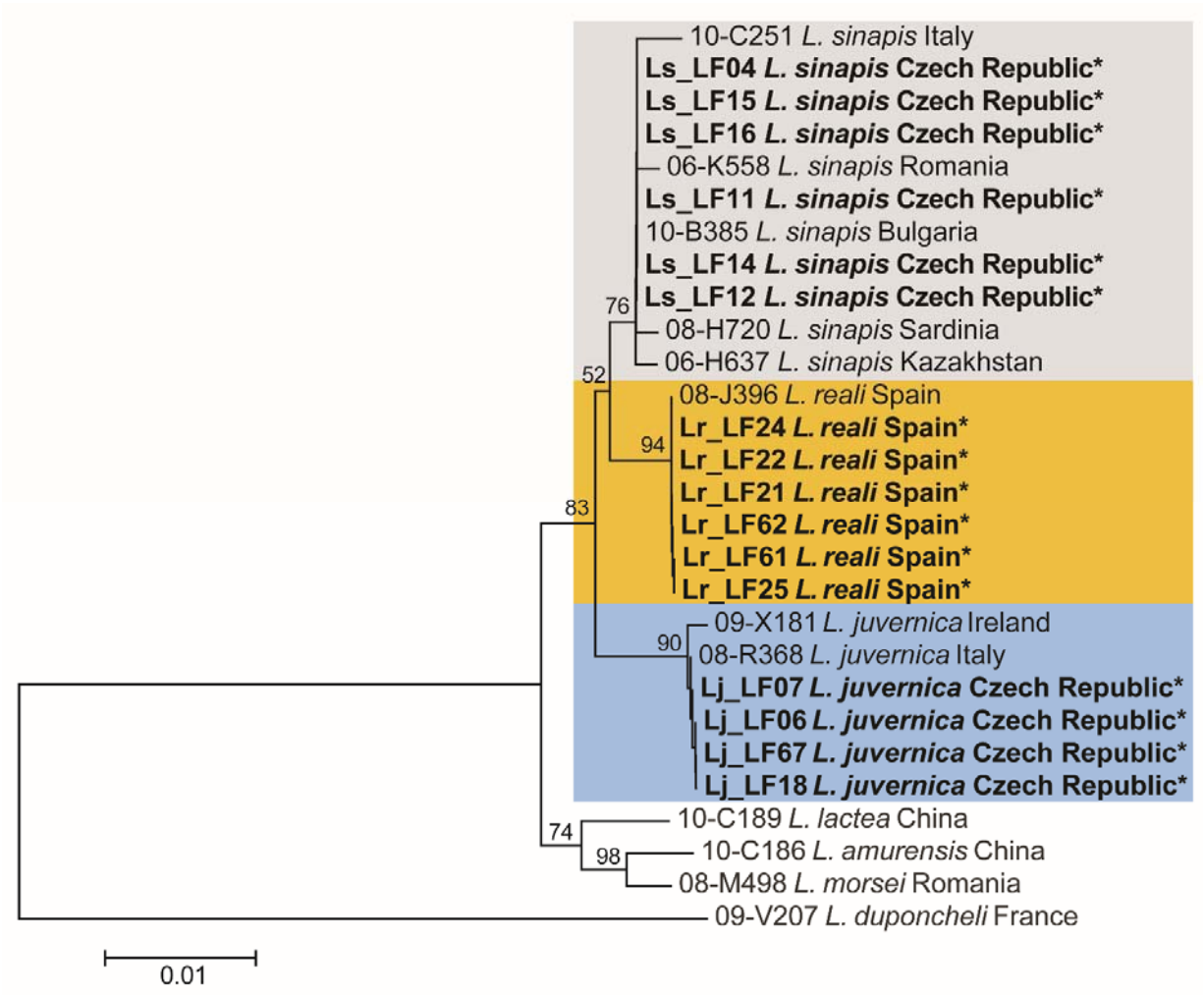

Figure S1 Neighbor-joining tree of nuclear *ITS2* haplotypes of *L. sinapis* (grey background), *L. reali* (orange background), and *L. juvernica* (blue background). Specimens sequenced and analysed in this study are indicated by an asterisk. *Leptidea amurensis*, *L. lactea*, *L. morsei*, and *L. duponcheli* were used as outgroup. For the origin of all specimens and GenBank accession numbers, see Additional file 5: Table S1. The scale represents 0.01 substitutions per site. Bootstrap supports (100 replicates) are shown next to the recovered nodes.

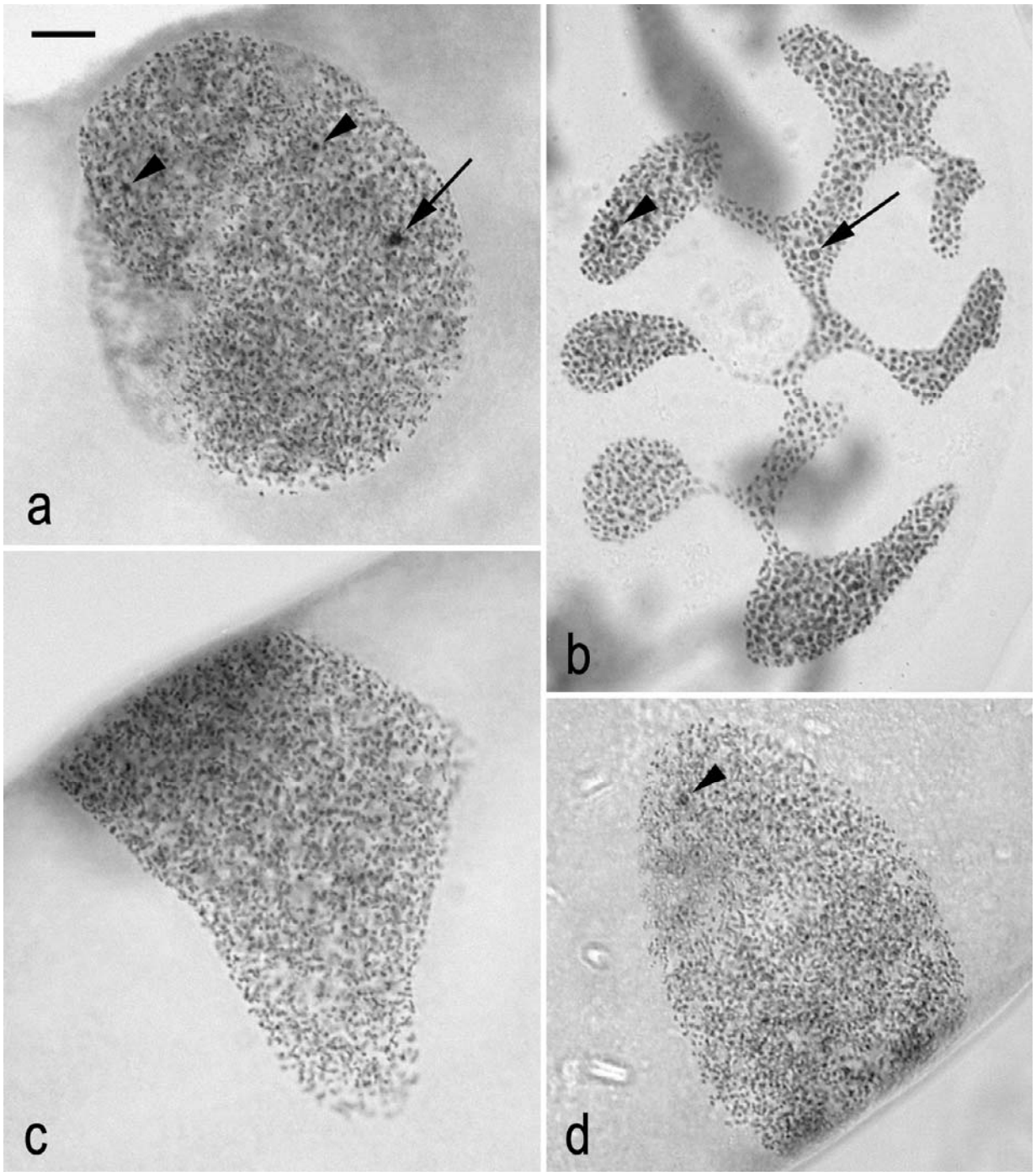

Figure S2 The status of sex chromatin in polyploid nuclei of three *Leptidea* species. The orcein-stained preparations were made from Malpighian tubule cells of the fifth instar larvae (**a**, **c**, **d**) and adult females (**b**). Black arrows indicate a larger deeply stained heterochromatin body, while arrowheads show smaller bodies. (**a**) A lower-ploidy female nucleus of *L. sinapis* with one larger and two smaller bodies. (**b**) A highly polyploid female nucleus of *L. sinapis* with two bodies, one larger and one smaller. (**c**) A male nucleus of *L. reali* without distinguishable heterochromatin bodies. (**d**) A male nucleus of *L. reali* with one smaller body. Scale bar = 10 µm.

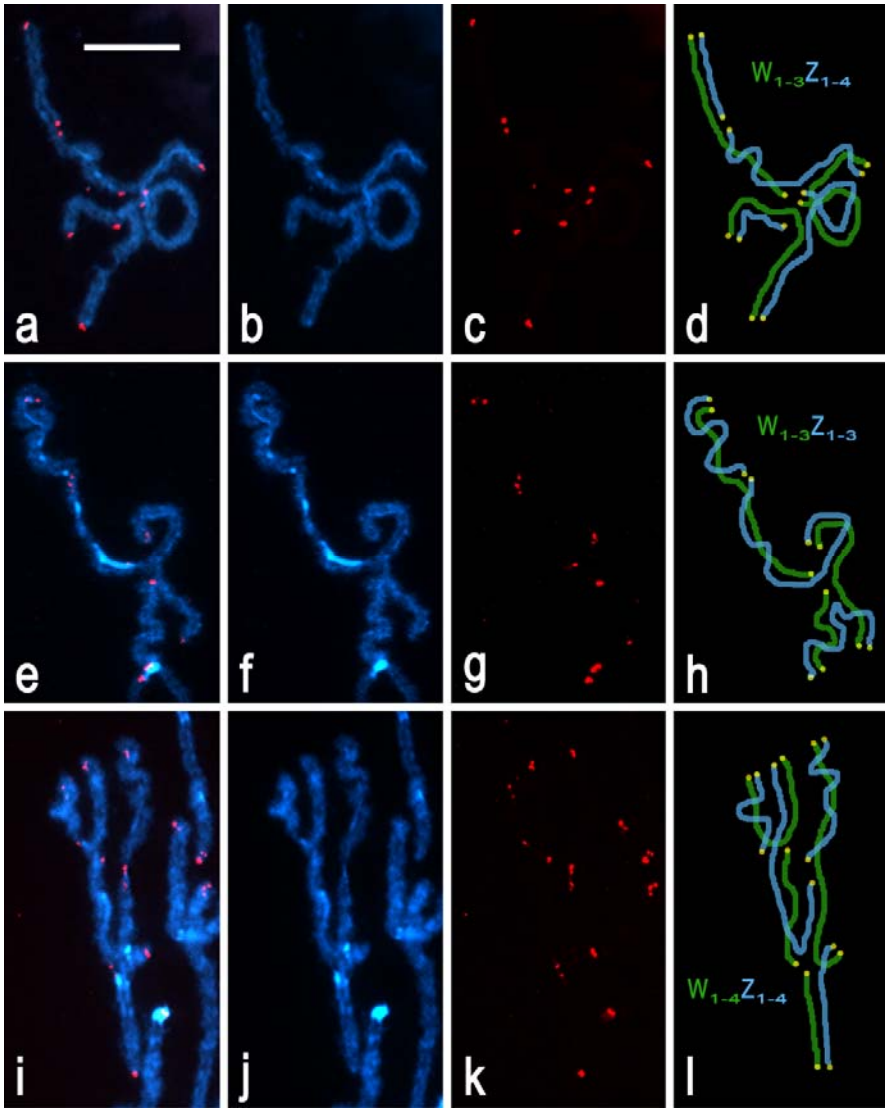

Figure S3 Analysis of sex chromosome multivalents of pachytene oocytes in *Leptidea juvernica* (a-d), *L. sinapis* (e-h), and *L. reali* (i-l) using FISH with the $(TTAGG)_n$ telomeric probe. Hybridization signals of the Cy3-dUTP-labelled telomeric probe (red) indicate chromosome ends. Chromosomes were counterstained with DAPI (blue). Figures (**a**-**d**), (**e**-**h**), and (**i**-**l**) show sex chromosome multivalents $W_{1-n}Z_{1-n}$: (**a**, **e**, **i**) merged images of the $(TTAGG)_n$ telomeric probe and DAPI staining; (**b**, **f**, **j**) DAPI images; note DAPI-highlighted heterochromatic segments of the W chromosomes; (**c**, **g**, **k**) hybridization pattern of the $(TTAGG)_n$ telomeric probe; (**d**, **h**, **l**) schematic drawings of the sex chromosome multivalents; yellow dots indicate the ends of individual chromosomes involved in the multivalents. Scale bar = 10 µm.

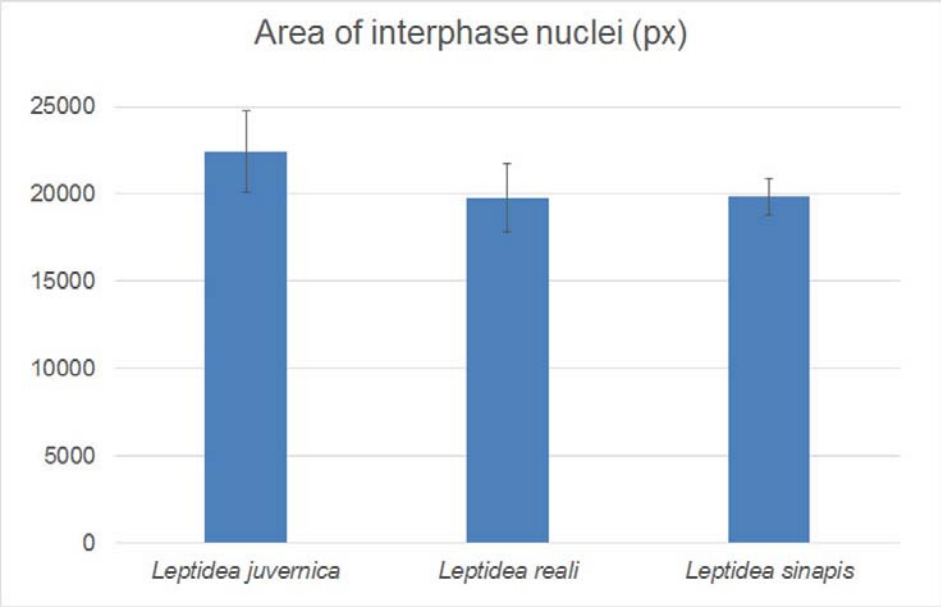

Figure S4 Comparison of interphase nuclei sizes in three *Leptidea* species. The y-axis shows the number of pixels. Micrographs of interphase nuclei were taken from DAPI-stained spread preparations of wing discs from three different larvae of each *Leptidea* species, using the same resolution. In these micrographs, we measured the area of 144 nuclei of *L. juvernica*, 154 nuclei of *L. reali*, and 130 nuclei of *L. sinapis*. The measurements were carried out using the software JMicroVision v1.2.7 [Roduit N: JMicroVision: Image analysis toolbox for measuring and quantifying components of high-definition images. Version 1.2.7. http://www.jmicrovision.com (accessed 27 March 2015)]. Calibration was performed using an image resolution so that the area of each nucleus was measured in pixels. The average size of nuclei was calculated for each species independently and then compared between species by one-way ANOVA using the software Statistica for Windows, version 8.0 (StatSoft, Inc., Tulsa, OK, USA). The comparison of interphase nuclei revealed no statistically significant between-species differences in their size ($F_{(2, 9)} = 0.6782$; $P = 0.5425$). The mean (± S.E.) area of interphase nuclei was 22434 ± 2296 pixels for *L. juvernica*, 19781 ± 1965 pixels for *L. reali*, and 19835 ± 1021 pixels for *L. sinapis*.

Table S1 List of specimens included in phylogenetic analyses. Sequences obtained in this study are in blue, the other sequences were downloaded from GenBank and are representative for all the *COI* and *ITS2* haplotypes of *Leptidea sinapis* , *L. reali* , and *L. juvernica* identified in a previous study [41]. The haplotype numbers correspond to those in [41].

Sample ID	Species	*COI* haplotype[1)]	*COI* accession number	*ITS2* haplotype[1)]	*ITS2* accession number	Locality	Country
RVcoll.08-R368	*L. juvernica*	**hj1**	KC865949	**hj1**	KC865858	Val di Tovo- Laghi	Italy
RVcoll.08-M322	*L. juvernica*	**hj2**	HQ004596	**hj1**	JF512789	Valea Belchia, Harghita	Romania
RVcoll.10-A259	*L. juvernica*	**hj6**	JF512651	**hj1**	JF512783	Gresse-en-Vercors, Isère	France
RVcoll.07-Z082	*L. juvernica*	**hj7**	JF512648	**hj1**	JF512768	South Altai, Uspenka	Kazakhstan
RVcoll.09-X181	*L. juvernica*	**hj9**	JF512715	**hj2**	JF512788	Kilternan, Dublin	Ireland
RVcoll.08-J396	*L. reali*	**hr1**	JF512603	**hr1**	JF512797	Viladrau, Barcelona	Spain
RVcoll.03-H535	*L. reali*	**hr6**	JF512617	**hr1**	JF512790	Saldes, Barcelona	Spain
RVcoll.07-E083	*L. reali*	**hr7**	JF512712	**hr1**	JF512795	Roccaraso, L'Aquila	Italy
RVcoll.10-B385	*L. sinapis*	**hs1**	JF512693	**hs1**	JF512817	Paril Village, Khadzhidimovo	Bulgaria
MF-63	*L. sinapis*	**hs14**	KC866102	**hs1**	KC865928	Riala	Sweden
MF-174	*L. sinapis*	**hs16**	KC866101	**hs1**	KC865919	Hodonín, South Moravia	Czech Republic
RVcoll.08-R436	*L. sinapis*	**hs24**	GU675857	**hs1**	KC865933	Ames, Novais	Spain
RVcoll.10-C245	*L. sinapis*	**hs5**	JF512697	**hs1**	JF512847	Příbram, Central Bohemia	Czech Republic
RVcoll.07-E237	*L. sinapis*	**hs8**	JF512597	**hs1**	JF512810	Novalesa-Moncenisio, Torino	Italy
RVcoll.08-H720	*L. sinapis*	**hs23**	KC865994	**hs2**	KC865883	Gairo	Sardinia
RVcoll.08-H769	*L. sinapis*	**hs3**	KC866005	**hs2**	KC865886	Fozzaninco	Corsica
RVcoll.06-H637	*L. sinapis*	**hs2**	JF513027	**hs3**	KC865931	Landman, Zyryanovsk	Kazakhstan
RVcoll.06-K558	*L. sinapis*	**hs1**	JF513036	**hs4**	KC865939	Bădeni, Cluj	Romania
RVcoll.10-C251	*L. sinapis*	**hs1**	JF512699	**hs5**	JF512832	Monte di Malo, Veneto	Italy
MF-F12	*L. juvernica*	**hj10**	KC866126			Riala	Sweden
GenBank	*L. juvernica*	**hj11**	EF599645			Barje	Slovenia

GenBank	*L. juvernica*	**hj12**	EF599643			Barje	Slovenia
GenBank	*L. juvernica*	**hj13**	EF599640			Vrhnika	Slovenia
RVcoll.08-Y008	*L. juvernica*	**hj3**	JF512578			Peterhof, St. Petersburg	Russia
RVcoll.11-G200	*L. juvernica*	**hj4**	KC865980			Narymski Mts.	Kazakhstan
RVcoll.11-G219	*L. juvernica*	**hj5**	KC865982			Balgyn	Kazakhstan
GenBank	*L. juvernica*	**hj8**	GU655014			Neustadt/Donau, Plattenberg, Bavaria	Germany
RVcoll.09-X183	*L. juvernica*	**hj9**	JF512716			Gortmore Point, Lough Derg, Tipperary	Ireland
RVcoll.10-C269	*L. reali*	**hr2**	JF512616			Cascia, Perugia	Italy
RVcoll.10-C270	*L. reali*	**hr3**	JF512704			Sibillini Mountains	Italy
RVcoll.08-H468	*L. reali*	**hr4**	GU676645			Hormiguera, Cantabria	Spain
MF-90	*L. reali*	**hr5**	KC866117			Pla de la Calma, Montseny	Spain
RVcoll.07-C210	*L. sinapis*	**hs10**	JF512592			Schitul Pahomie, Vâlcea	Romania
RVcoll.06-H631	*L. sinapis*	**hs11**	JF513025			Landman, Zyryanovsk	Kazakhstan
RVcoll.06-H632	*L. sinapis*	**hs12**	JF513047			Landman, Zyryanovsk	Kazakhstan
GenBank	*L. sinapis*	**hs13**	HM393183			Zahmer Kaiser, Aschinger Alm, Tyrol	Austria
RVcoll.10-C262	*L. sinapis*	**hs15**	KC866089			Corciano, Perugia	Italy
MF-155	*L. sinapis*	**hs17**	KC866100			Sant Celoni, Barcelona	Spain
RVcoll.11-K071	*L. sinapis*	**hs18**	KC866098			Mala Reka, Mavrovo	Macedonia
GenBank	*L. sinapis*	**hs19**	GU688515			Ruhpolding, Bavaria	Germany
RVcoll.10-B320	*L. sinapis*	**hs20**	KC866088			Studen Kladenets, Krumovgrad	Bulgaria
GenBank	*L. sinapis*	**hs21**	GU688533			Lenggries Isarauen, Bavaria	Germany
RVcoll.11-K078	*L. sinapis*	**hs22**	KC866104			Mala Reka, Mavrovo	Macedonia
RVcoll.11-J544	*L. sinapis*	**hs25**	KC866097			Manosque, Alpes-de-Haute-Provence	France
RVcoll.07-Z211	*L. sinapis*	**hs4**	JF513046			Saur Mts., Malyi Zhemeney	Kazakhstan
RVcoll.07-E250	*L. sinapis*	**hs6**	JF513034			NE Bézaudun-sur-Bine, Drôme	France
RVcoll.09-X562	*L. sinapis*	**hs7**	KC866082			Sorauren, Navarra	Spain
RVcoll.07-D938	*L. sinapis*	**hs9**	JF513026			Ciupercenii de Olteț, Gorj	Romania

Nz091	*L. amurensis*		JF512621			Bulgan	Mongolia
RVcoll.10-C186	*L. amurensis*		JF512622		JF512841	Jiexiu county, Shanxi	China
RVcoll.09-V207	*L. duponcheli*		JF512569		JF512852	Oraison, Alpes de Haute Provence	France
MF-1	*L. duponcheli*		KC866120			Skopje	Macedonia
RVcoll.10-C189	*L. lactea*		JF512717		JF512849	Qin Ling Shan, Madao, Liuba County, Shaanxi	China
RVcoll.10-C195	*L. lactea*		JF512718			Qin Ling Shan, Zhouzhi (Erqu) County, Shaanxi	China
RVcoll.07-Z124	*L. morsei*		JF512618			South Altai, Markakol	Kazakhstan
RVcoll.08-M498	*L. morsei*		HQ004591		JF512840	Bădeni, Cluj	Romania
Ls_LF04	*L. sinapis*		KM488575		KM488591	NP Podyjí, South Moravia	Czech Republic
Ls_LF11	*L. sinapis*		KM488578		KM488594	NP Podyjí, South Moravia	Czech Republic
Ls_LF12	*L. sinapis*		KM488579		KM488595	NP Podyjí, South Moravia	Czech Republic
Ls_LF14	*L. sinapis*		KM488580		KM488596	NP Podyjí, South Moravia	Czech Republic
Ls_LF15	*L. sinapis*		KM488581		KM488597	NP Podyjí, South Moravia	Czech Republic
Ls_LF16	*L. sinapis*		KM488582		KM488598	NP Podyjí, South Moravia	Czech Republic
Lr_LF21	*L. reali*		KM488584		KM488600	NP Montseny, Barcelona	Spain
Lr_LF22	*L. reali*		KM488585		KM488601	NP Montseny, Barcelona	Spain
Lr_LF24	*L. reali*		KM488586		KM488602	NP Montseny, Barcelona	Spain
Lr_LF25	*L. reali*		KM488587		KM488603	NP Montseny, Barcelona	Spain
Lr_LF61	*L. reali*		KM488588		KM488604	NP Montseny, Barcelona	Spain
Lr_LF62	*L. reali*		KM488589		KM488605	NP Montseny, Barcelona	Spain
Lj_LF06	*L. juvernica*		KM488576		KM488592	České Budějovice, South Bohemia	Czech Republic
Lj_LF07	*L. juvernica*		KM488577		KM488593	České Budějovice, South Bohemia	Czech Republic
Lj_LF18	*L. juvernica*		KM488583		KM488599	České Budějovice, South Bohemia	Czech Republic
Lj_LF67	*L. juvernica*		KM488590		KM488606	České Budějovice, South Bohemia	Czech Republic