-L (Hsiao et al. 1995) and ITS2-SR (5'-CTTAAACTCAGCGGGTAGTCCC-3') were used, a second read was done using internal primers ITS-C and ITS-D (both Blattner 1999).

PCR was carried out in a reaction volume of 25 μ l: 14.82 μ l ddH₂O, 2.5 μ l 10 \times buffer (Biodeal), 0.75 μ l of each primer (10 pm/ μ l), 0.04 μ l BSA (BioLabs), 2.5 μ l DMSO (Roth), 2.5 μ l dNTPs (Fermentas, each 2.5 μ l), 0.12 μ l *Taq*-Polymerase (Qiagen, 5u/ μ l), and 1 μ l template DNA solution. A touchdown PCR was carried out. After an initial denaturation (2 min at 94°C) five touchdown cycles were carried out. For the subsequent 25 cycles the following protocol was used: denaturation at 94°C (1 min), annealing at 52°C for 45 s, elongation at 72°C. The last step was a final elongation (10 min, 72°C).

After purification of the PCR products (Invitex, Berlin, Germany) the samples were sent to StarSeq (Mainz, Germany) for sequencing.

Sequence alignment and phylogenetic analysis

Sequences were edited using ChromasLite2000 (Technelysium, Helenvale, Australia) and aligned by hand using BioEdit (Hall 1999).

The dataset was analysed using three different approaches. First a maximum parsimony (MP) analysis was run on PAUP 4.0b10 (Swofford 2002) with equal weights, 1,000 closest sequence additions and tree bisection-reconnection (TBR) branch swapping, permitting ten trees to be held at each step. A strict consensus tree was computed. The trees were evaluated by a bootstrap analysis (Felsenstein 1985) with 1,000 replicates (using the same search strategy as the MP analysis) and MulTrees option in effect (but limiting the maximum tree number to 10,000).

For the maximum likelihood (ML) and Bayesian likelihood (BL) analysis, the optimal model of sequence

Table 1 List of plant material used for molecular analysis with relevant synonyms, GenBank accession numbers and voucher information for those accessions for which sequences were generated in this study (GenBank Acc. no. JF801910–JF801918)

Taxon ^a	Relevant synonyms	GenBank Acc. no.	Voucher
<i>Crepis alpestris</i> (Jacq.) Tausch		AJ633373	
<i>Crepis aurea</i> (L.) Cass ssp. <i>aurea</i>		EU363627	
<i>Crepis mollis</i> (Jacq.) Asch.		AJ633380	
<i>Helminthotheca aculeata</i> (Vahl) Lack		DQ451797	
<i>Helminthotheca comosa</i> (Boiss.) Holub	<i>Picris comosa</i> (Boiss.) B. D. Jacks. subsp. <i>comosa</i> ^b	AJ633323	
<i>Helminthotheca echioides</i> (L.) Holub		AF422123	
<i>Hyoseris radiata</i> L.		AF528494	
<i>Hypochaeris acaulis</i> (J. Rémy) Britton		AF528433	
<i>Hypochaeris achyrophorus</i> L.		AF528434	
<i>Hypochaeris angustifolia</i> (Litard. & Maire) Maire		AJ627258	
<i>Hypochaeris apargioides</i> Hook. & Arn.		AF528443	
<i>Hypochaeris arachnoides</i> Poir.	<i>Hypochaeris arachnoidea</i> (incorr. name) ^b	AJ627262	
<i>Hypochaeris clarionoides</i> (J. Rémy) Reiche		AF528446	
<i>Hypochaeris cretensis</i> (L.) Bory & Chaub.		AF528447	
<i>Hypochaeris gayana</i> (DC.) Cabrera		AF528451	
<i>Hypochaeris glabra</i> L.		AJ627264	
<i>Hypochaeris laevigata</i> (L.) Ces. & al.		AJ627265	
<i>Hypochaeris leontodontoides</i> Ball		AJ627266	
<i>Hypochaeris maculata</i> L. subsp. <i>Maculata</i>	<i>Hypochaeris grandiflora</i> Ledeb. ex Ledeb. ^b	AF528448	
<i>Hypochaeris meyeniana</i> (Walp.) Benth. & Hook. f.		AF528455	
<i>Hypochaeris microcephala</i> (Sch. Bip.) Cabrera		AJ627267	
<i>Hypochaeris oligocephala</i> (Svent. & Bramwell) Lack		AJ627268	
<i>Hypochaeris palustris</i> (Phil.) De Wild.		AF528456	
<i>Hypochaeris pampasica</i> Cabrera		AJ627269	
<i>Hypochaeris patagonica</i> Cabrera		AM932283	
<i>Hypochaeris radicata</i> L.		EF107656	
<i>Hypochaeris robertia</i> (Sch. Bip.) Fiori	<i>Robertia taraxacoides</i> (Loisel.) DC. ^{b,c}	Z93828	
<i>Hypochaeris rutea</i> Talavera		AJ627271	
<i>Hypochaeris salzmanniana</i> DC.		AJ627272	
<i>Hypochaeris scorzonerae</i> (DC.) F. Muell.		AF528462	
<i>Hypochaeris spathulata</i> (J. Rémy) Reiche		AF528464	
<i>Hypochaeris sessiliflora</i> Kunth in Humboldt & al.		AF528463	
<i>Hypochaeris taraxacoides</i> Ball		AF528466	
<i>Hypochaeris tenuifolia</i> (Hook & Arn.) Griseb.		AF528480	
<i>Hypochaeris thrincoides</i> (J. Rémy) Reiche		AF528467	
<i>Hypochaeris uniflora</i> Vill.		AF528481	
<i>Lactuca sativa</i> L.		L13957	
<i>Launaea lanifera</i> Pau		EU436699	
<i>Leontodon anomalus</i> Ball		DQ451753	
<i>Leontodon asperrimus</i> (Willd.) Ball		DQ451754	
<i>Leontodon berinii</i> (Bartl.) Roth		DQ451756	
<i>Leontodon boryi</i> DC.		DQ451757	
<i>Leontodon crispus</i> Vill.		DQ451761	
<i>Leontodon dubius</i> (Hoppe) Poir.	<i>Leontodon hispidus</i> L. subsp. <i>dubius</i> (Hoppe) Pawlowska	JF801914	Bozen/Trentino-Südtirol/Italy; Zidom CZ-20020808A-2

Table 1 (continued)

Taxon ^a	Relevant synonyms	GenBank Acc. no.	Voucher
<i>Leontodon farinosus</i> Merino & Pau		DQ451764	
<i>Leontodon graecus</i> Boiss. & Heldr.		DQ451765	
<i>Leontodon hispidus</i> L.		JF801910	Innsbruck-Land/Tirol/Austria; Zidorn 960813b
<i>Leontodon incanus</i> (L.) Schrank		JF801915	Innsbruck-Land/Tirol/Austria; Zidorn CZ-20030529B-1
<i>Leontodon intermedius</i> Huter		JF801913	Potenza/Basilicata/Italy; Zidorn CZ-20020403 C-1
<i>Leontodon kulczynskii</i> Popov	<i>Leontodon kulczynskii</i> Popov ^b	DQ451773	
<i>Leontodon maroccanus</i> (Pers.) Ball.	<i>Thrinicia maroccana</i> Pers.	DQ451778	
<i>Leontodon rhagadioloides</i> (L.) Enke & Zidorn ^d	<i>Hedypnois glabra</i> (incorr. name) ^{b,e}	AJ633308	
<i>Leontodon rhagadioloides</i> (L.) Enke & Zidorn ^d	<i>Hedypnois rhagadioloides</i> (L.) F.W.Schmidt ^b	AJ633307	
<i>Leontodon rigens</i> (Aiton) Paiva & Ormonde		DQ451789	
<i>Leontodon rosanii</i> (Ten.) DC ^f	<i>Leontodon rosani</i> (incorr. name) ^b , <i>Leontodon hirtus</i> L. ^a	DQ451792	
<i>Leontodon siculus</i> (Guss.) Nyman		JF801917	Messina/Sicilia/Italy; Zidorn CZ-20070413 C-1
<i>Leontodon siculus</i> (Guss.) Nyman		JF801912	Messina/Sicily/Italy; Zidorn CZ-20100510E-1
<i>Leontodon siculus</i> (Guss.) Nyman		JF801911	Catania/Sicily/Italy; Zidorn CZ-20100514A-1
<i>Leontodon saxatilis</i> Lam.	<i>Thrinicia saxatilis</i> (Lam.) Holub & Moravec	DQ451794	
<i>Leontodon saxatilis</i> subsp. <i>rothii</i> Maire	<i>Leontodon longirostris</i> (Finch & P.D.Sell) Talavera ^b <i>Thrinicia saxatilis</i> subsp. <i>hispida</i> (Roth) Holub & Moravec	DQ451776	
<i>Leontodon tenuiflorus</i> (Gaudin) Rchb.		JF801916	Trento/Trentino-Südtirol/Italy; Zidorn CZ-20040521A-1
<i>Leontodon tingitanus</i> (Boiss. & Reut.) Ball	<i>Thrinicia tingitana</i> Boiss. & Reut.	DQ451795	
<i>Leontodon tuberosus</i> L.		AF528487	
<i>Leontodon villarsii</i> (Willd.) Loisel ^f	<i>Leontodon hirtus</i> L. ^a	JF801918	Hautes Alpes/Provence-Alpes-Côte d'Azur/France; Zidorn CZ-20090716 C-1
<i>Picris angustifolia</i> DC.		DQ451799	
<i>Picris asplenioides</i> L.	<i>Picris coronopifolia</i> (Desf.) DC. ^b	DQ451801	
<i>Picris asplenioides</i> ssp. <i>saharae</i> (Coss. & Kralik) Dobignard	<i>Picris saharae</i> (Coss. & Kralik) Hochr. ^b	DQ451807	
<i>Picris burbridgeae</i> S. Holzapfel		EU352247	
<i>Picris cupuligera</i> (Durieu) Walp.		DQ451803	
<i>Picris hieracioides</i> L.		AF528490	
<i>Picris hispanica</i> (Willd.) P.D.Sell		DQ451808	
<i>Picris nuristanica</i> Bormm.		DQ451810	
<i>Picris pauciflora</i> Willd.		DQ451811	
<i>Picris rhagadioloides</i> (L.) Desf.		DQ451815	
<i>Picris scabra</i> Forssk.		DQ451812	
<i>Picris scabra</i> subsp. <i>abyssinica</i> (Sch. Bip.) Smalla	<i>Picris abyssinica</i> Sch.Bip. ^b	DQ451798	
<i>Picris squarrosa</i> Steetz		DQ451813	
<i>Picris strigosa</i> M.Bieb.		DQ451814	
<i>Picris willkommii</i> (Sch.Bip. ex Willk.) Nyman		DQ451805	

Table 1 (continued)

Taxon ^a	Relevant synonyms	GenBank Acc. no.	Voucher
<i>Prenanthes purpurea</i> L.		AJ633343	
<i>Scorzoneroides autumnalis</i> (L.) Moench	<i>Leontodon autumnalis</i> L. ^b	AJ633313	
<i>Scorzoneroides cantabrica</i> (Widder) Holub	<i>Leontodon cantabricus</i> Widder ^b	DQ451758	
<i>Scorzoneroides carpetana</i> (Lange) Greuter	<i>Leontodon carpetanus</i> Lange ^b	DQ451759	
<i>Scorzoneroides cichoriacea</i> (Ten.) Greuter	<i>Leontodon cichoraceus</i> (incorr. name) ^{b,g}	DQ451760	
<i>Scorzoneroides crocea</i> (Haenke) Holub	<i>Leontodon croceus</i> Haenke ^b	DQ451762	
<i>Scorzoneroides duboisii</i> (Sennen) Greuter	<i>Leontodon duboisii</i> Sennen ^b	DQ451763	
<i>Scorzoneroides garnironii</i> (Emb. & Maire) Greuter & Talavera		GQ494878	
<i>Scorzoneroides helvetica</i> (Mérat) Greuter	<i>Leontodon helveticus</i> Mérat ^b	DQ451768	
<i>Scorzoneroides kralikii</i> (Pomel) Greuter & Talavera		GQ494871	
<i>Scorzoneroides laciniata</i> (Bertol.) Greuter	<i>Leontodon laciniatus</i> (Bertol.) Bomm. ^b	DQ451774	
<i>Scorzoneroides montaniformis</i> (Widder) Gutermann	<i>Leontodon montaniformis</i> Widder ^b	DQ451780	
<i>Scorzoneroides montana</i> (Lam.) Holub	<i>Leontodon montanus</i> Lam. ^b	DQ451781	
<i>Scorzoneroides muelleri</i> (Sch.Bip.) Greuter & Talavera	<i>Leontodon muelleri</i> (Sch.Bip.) Fiori ^b	DQ451786	
<i>Scorzoneroides nevadensis</i> (Lange) Greuter	<i>Leontodon nevadensis</i> Lange ^b	DQ451784	
<i>Scorzoneroides oraria</i> (Maire) Greuter & Talavera		GQ494880	
<i>Scorzoneroides palisiae</i> (Izuzq.) Greuter & Talavera	<i>Leontodon palisiae</i> Izuzq. ^b	DQ451787	
<i>Scorzoneroides pseudotaraxaci</i> (Schur) Holub		GQ494905	
<i>Scorzoneroides pyrenaica</i> (Gouan) Holub	<i>Leontodon pyrenaicus</i> Gouan ^b	DQ451788	
<i>Scorzoneroides rilaensis</i> (Hayek) Holub	<i>Leontodon rilaensis</i> Hayek ^b	DQ451791	
<i>Scorzoneroides salzmannii</i> (Sch. Bip.) Greuter & Talavera		GQ494875	
<i>Urospermum dalechampii</i> (L.) F.W.Schmidt		DQ451820	
<i>Urospermum picroides</i> (L.) F.W.Schmidt		DQ451821	

^a Current name according to ICN International Cichorieae Network et al. (2009+)

^b Name found in GenBank

^c Placement in genus *Robertia* suggested by molecular evidence

^d New name from this paper

^e Voucher consulted and determined as *Hedypnois cretica* (L.)Dum. Cours. which is a synonymy of *Hedypnois rhagadioloides* (L.) F.W. Schmidt

^f Here we disagree with the ICN nomenclature due to molecular and cytological evidence, see discussion for details

^g *Leontodon cichoriaceus* (Ten.) Sanguin

evolution that best fits the sequence data (GTR + G + I; Tavaré 1986) was calculated under the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) using modeltest 3.7 (Posada and Crandall 1998).

Second, an ML analysis was conducted using RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008), ML search option (GTR + G + I) and 10,000 bootstrap replicates (model GTRCAT as implemented in RAxML for the rapid bootstrap algorithm).

A third analysis was run on MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using gamma distribution rate variation among sites and 1,000,000 generations of the MCMC chains in two independent runs of four chains apiece; otherwise the default parameters were used. The first 25,000

trees were discarded as burn-in; the rest was used to calculate a 50% majority rule consensus tree.

The strict consensus tree of the MP analysis was compared to the 50 % majority rule tree of the BL analysis, and the best ML tree found by RAxML. Trees were drawn using FigTree v1.2.2 (Rambaut, 2008) and Adobe Illustrator (Adobe Systems, San Jose, CA).

Phytochemical methods

Plant material

Details on the origin of plant material used for new phytochemical investigations are available in Table 2.

Table 2 Material for phytochemical analysis with relevant synonymy and voucher information

Taxon	Synonyms ^a	Voucher
<i>Crepis aurea</i> (L.) Cass.		CZ-20090614A-1, Innsbruck, Hechenberg, below Kirchbergalm; Innsbruck-Stadt/Tirol/Austria, N 47°16'54.2", E 11°16'55.1", alt.: 1300 m, IB 33276
<i>Hedypnois cretica</i> (L.) Willd.	<i>Hedypnois rhagadioloides</i> (L.) F. W. Schmidt	CZ-20090417A-1, between Vélez Rubio and Santa Maria de Nieva/Almeria/Andalucia/Spain, N 37°37'26.8", W 02°00'53.6", alt.: 890 m, 17.04.2009 IB 33277
<i>Hypochaeris cretensis</i> Benth. & Hook.f.		CZ-20010717B-1, S Monte Amara, Maiella/L'Aquila/Abruzzo/Italy, N 42°06'00.6", E 14°03'33.0", alt.: 1540 m, 17.07.2001 IB 33279
<i>Hypochaeris laevigata</i> Ces., Passer. & Gib		CZ-20100511A-1, wind park near Vizzini/Catania/Sicily/Italy, N 37°10'21.1", E 14°47'36.4", alt.: 770 m, 11.05.2010 IB 33283
<i>Hypochaeris maculata</i> L.		CZ-20090718A-3, between Morinesio and Monte Nebin/Cuneo/Piemonte/Italy, N 44°31'35.2", E 07°08'36.2", alt.: 1910 m, 17.08.2009 IB 33286
<i>Leontodon siculus</i> (Guss.) Nyman		CZ-20100514A-1, Nebrodi between Randazzo and Santa Maria del Bosco/Catania/Sicily/Italy, N 37°54'32.5", E 14°56'32.8", alt.: 920 m, 14.05.2010 IB 33284
<i>Leontodon villarsii</i> (Willd.) Loisel.	<i>Leontodon hirtus</i> L.	CZ-20090716 C-1, Mont Dauphin/Hautes Alpes/Provence-Alpes-Côte d'Azur/France, N 44°40'15.6", E 06°37'43.1", alt.: 1050 m, 16.07.2009 IB 33285
<i>Picris hieracioides</i> L. subsp. <i>villarsii</i> (Jord.) Nyman	<i>Picris hieracioides</i> subsp. <i>umbellata</i> (Schrank) Ces.	CZ-20010801A-1, Ötztal near Zwieselstein/Tirol/Austria, N 46°56'19.2", E 11°01'31.4", alt.: 1480 m, 01.08.2001 IB 33281
<i>Prenanthes purpurea</i> L.		MP-20100802A-1, Innsbruck, between Planötzenhof and Höttinger Bild, Innsbruck/Tyrol/Austria, N 47°16'35.2", E 11°22'24.4", alt.: 820 m, 02.08.2010 IB 33282
<i>Robertia taraxacoides</i> DC.	<i>Hypochaeris robertia</i> (Sch. Bip.) Fiori	CZ-20010716B-1, SW Corno Grande/L'Aquila/Abruzzo/Italy, N 42°27'54.5", E 13°33'29.6", alt.: 2350 m, 16.07.2001 IB 33280
<i>Urospermum picroides</i> (L.) F.W.Schmidt		CZ-20090417A-4, between Vélez Rubio and Santa Maria de Nieva/Almeria/Andalucia/Spain, N 37°37'26.8", W 02°00'53.6", alt.: 890 m, 17.04.2009 IB 33278

^a Current names according to ICN International Cichorieae Network et al. (2009+), if differing from taxon name

HPLC-analyses

Phenolics were detected in air-dried, ground plant material using the HPLC-systems described in Zidorn and Stuppner (2001), with the following change in procedure: extracts were prepared using three 30-min cycles of ultrasonication instead of three 5-min cycles using an Ultraturax apparatus. Hypocretenolides—including sub-aerial parts of *L. siculus* and *L. villarsii*—were analysed using the methodology described in Zidorn et al. (2007).

Results

DNA Analysis

The topologies of the ML and BL 50% majority rule consensus trees are congruent (Fig. 1), whereas the MP strict consensus tree shows a slightly different topology, especially concerning the position of the genera *Robertia* and *Urospermum* as well as the monophyly of the genus *Hypochaeris* (Fig. 2). In the text, bootstrap values are given first for ML, second for posterior probabilities (pp) of the BL, and third bootstrap values for MP (ML/BL/MP). The

minus sign denotes weak (below 75%) support by bootstrap or posterior probabilities.

The backbone of the generic delimitation within the subtribe is supported only partly by ITS sequences. The Hypochaeridinae genera *Helminthotheca* (100/1.00/100), *Picris* (95/1.00/87), *Urospermum* (100/1.00/80) are monophyletic, as is *Scorzoneroides* (100/1.00/100; Figs. 1,2). *Robertia* is a monotypic genus and of unclear phylogenetic association. In the ML and BL analyses, it is sister to a clade consisting of *Leontodon*, *Helminthotheca* and *Picris* (Fig. 1), whereas in the MP analysis it is sister to all *Hypochaeridinae* genera except for *Prenanthes* and *Urospermum*.

The genus *Hypochaeris*, however, consists of two main clades [H I (99/1.00/97) and H II (94/1.00/90); Figs. 1,2]. Both the ML and BL analyses (Fig. 1) recover a monophyletic genus *Hypochaeris* (Fig. 1). By contrast, MP analysis results in two independent clades, H I and H II—the first constitutes the sister group to the genera *Leontodon*, *Helminthotheca*, *Picris*, and *Scorzoneroides*; while the second is the sister group to *Hypochaeris* clade H I and these genera (Fig. 2). None of these affiliations is supported.

The species of the former genus *Leontodon* s.l. (sensu Widder 1975) form three clades intercalated by species not traditionally included in *Leontodon* s.l.: clade 1, identical to the genus *Scorzoneroides* (*Leontodon* sections *Kalbfussia* and

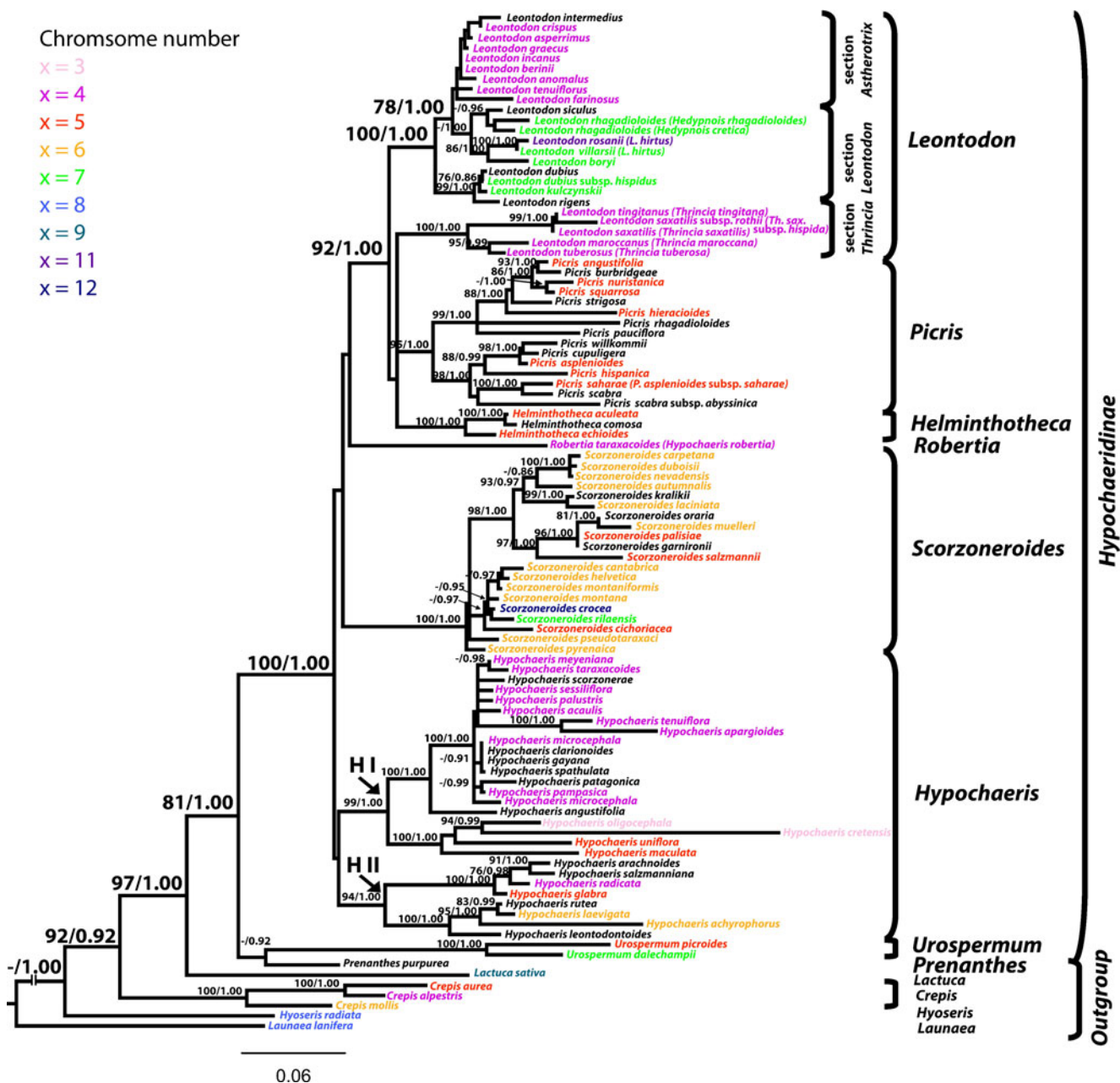


Fig. 1 Maximum likelihood (ML) phylogram. Bootstrap support values of ML and posterior probabilities of the Bayesian Likelihood (BL) analysis are given above branches (ML/BL). H I and H II denote clades

Oporinia) sensu Samuel et al. (2006), clade 2 equaling *Leontodon* section *Thrinicia* sensu Widder (1975), and clade 3 comprising *Leontodon* sections *Asterothrix* and *Leontodon* sensu Widder (1975) (Figs. 1,2). The *Scorzoneroideis* clade is well supported by both bootstrap values and posterior probabilities (100/1.00/100, Figs. 1,2). *Leontodon* sections *Asterothrix* and *Leontodon* sensu Widder (1975) form a clade which also includes members of the genus *Hedypnois* (Figs. 1,2). The closest sister taxon to members of *Hedypnois* is *L. siculus* (−/0.96/−). Considering the pronounced morphological differences between *Hedypnois* and *L. siculus* on the one hand and the

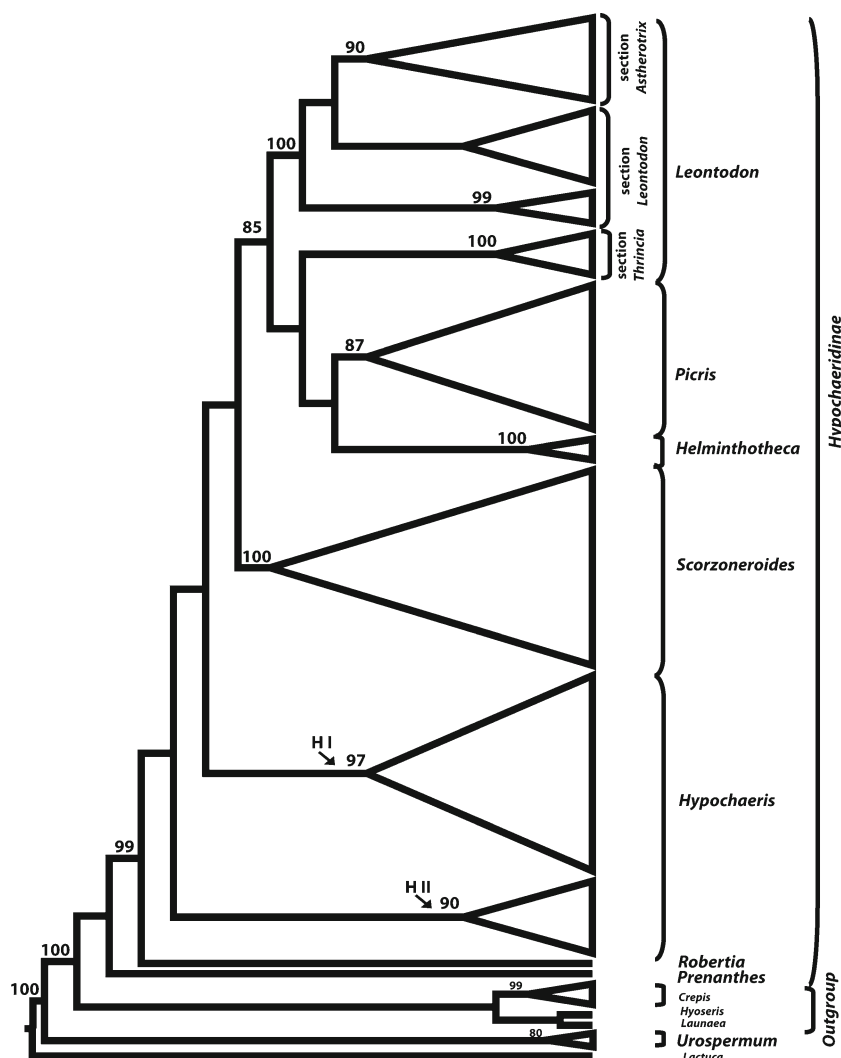
discussed in the text. Basic chromosome numbers are indicated with coloured codes

very close similarity of *L. siculus* and *L. hispidus* on the other hand this finding was unexpected. Therefore, a total of three accessions of *L. siculus* was sequenced (Table 1). However, all accessions had identical sequences and, thus, these accessions are represented by only one branch in the phylogram in Fig. 1.

The two investigated *Hedypnois* species and *L. siculus* are sister to a group comprising the *Leontodon* species *L. boryi*, *L. rosani*, and *L. villarsii* (−/1.00/−).

The species of *Leontodon* section *Thrinicia* (100/1.00/100) cluster together with the genera *Picris* and *Helminthochea* (Figs. 1,2). This clade, however, is not supported (Figs. 1,2).

Fig. 2 Maximum parsimony (MP) 50% majority rule consensus tree. Bootstrap support values given above branches. Clades contain species according to Fig. 1



Phytochemical analysis

Phytochemical data newly acquired in the course of this study are summarised in Table 3. In addition to some new sources of caffeic acid derivatives and flavonoids, two more sources of hypocretenolides were discovered. In extracts of *L. siculus*, the same set of sesquiterpene lactones was found as in *L. hispidus*: 14-hydroxyhypocretenolide, 11 β ,13-dihydro-14-hydroxyhypocretenolide, 14-hydroxyhypocretenolide- β -glucopyranoside, 11 β ,13-dihydro-14-hydroxyhypocretenolide- β -glucopyranoside, and 14-hydroxyhypocretenolide- β -glucopyranoside-4'-14''-hydroxyhypocretenoate. On the other hand, *L. villarsii* extracts contained the same array of sesquiterpene lactones as reported for *L. rosani* (Zidorn et al. 2007: 15-hydroxyhypocretenolide, 11 β ,13-dihydro-15-hydroxyhypocretenolide, 15-hydroxyhypocretenolide- β -glucopyranoside, and 11 β ,13-dihydro-15-hydroxyhypocretenolide- β -glucopyranoside).

Moreover, literature data on caffeic acid derivatives in the Hypochaeridinae are compiled systematically for the first

time in Table 4. Reviews on flavonoids (Sareedenchai and Zidorn 2010) and sesquiterpene lactones (Zidorn 2006, 2008b) were compiled earlier and are supplemented here with some new data (Table 3, Figs. 3–5).

The most important chemosystematic markers and their distributions within the Hypochaeridinae are depicted in Figs. 3–5. Caffeoyltartaric acid derivatives (Fig. 3) are distributed widely within the Hypochaeridinae and have been detected in all of its major clades and also in a considerable number of taxa. Isoetin derivatives (Fig. 4), are a rare class of flavonoids with a hard-to-interpret general distribution within the plant kingdom. However, in the Cichorieae tribe of the Asteraceae family, isoetin derivatives have been reported from a number of taxa and have also been found in all major clades within the Hypochaeridinae. Hypocretenolides (Fig. 5) an unusual sub-class of guaianolide-type sesquiterpene lactone derivatives featuring a 12,5- instead of a 12,6-lactone ring have—with one exception, *Crepis aurea*—so far only been reported from members of the Hypochaeridinae. Within the Hypochaeridinae, hypocretenolides with no hydroxylation in

Table 3 New phytochemical data acquired in the course of this investigation. *F* Flowering heads, *L* leaves, *R* rhizomes and roots; *CAF* caffeic acid; *CGA* chlorogenic acid, *DCA* 3,5-dicaffeoylquinic acid, *CTA* caffeoyl tartaric acid, *CCA* cichoric acid, *LUT* luteolin, *L7GC* luteolin 7-O-glucoside, *L7GU* luteolin 7-O-glucuronide, *L4'GC* luteolin 4'-O-glucoside, *14-OH-HYPs* 14-hydroxyhypocretenolides (14-hydroxyhypocretenolide, 11 β ,13-dihydro-14-hydroxyhypocretenolide, 14-hydroxyhypocretenolide-

β -glucopyranoside, 11 β ,13-dihydro-14-hydroxyhypocretenolide- β -glucopyranoside, and 14-hydroxyhypocretenolide- β -glucopyranoside-4'-14''-hydroxyhypocretenoate), *15-OH-HYPs* 15-hydroxyhypocretenolides (15-hydroxyhypocretenolide, 11 β ,13-dihydro-15-hydroxyhypocretenolide, 15-hydroxyhypocretenolide- β -glucopyranoside, and 11 β ,13-dihydro-15-hydroxyhypocretenolide- β -glucopyranoside)

Taxon	Organ	Caffeic acid derivatives				Flavonoids				Sesquiterpenoids	
		CAF	CGA	DCA	CTA	CCA	LUT	L7GC	L7GU	L4'GC	14-OH-HYPs
<i>Hedypnois cretica</i>	F		+	+	+	+	+	+	+		
<i>Hypochaeris cretensis</i>	F	+	+	+	+	+					
<i>Hypochaeris maculata</i>	F	+	+	+	+	+					
<i>Hypochaeris laevigata</i>	F	+	+	+	+	+	+	+	+		
<i>Leontodon rosani</i>	L		+	+	+	+					
<i>Leontodon siculus</i>	F		+	+			+	+	+	+	
	R									+	
<i>Leontodon villarsii</i>	L		+	+	+	+					
	R										+
<i>Picris hieracioides</i> subsp. <i>villarsii</i>	F	+	+	+	+	+					
<i>Prenanthes purpurea</i>	F + L		+	+	+	+					
<i>Robertia taraxacoides</i>	F		+	+			+	+			
<i>Urospermum picroides</i>	F		+	+							

either position C-14 or C-15 were reported from *Hypochaeris cretensis*. Hypocretenolides with a hydroxyl group in either position C-14 or C-15 were reported from *Crepis aurea* and the clade comprising *Hedypnois cretica*, *Hedypnois rhagadioloides*, *Leontodon boryi*, *L. hispidus*, *L. kulczinskii*, *L. rigens*, *L. rosani*, *L. siculus*, and *L. villarsii*.

Discussion

As the results of the ITS based molecular phylogeny show the need to reevaluate some of the generic circumscriptions within *Hypochaeridinae*, especially in the genus *Leontodon*, we will discuss some changes in respect to molecular, phytochemical as well as morphological characters. This also takes into account evidence from previous molecular studies (e.g. Samuel et al. 2003, 2006; Kilian et al. 2009).

DNA analysis

Leontodon

The genus *Leontodon* s.l. Widder (1975) is scattered among three well supported clades: the first clade includes the genus *Hedypnois* (type species: *Hedypnois rhagadioloides*)

and *Leontodon* sections *Asterothrix* and *Leontodon* and thus also the type species of the genus, *Leontodon hispidus* L.; the second clade comprises only *Leontodon* section *Thrinacia*; and third only the genus *Scorzoneroides* (former *Leontodon* sections *Kalbfussia* and *Oporinia*). Due to the fact that the other *Leontodon* sections are related more closely to the genera *Helminthotheca*, *Hypochaeris*, *Picris*, and *Robertia* than to *Scorzoneroides*, *Scorzoneroides* has to be classified as a genus distinct from *Leontodon* as already proposed by Samuel et al. (2006). Nomenclatural consequences for species from the Euro-Mediterranean were drawn by Greuter et al. (2006). The separation of *Scorzoneroides* is also supported by cytological evidence as the prevailing basic chromosome number is $x=5$ (Fig. 1)—a number so far not reported from *Leontodon* species.

Leontodon boryi, *L. rosani*, and *L. villarsii* constitute the sister clade to *Hedypnois* and *L. siculus* (Fig. 1). These species are presumably of hybrid origin—a hypothesis supported by their intermediate position (with respect to their supposed parental species) in the phylogeny as well as by their aberrant karyotype (Samuel et al. 2006). The species of *Leontodon* section *Asterothrix* have $2n=8$ chromosomes, the species of *Leontodon* section *Leontodon* $2n=14$. *Hedypnois rhagadioloides* forms a polymorphic complex with varying chromosome numbers ($2n=8, 11, 12, 13, 14, 16$; Nordenstam 1971; Carr et al. 1999). *Leontodon boryi* and *L.*

Table 4 Overview of reported occurrences of caffeic acid derivatives in the Hypochaeridinae. *CAF* caffeic acid; *CGA* chlorogenic acid; *iso-CGA* isochlorogenic acid; *DCA* unspecified dicaffeoylquinic acid

derivative; *3,5-DCA* 3,5-dicaffeoylquinic acid; *4,5-DCA* 4,5-dicaffeoylquinic acid; *PHA* phaselic acid; *CTA* caffeoyl tartaric acid; *CCA* cichoric acid

Taxon	CAF	CGA	iso-CGA	DCA	3,5-DCA	4,5-DCA	PHA	CTA	CCA	Reference
<i>Hedypnois cretica</i>		+			+			+	+	Present study
<i>Helminthotheca echioides</i>	+		+	+						Rios et al. 1992
<i>Hypochaeris cretensis</i>	+	+			+			+	+	Present study
<i>Hypochaeris maculata</i>	+	+			+			+	+	Present study
<i>Hypochaeris laevigata</i>	+	+			+			+	+	Present study
<i>Hypochaeris radicata</i>	+	+	+		+			+	+	Rios et al. 1992; Zidorn et al. 2005
<i>Leontodon</i> sect. <i>Asterothrix</i> (except <i>L. crispus</i>)		+			+	+		+	+	Zidorn 1998, 2008a
<i>Leontodon crispus</i>		+			+		+			Zidorn 1998, Zidorn et al. 2006
<i>Leontodon</i> sect. <i>Leontodon</i>		+			+	+		+	+	Zidorn 1998, 2008a
<i>Leontodon siculus</i>		+			+					Present study
<i>Leontodon rosani</i>		+			+			+	+	Present study
<i>Leontodon villarsii</i>		+			+			+	+	Present study
<i>Leontodon</i> sect. <i>Thrinicia</i>		+			+	+		+	+	Zidorn 1998
<i>Leontodon saxatilis</i>			+							Rios et al. 1992
<i>Picris hieracioides</i> subsp. <i>villarsii</i>	+	+			+			+	+	Present study
<i>Prenanthes purpurea</i>		+			+			+	+	Present study
<i>Robertia taraxacoides</i>		+			+					Present study
<i>Scorzoneroides</i> div. taxa		+			+	+		+	+	Zidorn 1998, 2008a; Zidorn and Stuppner 2001
<i>Urospermum dalechampii</i> & <i>Urospermum picroides</i>	+	+	+	+						Rios et al. 1992
<i>Urospermum picroides</i>		+			+					Present study

villarsii have a chromosome number of $x=7$, whereas *L. rosani* has a chromosome number of $x=11$ (Lippi and Garbari 2004; Samuel et al. 2006; Fig. 1). The former two taxa could result from two ancestral taxa with $2n=14$ (Samuel et al. 2006). *Leontodon rosani* is thought to have originated from an *L. villarsii*-like ancestor ($x=7$) and a member of *Leontodon* section *Asterothrix* ($x=4$) (Pittoni 1974; Samuel et al. 2006). However, an ancestor from *Hedypnois* instead of a parent from either former *Leontodon* section *Asterothrix* ($x=4$) or former *Leontodon* section *Leontodon* ($x=7$) is also a possibility as some *Hedypnois* taxa can possess either $x=4$ or $x=7$.

As *Leontodon* section *Leontodon* is paraphyletic in its current circumscription, we propose to incorporate *Leontodon* section *Asterothrix* as well as the former genus *Hedypnois* into *Leontodon* section *Leontodon*, which is then well supported (100/99/1.00, Figs. 1,2)

The species found in the clade *Thrinicia* all belong to *Leontodon* section *Thrinicia* sensu Widder (1975) and share the basic chromosome number $x=4$ (Fig. 1). Stebbins et al. (1953) assumed a trend towards a reduction of chromosome

numbers within the tribe Cichorieae (also see Babcock 1947a,1947b). Therefore, section *Thrinicia* was considered to be derived within *Leontodon* s.l. due to its low chromosome number ($x=4$; Izuzquiza and Feliner 1991). Molecular and karyological studies in other genera of the Cichorieae suggest that chromosome number reductions as well as increases can coexist within one genus (e.g. *Crepis*; Enke and Gemeinholzer 2008; Enke et al. 2011) and are therefore not always suitable to infer phylogenetic relationships. The question of whether *Thrinicia*, as part of the sister group of the clade encompassing *Hedypnois* and *Leontodon* sections *Asterothrix* and *Leontodon*, constitutes a separate genus or a section of *Leontodon* needs further investigation.

Hypochaeris

Analysis of the nuclear marker ITS did not provide strong support for or against the monophyly of the genus *Hypochaeris* (Figs. 1,2). Members of *Hypochaeris* clustered in two clades H I [Figs. 1,2; including *H. maculata* (L.) Bernh., the designated type species of a putative segregate genus

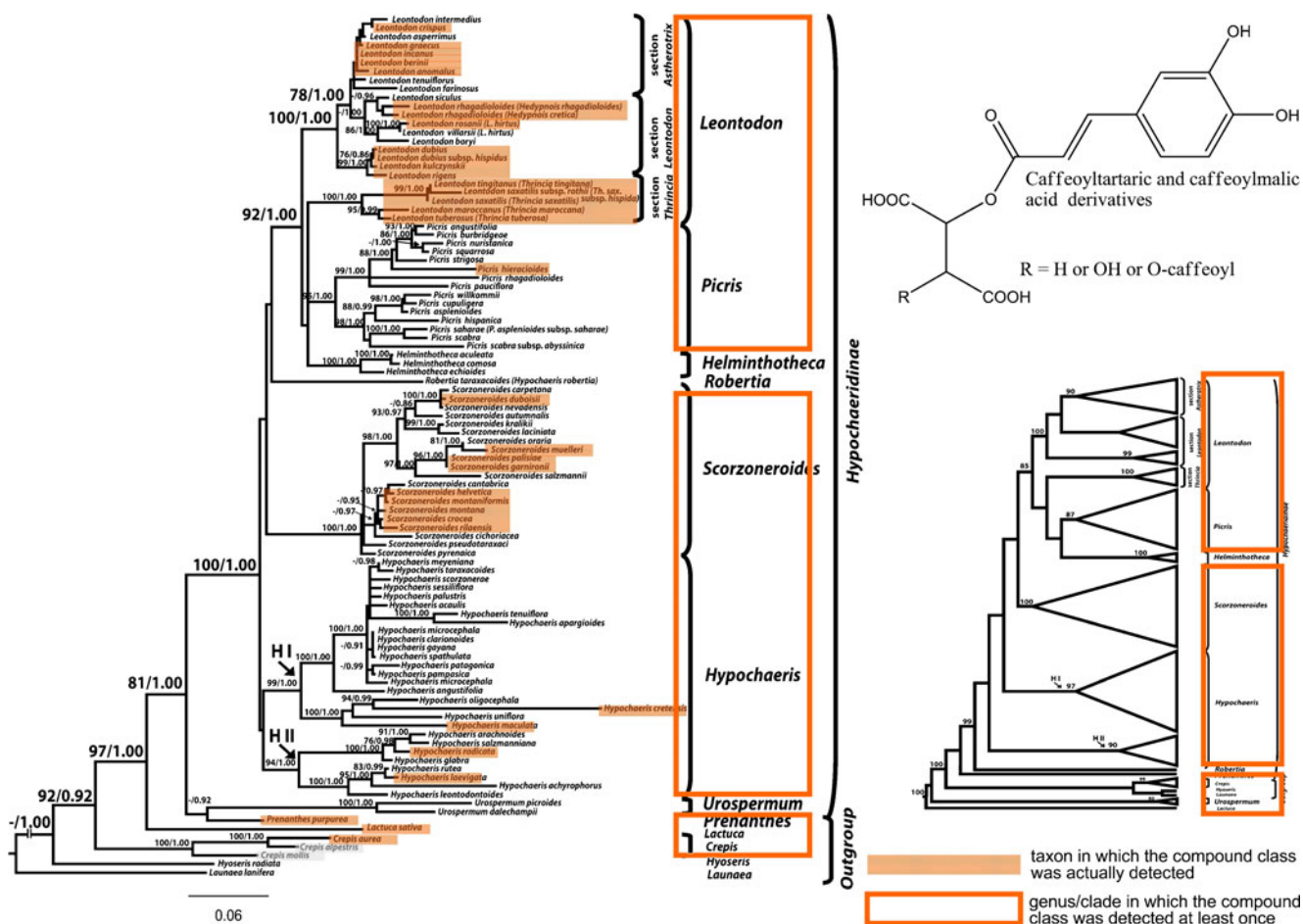


Fig. 3 Overview of the distribution of caffeoyl tartaric acid derivatives within the phylogenetic context of the Hypochaeridinae

Trommsdorffia Bernh.] and H II (Figs. 1, 2; including the *Hypochaeris* type species *Hypochaeris radicata* L.). Both clades H I and H II are supported by nuclear and plastid markers (Cerbah et al. 1998, Samuel et al. 2003). This holds true also for the bifurcation that separated the two groups in the study by Samuel et al. (2003). The nuclear ITS data and the combined phylogeny of ITS and the plastid markers *trnL* and *matK* supported the monophyly of *Hypochaeris* in the study of Samuel et al. (2003). An individual assessment of each plastid marker suggests the following conclusions: *matK* (Samuel et al. 2003) did not resolve the relationship between *Hypochaeris* clades H I, H II, and *Scorzoneroideis*; the *trnL* intron and the *trnL/trnF* spacer region (Samuel et al. 2003) supported two independent genera *Trommsdorffia* and *Hypochaeris* congruent with clades *Hypochaeris* H I and H II, respectively.

Interestingly, an investigation on the geographical origin of *Hypochaeris* by Tremetsberger et al. (2005) based on ITS sequences, which included only one *Leontodon* species (*L. saxatilis*), did not support the monophyly of *Hypochaeris* s. l. *Trommsdorffia* was also treated as a separate genus by Tzevelev and Fedorov (2003).

Hypochaeris clade H I comprises the South American species of *Hypochaeris* section *Achyrophorus* as well as the European and Asian species of the *Hypochaeris* sections *Achyrophorus* and *Metabasis*. This is corroborated by the findings of Samuel et al. (2003). So far, only species with $x=4$ have been reported for the South American members of H I (Fig. 1; see also Weiss et al. 2003, Weiss-Schneeweiss et al. 2003). For the subclade of clade H I, which encompasses the old world members of clade H I, chromosome numbers $x=3$ and $x=5$ have been reported (Fig. 1).

Hypochaeris clade H II includes the old world *Hypochaeris* sections *Hypochaeris* and *Seriola*. These sections feature a pappus of two rows of hairs, whereas the other European sections *Achyrophorus* and *Metabasis* feature either one row of hairs or fimbriate scales. The basic chromosome numbers reported are $x=4$, $x=5$, and $x=6$ (Fig. 1).

Whether the genus *Hypochaeris* is monophyletic or not should be the subject of further molecular and morphological analyses with an extensive taxon sampling before any taxonomic conclusions should be drawn.

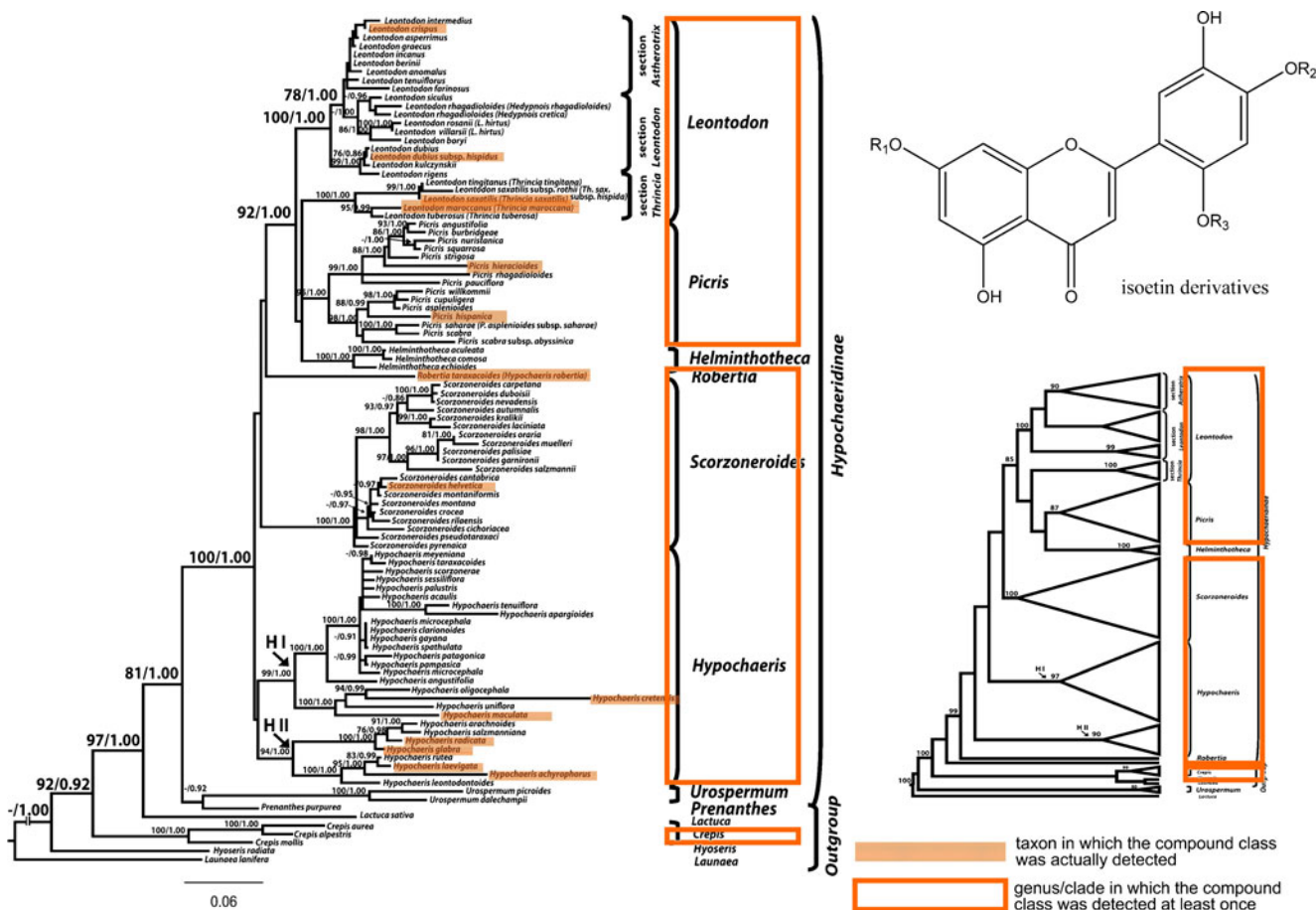


Fig. 4 Overview of the distribution of isoetin derivatives within the phylogenetic context of the Hypochaeridinae

Remaining taxa

The exact position of *Robertia taraxacoides* (synonym: *Hypochaeris robertia*) within the *Hypochaeridinae* remains uncertain; nonetheless, the results presented here suggest reinstating the monotypic genus *Robertia* DC. instead of merging *Robertia* with *Hypochaeris* (synonym: *Hypochaeris robertia*). This was also suggested by Siljak-Yakovlev et al. (1994) based on cytogenetic studies.

In contrast to *Hypochaeris* and *Leontodon* s.l., the genera *Helminthotheca*, *Picris*, *Prenanthes* and *Urospermum* can be maintained in their current circumscription. *Picris* and *Helminthotheca*, in particular, are monophyletic and share a basic chromosome number $x=5$ (Fig. 1).

Phytochemical analysis

As discussed in some detail elsewhere (Zidorn 2008b), a major problem with the application of literature data to the phytochemical characterization of taxa is the diverging degree of coverage and the varying quality of the phytochemical data in the literature. This problem is also present in the Hypochaeridinae. However, many of the published data in

the Hypochaeridinae are derived from one of the authors of this study (C.Z.) and thus were produced using analytical procedures comparable to those used to generate the new phytochemical data here, in particular data on phenolic compounds contained in some species of the Hypochaeridinae.

Caffeic acid derivatives

The Cichorieae are generally a rich source of caffeic acid derivatives. However, while some compounds, such as chlorogenic acid and 3,5-dicaffeoylquinic acid, are virtually ubiquitous, others, such as as caffeoyltartaric acid and cichoric acid, have a more restricted distribution. In two extensive studies of the genera *Crepis* and *Hieracium*, respectively, caffeoyl tartaric acid and cichoric acid were found in nearly all investigated taxa of *Crepis* but in none of *Hieracium* (Zidorn et al. 2002, 2008). Thus, in the present account, we investigated whether caffeic acid derivatives might also serve in the Hypochaeridinae as chemosystematic markers to either characterize the group as a whole or to distinguish between sub-groups within the Hypochaeridinae. As is evident from Table 4, the currently known distribution of caffeic acid derivatives in the Hypochaeridinae

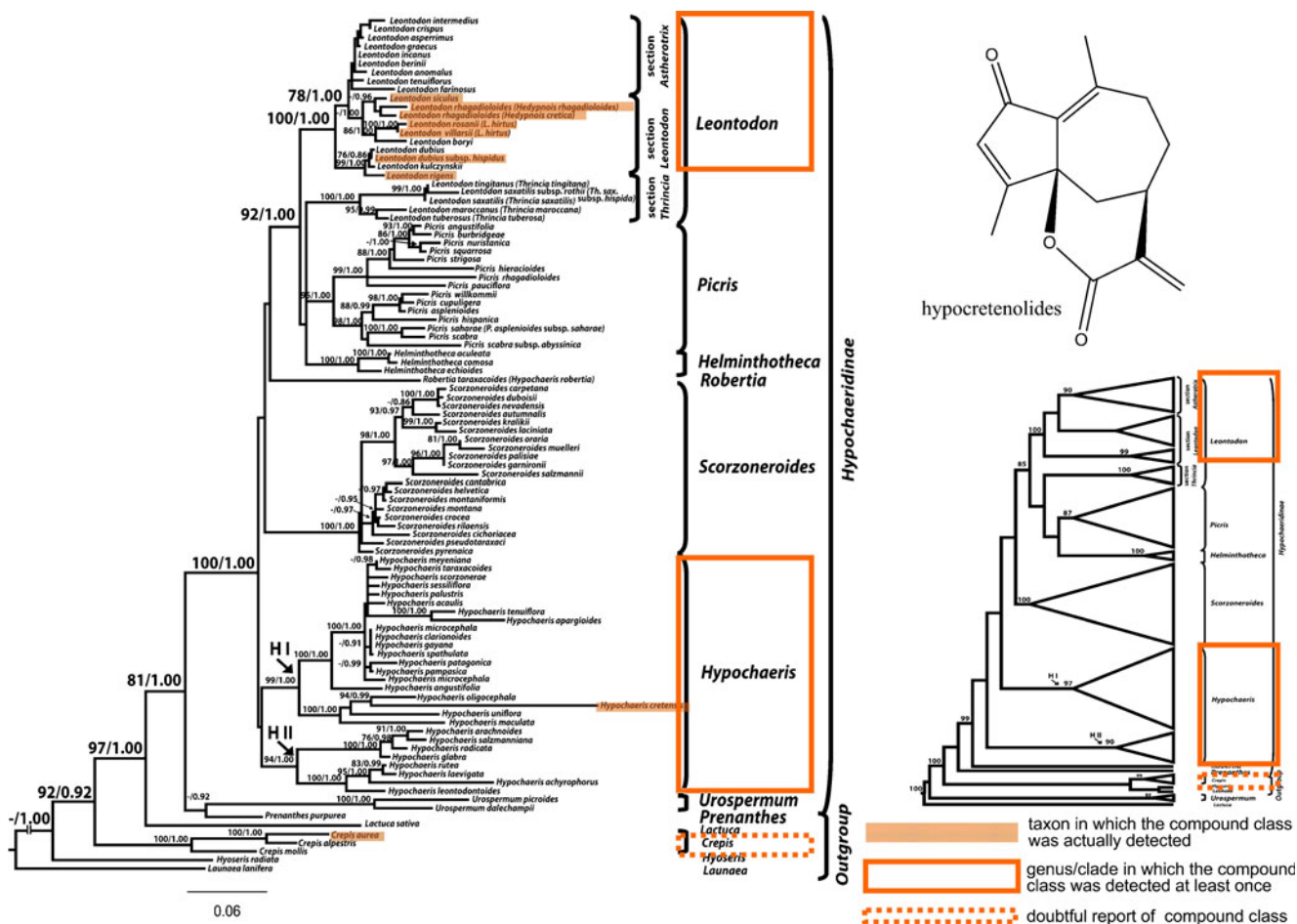


Fig. 5 Overview of the distribution of hypocretenolides within the phylogenetic context of the Hypochaeridinae

does not give a clear cut picture. The most parsimonious explanation for the observed pattern is that both caffeoyl quinic and caffeoyl tarataric were part of the secondary metabolite profile of the common ancestor of the Hypochaeridinae, and that the ability to synthesise caffeoyl tartaric acid derivatives was lost multiple times independently by some members of the subtribe. This loss is linked most probably to one or a few enzymes only. A similar mechanism was postulated by Wink (2003) to explain the distribution of particular classes of alkaloids in the Fabaceae.

Flavonoids

It is generally known that flavonoids are poor chemosystematic markers at higher taxonomic levels but excellent markers at the species level and below. Nonetheless, luteolin, the most common aglycon in the Asteraceae, seems also to be the most common aglycon in the Cichorieae tribe and the Hypochaeridinae subtribe. One aglycon of particular interest that occurs also in some members of the Hypochaeridinae is isetin. This otherwise rare flavonoid has been

found in some genera of the Cichorieae, *Hieracium*, and a number of genera of the Hypochaeridinae (see above).

Sesquiterpene lactones

Sesquiterpene lactones are widespread in the Asteraceae and also in the Cichorieae. However, compared to other taxa within the Asteraceae, the diversity of sesquiterpene lactone ring structure diversity is rather poor in the Cichorieae. As opposed to other taxa in the Asteraceae, many compounds in this tribe are sesquiterpene lactone glucosides, and the structural diversity is due also to substitution patterns of a few ring systems—eudesmane, germacrane, and guaiane in particular—substituted with sugar and acyl moieties. In general, and also in the Hypochaeridinae, sesquiterpene lactones are useful chemosystematic markers. Like in other subtribes of the Cichorieae, costus lactone, hieracin, and lactucin type guaianolides play a dominant role. Compound classes specific to some members of the Hypochaeridinae are urospermal type melampolides and hypocretenolides. Urospermal type melampolides are restricted to the genus

Urospermum. Hypocretenolides have been found within the Hypochaeridinae in *Hedypnois cretica*, *Hypochaeris cretensis*, and various members of *Leontodon* s.str. Outside the Hypochaeridinae, these compounds have so far only in *Crepis aurea* (Kisiel 1994; Zidorn 2008b). However, this occurrence, based on a report by Kisiel (1994) and on plant material grown in a botanical garden from seeds obtained from another botanical garden is, at present, not backed up by a voucher specimen (W. Kisiel personal communication). Given the fact that incorrectly assigned seed samples are not uncommon, and the close similarity of *Crepis aurea* and glabrous forms of *Leontodon hispidus* in the vegetative state, the occurrence of hypocretenolides in *C. aurea* might be erroneous. Our HPLC/MS investigations of the sub-aerial parts of *C. aurea* of Tyrolean origin suggested the presence of a number of sesquiterpene lactone glucosides, but none of the hypocretenolides reported from this species was detectable. It is of course impossible to rule out that *C. aurea* comprises different chemotypes with different patterns of sesquiterpene lactones, possibly separated from each other geographically. Nonetheless, based on the fact that no hypocretenolides were detected by Kisiel (1994) in any other *Crepis* species during their meticulous phytochemical investigations of the genus *Crepis* (reviewed in Zidorn 2008b) and based on the only remote phylogenetic relationship of *C. aurea* with the clade comprising all other hydroxyhypocretenolide containing taxa, we currently consider the occurrence of 14-hydroxyhypocretenolides in *C. aurea* as rather unlikely.

Conclusions

A careful reexamination of ITS data of members of the Hypochaeridinae subtribe of the Asteraceae family revealed that the recently redrawn generic limits are still not fully satisfactory. The genus *Leontodon* was also based on molecular evidence split into two genera—*Leontodon* and *Scorzoneroides*—by Samuel et al. (2006). Though the split proposed by Samuel et al. (2006) was corroborated by our data, *Leontodon* has to be redefined again. Rather unexpectedly, when only considering morphological features, members of the genus *Hedypnois* cluster together intricately with members of *Leontodon* section *Leontodon*. Conclusively, *Hedypnois* was assigned to *Leontodon* s.str. This reassignment is well supported by phytochemical data with *Hedypnois* and *Leontodon* section *Leontodon* sharing the occurrence of hypocretenolides—an otherwise rare type of sesquiterpene lactones.

New combinations

Taxonomy and nomenclature of the new combinations are based on the ICN International Cichorieae Network et al.

(2009+). Here, we give only new names within the genus *Leontodon* for the taxa formerly assigned to the genus *Hedypnois*.

Leontodon L

Type species: *L. hispidus* L

= *Hedypnois* Mill. **syn. nov.** (Gard. Dict. Abr., ed. 4: 606, 1754; Type species: *Hedypnois annua* Ferris, = *Hedypnois rhagadioloides* (L.) F.W. Schmidt)

Leontodon arenicola (Sennen & Mauricio) Enke & Zidorn **comb. nov.** (Basionym: *Hedypnois arenicola* Sennen & Mauricio in frère Sennen, Diagn. Nouv.: 236. 1936)

Leontodon caspicus (Hornem.) Enke & Zidorn **comb. nov.** (Basionym: *Hedypnois caspica* Hornem., Suppl. Hort. Bot. Hafn.: 91. 1819)

Leontodon rhagadioloides (L.) Enke & Zidorn **comb. nov.** subsp. *rhagadioloides* (Basionym: *Hyoseris rhagadioloides* L., Sp. Pl.: 809. 1753; ≡ *Hedypnois rhagadioloides* (L.) F.W.Schmidt; = *Hedypnois cretica* (L.) Dum.Cours. subsp. *cretica*)

Leontodon rhagadioloides subsp. *tubaeformis* (Ten.) Enke & Zidorn **comb. nov.** (Basionym: *Hyoseris tubaeformis* Ten., Fl. Napol. 1: XLVI. 1811; ≡ *Hedypnois cretica* subsp. *tubaeformis* (Ten.) Nyman)

Leontodon schousboei Enke & Zidorn **nom. nov.** (Replaced synonym: *Hyoseris arenaria* Schousb. in Kongel, Danske Vidensk.-Selsk. Skr. 1: 197. 1800 ; ≡ *Hedypnois arenaria* (Shousboe) DC.; non *Leontodon arenarius* (Gaudich) Albov in Revista Mus. La Plata, 7: 376. 1896)

Acknowledgements The authors wish to thank Renate Spitaler (Innsbruck) and Jonas Zimmermann (Berlin) for proof reading, the latter also for help with the molecular work; Michaela Posch, Birthe Schubert, and Judith Strauch (all Innsbruck) for phytochemical assistance; Serhat Cicek (Innsbruck) for HPLC/MS measurements; and Eckhard von Raab-Straube, Ralf Hand and Wolf-Henning Kusber (all Berlin) for advice with regards to botanical nomenclature. This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF, project P20278-B16).

References

- Babcock, E. B. (1947a). *The Genus Crepis, part one, The Taxonomy, Phylogeny, Distribution and Evolution of Crepis*: University of California Publications. 21.
- Babcock, E. B. (1947b). *The Genus Crepis, part two, Systematic Treatment*: University of California Publications. 22.
- Bailly, F., & Cotellet, P. (2005). Anti-HIV activities of natural antioxidant caffeic acid derivatives: toward an antiviral supplement diet. *Current Medicinal Chemistry*, 12, 1811–1818.
- Blattner, F. R. (1999). Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques*, 27, 1180–1186.
- Carr, G. D., King, R. M., Powell, A. M., & Robinson, H. (1999). Chromosome numbers in Compositae, XVIII. *American Journal of Botany*, 86, 1003–1013.

- Cerbah, M., Souza-Chies, T., Jubier, M. F., Lejeune, B., & Siljak-Yakovlev, S. (1998). Molecular phylogeny of the genus *Hypochaeris* using internal transcribed spacers of nuclear rDNA: Inference for chromosomal evolution. *Molecular Biology and Evolution*, *15*, 345–354.
- Enke, N., Fuchs, J., & Gemeinholzer, B. (2011). Shrinking genomes? Evidence from genome size variation in *Crepis* L. (Cichorieae, Compositae). *Plant Biology*, *13*, 185–193.
- Enke, N., & Gemeinholzer, B. (2008). Babcock revisited: new insights into generic delimitation and character evolution in *Crepis* L. (Compositae: Cichorieae) from ITS and *matK* sequence data. *Taxon*, *57*, 756–768.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*, 783–791.
- Greuter, W., Gutermann, W., & Talavera, S. (2006). A preliminary conspectus of *Scorzoneroides* (Compositae, Cichorieae) with validation of the required new names. *Willdenowia*, *36*, 689–692.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.
- Hsiao, C., Chatterton, N. J., Asay, K. H., & Jensen, K. B. (1995). Molecular phylogeny of the Pooideae (Poaceae) based on nuclear rDNA (ITS) sequences. *Theoretical and Applied Genetics*, *90*, 389–398.
- ICN International Cichorieae Network, General editors Hand, R., Kilian, N., & Raab-Straube, E. von (2009+). Cichorieae Portal. Published at <http://wp6-chichorieae-e-taxonomy.eu/portal/> [accessed 15.03.2011]
- Izuzquiza, A., & Feliner, G. N. (1991). Cytotaxonomic notes on the genus *Leontodon* (Asteraceae, Hypochoeridinae). *Willdenowia*, *21*, 215–224.
- Kilian, N., Gemeinholzer, B., & Lack, H. W. (2009). Tribe Cichorieae Lam. & DC. In V. A. Funk, A. Susanna, T. Stuessy, & R. Bayer (Eds.), *Systematics, Evolution and Biogeography of the Compositae*. Austria: IAPT, Vienna.
- Kisiel, W. (1994). *Hypocretenolides* from *Crepis aurea*. *Fitoterapia*, *65*, 381.
- Lippi, M. M., & Garbari, F. (2004). *Leontodon villarsii* (Willd.) Loisel. and *L. rosani* (Ten.) DC. (Asteraceae): nomenclatural, palynological, karyological, and micromorphological aspects. *Plant Biosystems*, *138*, 165–174.
- Nordenstam, B. (1971). Cytogeography of the genus *Hedypnois* (Compositae). *Botaniska Notiser*, *124*, 483–489.
- Pittoni, H. (1974). Behaarung und Chromosomenzahlen sternhaariger *Leontodon*-Sippen. *Phyton (Austria)*, *16*, 165–188.
- Posada, D., & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818.
- Rambaut, A. (2008). Figtree v1.2.2. Institute of Evolutionary Biology, University of Edinburgh. Available at <http://tree.bio.ed.ac.uk/software/figtree>
- Rios, J. L., Giner, R. M., Cuellar, M. J., Recio, M. C., & Serrano, A. (1992). Phenolics from some species of subtribe Leontodontinae. *Planta Medica*, *58*(Suppl. 1), A701.
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574.
- Samuel, R., Gutermann, W., Stuessy, T. F., Ruas, C. F., Lack, H. W., Tremetsberger, K., Talavera, S., Hermanowski, B., & Ehrendorfer, F. (2006). Molecular phylogenetics reveals *Leontodon* (Asteraceae, Lactuceae) to be diphyletic. *American Journal of Botany*, *93*, 1193–1205.
- Samuel, R., Stuessy, T. F., Tremetsberger, K., Baeza, C. M., & Siljak-Yakovlev, S. (2003). Phylogenetic relationships among species of *Hypochaeris* (Asteraceae, Cichorieae) based on ITS, plastid *trnL* intron, *trnL-F* spacer, and *matK* sequences. *American Journal of Botany*, *90*, 496–507.
- Sareedenchai, V., & Zidorn, C. (2010). Flavonoids as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Biochemical Systematics and Ecology*, *38*, 935–957.
- Siljak-Yakovlev, S., Bartoli, A., Roitman, G., Barghi, N., & Mugnier, C. (1994). Etude caryologique de trois espèces d'*Hypochaeris* originaires d'Argentine: *H. chiliensis* (H. B. K.) Hieron, *H. megapotamica* Cabr. et *H. microcephala* (Sch. Bip.) Cabr. var. *albiflora* (O.K.) Cabr. *Canadian Journal of Botany*, *72*, 1496–1502.
- Stamatakis, A. (2006). RAXML-VI-HPC: Maximum-Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.
- Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology*, *57*, 758–771.
- Stebbins, G. L., Jenkins, J. A., & Walters, M. S. (1953). Chromosomes and phylogeny in the Compositae, tribe Chichorieae. *University of California Publications in Botany*, *26*, 401–429.
- Swofford, D. L. (2002). *PAUP*: Phylogenetic analyses using parsimony (* and other methods)*, version 4.0beta. Sunderland: Sinauer.
- Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, *17*, 57–86.
- Tremetsberger, K., Weiss-Schneeweiss, H., Stuessy, T., Samuel, R., Kadlec, G., Angeles Ortiz, M., & Talavera, S. (2005). Nuclear ribosomal DNA and karyotypes indicate a NW African origin of South American *Hypochaeris* (Asteraceae, Chichorieae). *Molecular Phylogenetics and Evolution*, *35*, 102–116.
- Tzevelev, N. N., & Fedorov, A. A. (2003). *Flora of Russia: The European Part and Bordering Regions*. Rotterdam: A.A. Balkema.
- Weiss, H., Stuessy, T. F., Grau, J., & Baeza, C. M. (2003). Chromosome reports from South American *Hypochaeris* (Asteraceae). *Annals of the Missouri Botanical Garden*, *90*, 56–63.
- Weiss-Schneeweiss, H., Stuessy, T. F., Siljak-Yakovlev, S., Baeza, C. M., & Parker, J. (2003). Karyotype evolution in South American species of *Hypochaeris* (Asteraceae, Lactuceae). *Plant Systematics and Evolution*, *241*, 171–184.
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols. A guide to methods and applications* (pp. 315–322). San Diego: Academic.
- Widder, F. J. (1975). Die Gliederung der Gattung *Leontodon*. *Phyton (Austria)*, *17*, 23–29.
- Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, *64*, 3–19.
- Zidorn, C. (1998). *Phytochemie, Pharmakologie, Chemotaxonomie und Morphologie von Leontodon hispidus L. s.l.* Shaker Verlag, Aachen. Also as PhD thesis. Innsbruck: University of Innsbruck.
- Zidorn, C. (2006). Sesquiterpenoids as chemosystematic markers in the subtribe Hypochaeridinae (Lactuceae, Asteraceae). *Biochemical Systematics and Ecology*, *34*, 144–159.
- Zidorn, C. (2008a). Plant Chemosystematics. In M. Waksman-Hajnos, J. Sherma, & T. Kowalska (Eds.), *Thin Layer Chromatography in Phytochemistry* (pp. 77–101). Boca Raton: Taylor & Francis.
- Zidorn, C. (2008b). Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Phytochemistry*, *69*, 2270–2296.
- Zidorn, C., Gottschlich, G., & Stuppner, H. (2002). Chemosystematic investigations on phenolics from flowerheads of central European taxa of *Hieracium sensu lato* (Asteraceae). *Plant Systematics and Evolution*, *231*, 39–58.
- Zidorn, C., Pschorr, S., Ellmerer, E. P., & Stuppner, H. (2006). Occurrence of equisetumpyrone and other phenolics in *Leontodon crispus*. *Biochemical Systematics and Ecology*, *34*, 185–187.

- Zidorn, C., Schubert, B., & Stuppner, H. (2005). Altitudinal differences in the contents of phenolics in flowering heads of three members of the tribe Lactuceae (Asteraceae) occurring as introduced species in New Zealand. *Biochemical Systematics and Ecology*, 33, 855–872.
- Zidorn, C., Schubert, B., & Stuppner, H. (2008). Phenolics as chemosystematics markers in and for the genus *Crepis* (Asteraceae, Cichorieae). *Scientia Pharmaceutica*, 76, 743–750.
- Zidorn, C., Spitaler, R., Grass, S., Mader, J., Müller, T., Ellmerer, E. P., & Stuppner, H. (2007). Four new hypocretenolides (guaian-12,5-olides) from *Leontodon rosani* (Asteraceae, Cichorieae). *Biochemical Systematics and Ecology*, 35, 301–307.
- Zidorn, C., & Stuppner, H. (2001). Chemosystematics of taxa from the *Leontodon* section *Oporinia*. *Biochemical Systematics and Ecology*, 29, 827–837.