The wild vine (*Vitis vinifera ssp. silvestris*) in the riparian woods of Donau and March (Austria): evaluation of genetic divergence, potential on grape damaging viruses, bacterials and soilborne vectors

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Summary

In the riparian woods of the Donau (Danube) east of Vienna, 27 specimens of *Vitis vinifera ssp. silvestris* were discovered and 32 near the Austrian border to Slovakia in the floodplains of the March. Male specimens are a bit more frequent. Only one hermaphrodite was registrated. Near Orth/Donau *Populus sp*., *Crataegus monogyna* and *Cornus sanguinea* were the most important hosts of the wild vine. Near Marchegg it grows mostly on *Quercus* species.

The viruses GLRaV I and SLRV could be detected near Orth/Donau, SLRV for the first time as a pathogen of *Vitis* in Austria. Compared with the nearby vine growing region Carnuntum the infestation with viruses was low. No viruses were registrated at Marchegg.

Agrobacterium vitis, the major cause of crown gall, was not detected.

*Longidorus attenuatus**, *L. elongatus**, *L. intermedius*, *L. juvenilis*, *L. macrosoma**, *L. poessneckensis, Xiphinema diversicaudatum**, *X. histriae und X. pachtaicum* were the root parasitic longidorid nematodes, that were discovered in the rhizosphere of the wild vine (virus vectors are characterised by an asterisk). *X. histriae* was found for the first time since the original description.

Genetically the grape from the Donau riparian woods are clearly seperated from the one of the March. There are two genetically and morphologically distinguishable groups of wild vine in the floodplains of the Donau. A close linkage to the economical useful Vitis vinifera cultivars could not be observed but there do not exist a principal barrier of alleles between vinifera and silvestris genotypes.

Keywords: grapevine, virus disease, agrobacteria, nematodes, microsatellite, genetic variability, host plant

Introduction

In the river floodplains of Donau (Danube) and March the wild grapevine *Vitis vinifera ssp. silvestris* is native (Jacquin 1762, Kirchheimer 1955) and was there very frequent, but since the beginning of the 20th century their number rapidly declined. Kirchheimer (1955) described 25 specimens near Orth and Eckartsau and some at Regelsbrunn and Stopfenreuth. Wine of acceptable quality was produced until 1911 from these native wild vines. Kirchheimer was sure, that the wild grape of the March river plains near Marchegg were extinct ‘since some decades’.

The riparian woods of Donau and March near the Austrian border to Slovakia are now partly belonging to the “Nationalpark Donauauen”, partly to a protection area of the World Wildlife Fund. Both organisations as well as the “Österreichische Bundesforste “are interested to preserve *Vitis v. ssp. silvestris.* In 1996 they started a recultivation program for the wild vines using the single individuals that still remained there.

Arnold et al. (1998) investigated the occurrence of wild vines in Europe and listed two female specimens for Marchegg (the wild vine there was obviously never extinct), eight near Orth/Donau (two females, six males) and six in the Lobau area near Vienna (five females, one male).

Several vines from Orth/Donau and Marchegg were chosen for a genetical and phytopathological evaluation. Leaf samples of the vines were used to prepare DNA and to analyse their genetic profile. Their microsatellite (SSR) allelic profile was applied to estimate

the heterozygousity and to search for linkages to the todays grapevine cultivars. Occurrence and geographical distribution, sex and hostplants of these liana were registered. Their bacterial and viral pathogens were analyzed. Furthermore soil samples of the rhizosphere of the vines were taken to find out which longidorid nematodes – some of them are virus vectors for vine viruses – are occuring and are propably associated with grapevine.

Methods

The position of each sampling site was registrated using GPS. Samples from the roots and tendrils from all specimens were taken for genetic comparison and for the identification of viral and bacterial (*Agrobacterium vitis*) pathogens. DAS-Elisa tests were done for the following viruses: Grapevine Fanleaf virus (GFLV), Arabis Mosaic virus (ArMV), Raspberry Ringspot virus (RpRSV „ch“ and „g“), Strawberry Latent Ringspot virus (SLRV), Tomato Ringspot virus (TomRSV „ch“ and „pybm“), Alfalfa mosaic virus (AMV), Tobacco Ringspot virus (TRSV), Grapevine Fleck virus (GFkV), Grapevine Virus A (GVA) and Grapevine Leafroll associated virus (GLRaV I, III, VI). The procedure of testing is described in Flak & Gangl 1994.

For identification of *Agrobacterium vitis* we used the methode of DNA isolation and PCR described by Schulz et al. (1993), exept the prolongation of the extension time. We receive the primers acs1, acs2 and vis1, vis2 from VITOLAB.

For the record of the soilborne virus vectors soil samples mainly from the rhizosphere of the wild vine were taken. To minimize root destruction, for the soil samples a cylindrical 22 mm diameter soil auger was used. The samples were from a depth of 0 down to 90 cm and had a volume of ca. 340 cm^3. For the extraction an Oostenbrink-Elutriator was used (sampling sieve width: 150 μm). All nematodes were extracted and the one from the family Longidoridae were identified on the species level. For the identification the following polytomous keys were used: genus *Longidorus*: Chen et al. 1997 and Supplement 1 - Loof & Chen 1999 -, *Xiphinema* with exclusion of *X. americanum s. l.*: Loof & Luc 1990 and

Supplement 1 - Loof & Luc 1993 - and 2 - Loof et al. 1996. Preparation and determination of the nematodes is described in Tiefenbrunner 1999 and Gangl et al. 2000.

The genetic profile was gained by genotyping all individuals with 18 SSR markers. As the first step DNA was extracted from young leaves of natural grown shoots by following the protocol described by Thomas *et al*. (1993) modified by Regner *et al*. (1998). PCR conditions were applied as already used for identification of grapevines (Regner et al. 2000). The amplified allelic fragments were separated on a 6% polyacrylamide gel on the H373 Sequencer (Applied Biosystems). The fluorochrome labelled primers (Fam, Tet, Hex) allowed an automatic estimation of the length by using GenScan 350 Tamra as an internal standard. The following SSR loci are involved in the study: The VVS2 marker was developed by Thomas and Scott (1993) and the VVMD5, 6, 7, 8, 24, 25, 27, 28, 36, markers by Bowers et al. (1996) as well as by Bowers and Meredith (1999). The VRZAG 7, 12, 15, 21, 62, 67, 79 markers (Sefc et al., 1999) were obtained from investigations into simple sequence repeats of *Vitis riparia*. Only the VMC 62 marker is not from the public domain as this marker is still not published and is part of the Vitis Microsatellite Consortium database. The data were analysed by using Microsat program and the multivariate comparison was drawn with the Scramble 3.0 program (www.visualdataflow.de/biologies). For the multivariate presentation this program computes a distance matrix and minimises the quantity:

1) $$\Sigma\Sigma \left| (a_{ij}-b_{ij})/a_{ij} \right| ,$$

where a_{ij} is the distance matrix value, b_{ij} is the corresponding distance of the two objects i and j in the graphic. We did not consider the linkage of gene loci by computing the distance matrix.

Additional statistics was done with Statgraphics 4.0 plus (Manugistics, Inc.).

Fig. 1 and fig. 2 were produced by applying the program Austrian MAP (data from Bundesamt für Eich- und Vermessungswesen, software from DaimlerChrysler Aerospace).

Results and discussion

In the riparian woods of the Danube we tracked 27 specimens of the wild vine, 23 at Orth/Donau and 4 at Regelsbrunn. Additionally we registrated 32 individuals at Marchegg in the March floodplains. The locations with a wild vine record are printed in fig. 1 and fig. 2. Near Marchegg *Vitis* is more nested, whereas the species is relatively homogeneous distributed at Orth/Donau.

Kirchheimer 1955 wrote that the wild vine specimens are overwhelmingly male (the subspecies normally is not hermaphroditic). On the Danube river banks we registrated 11 female and 13 male plants. One was clearly a hermaphrodite. In two vine plants we could not differentiate the sex, due to the fact that we did not find inflorescences. In Marchegg most specimens did not develop inflorescences at all (or maybe they were destroyed by parasites), so we could only determine the sex of eight specimens. Two were females and six males. All in all the relation of the sexes seems to be relatively balanced.

Hostplants: *Vitis vinifera ssp. silvestris* grows on a lot of different hostplants (tab. 1). Near the March river, oak species dominate clearly, together with *Acer campestre, Populus spp., Carpinus betulus*, *Crataegus monogyna*, *Fraxinus excelsior* and *Ulmus laevis*. In the river forests of the Donau *Cornus sanguinea* and *Crataegus monogyna* dominate, beside them *Populus spp*., *Acer campestre*, *Fraxinus excelsior* and *Alnus glutinosa.* Here the specimens of the wild vine are larger and normally grow on more than one tree or bush and hence they grow also on a higher diversity of host plants. It seems that the wild genotype grows more vital if the host plant even grows faster. So the mightiest wild vine can be seen growing on *Populus* species.

Pathogens (viruses and *Agrobacterium vitis*): In the vine growing region Carnuntum located near the riparian woods of the Donau and borders on the sampling sites near Regelsbrunn (Gangl et al. 2001), several grape vine viruses and the bacterium *Agrobacterium vitis* are common: GLRaV I was found in 31% of all analyzed vines, GLRaV III in more than 5% and GFkV in 11%. GFLV and ArMV were registrated too. *Agrobacterium vitis* was detected in more than 8% of all samples. So we had no reason to doubt, that this viruses and the bacterioses would be common in the wild vine, too. Surprisingly this is not the case. We did

not find any indication for viral infections in any tendril and no positive result for *Agrobacterium vitis* in any root. Confirming these results we analyzed other parts of the plants, e.g. leaves, inflorescences, ranks and roots. Indeed, six inflorescences were GLRaV I positive and one root SLRV positive. Hence for the vigorous plants of *Vitis vinifera ssp. silvestris* climbing on trees a larger number of samples from diverse parts of the plants must be taken.

The evidence of SLRV is the first infection of this virus recorded from grapevine in Austria. It seems that the viral spectrum of wild and cultivated vines differs and therefore interactions cannot be excluded between them and economically important plantations of *Vitis vinifera*.

In the rhizosphere of the SLRV positive plant *Xiphinema diversicaudatum* was detected. This *Xiphinema* species is known as a vector of the nepovirus SLRV.

Longidorid parasites: In the Donau river plains 38 soil samples (2 near Stopfenreuth, 4 near Regelsbrunn and 32 at Orth) were taken during the years 2001 and 2002 and 31 near Marchegg in the March riparian woods. Most of them were from the rhizosphere of wild grapevine and some from meadow- or forest soil nearby. Eleven were classified as Longidoridae nematodes, but two could not be identified due to the lack of specimens and preservation quality (e.g. they were partly distorted). From the nine remaining, six belong to the genus *Longidorus* and three to *Xiphinema*. They are listed in tab. 2. Four species are vectors of viruses: *L. attenuatus*, *L. elongatus*, *L. macrosoma* and *X. diversicaudatum*. *L. elongatus* is common in the vine growing region Carnuntum, too.

In the riparian woods of the March *L. intermedius* clearly dominates. *X. diversicaudatum* is very common, too, as well as *L. poessneckensis*. Both *Longidorus* species are surely not associated with the wild vine, because they are abundant in samples that were not from the rhizosphere of the wild vine. *X. diversicaudatum* is also not strictly associated with the wild vine. It is common near the river banks or the banks of its branches.

In the floodplain forests of the Donau there is no Longidorid species that dominates clearly. Nevertheless some are relatively common: *L. macrosoma*, *X. diversicaudatum*, *L.*

poessneckensis and *L. attenuatus.* The 28 specimens of *X. histriae* are from three samples and two sites. In one site only one individual was found. Although the number of specimens is large, the species seems to be seldom with a very small distribution. It was only common in the rhizosphere of a wild pear tree.

L. macrosoma seems to be assoziated with grape vine but was also discovered in meadows near the locations of wild vine. Beside *L. macrosoma*, also *X. diversicaudatum* was registrated in meadows, whereas *L. attenuatus* and *L. poessneckensis* are strictly restricted to the floodplain forests.

A biometrical description of the species will be published elsewhere.

Genetic profile: The multivariate genetical comparison clearly separates the specimens of the Donau riparian woods from that one of the March floodplains. The differences within the genetical distances are not large but nevertheless the March specimens cluster together (fig. 3). Furthermore there is a subgroup of the Donau specimens, that are mutually neighbours and can genetically be distinguished from all the others (e.g. the smallest genetical distance values are the one to the other members of the subgroup). Interestingly this subgroup is also morphologically different to the other Donau specimens. The reasons for this genetical separation indicates to another population. They may be based on introduction of other alleles by foreign pollination or seeds.

Because there are local subgroups we analysed the question, wether there is a coherence between geographical and genetical distance in the whole, so that individuals which are nearer one to another are also more related. The result of the regression and correlation analysis is, that this is not the case. Clearly there is not a statistically significant relationship between genetical distance and the distance in space at the 90% or higher confidence level.

Comparing the alleles of wild vines with cultivars of economical importance in vineyards of the same region we could recognize several characteristics. The degree of heterozygous alleles at the amplified loci is much less than in V. vinifera not exceeding more than 50%. The loci VVMD 7, VVMD 27,VVMD 36 and VRZAG 15 show very frequently alleles untypical

for *Vitis vinifera*. Some of them were also found in wild vine samples of Germany, Croatia and Turkey (data not shown). For instance all alleles at VVMD 7 longer than 260bp indicate a non *V. vinifera* genetic. On the other side genetic divergence allows to differentiate all samples of wild vine found in close distance. Therefore wild vines of Eastern Austria show high variability despite the higher amount of homologues alleles.

Legends:

Tab. 1: Frequency of host plants of *Vitis v. ssp. silvestris.*

Tab. 2: Number of specimens of the soil nematodes of the riparian woods of Donau and March. Most samples are from the rhizosphere of *Vits v. ssp. silvestris*. Virus vector species are characterized by an asterisk. Sampling sieve width: 150 μm.

Tab. 3: Additional information to the maps of the Donau and March riparian woods: viruses and longidorid nematodes.

Table 4: Additional information to the maps of the Donau and March riparian woods: Alleles of analysed wild vines.

Fig. 1: Map of the Donau riparian woods near Orth/Donau. Using GPS for location, the wild vine specimens are shown. Red circles: females, green circles: males, lilac circle (specimen H): hermaphrodite, yellow circles: sex not known. Frame width: 1 minute.

Fig. 2: Map of the March riparian woods near Marchegg. Using GPS for location, the wild vine specimens are shown. Red circles: females, green circles: males, yellow circles: sex not known. Site of B' is equal to B and of M' to M. Frame width: 1 minute.

Fig. 3: Multivariate genetical comparison of the *Vitis v. ssp. silvestris* specimens from the Donau and March riparian woods. The red circle borders the march specimens (small letters), whereas the blue circle surrounds a group of one location (see fig. 1) of Donau-

specimens, that differs morphologically from the other ones. Donau-specimens are symbolized by capital letters.

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Table 1

	March Marchegg	**Danube** Orth & Regelsbrunn
Acer campestre	9	7
Alnus glutinosa	1	5
Carpinus betulus	6	1
Corelus avellana	2	2
Cornus mas	0	3
Cornus sanguinea	2	16
Clematis vitalba	0	1
Crataegus monogyna	5	14
Fraxinus excelsior	6	7
Ailanthus altissima	0	1
Rhamnus frangula	0	1
Humulus lupulus	0	1
Ligustrum vulgare	0	1
Pyrus pyraster	0	1
Populus spp.	7	7
Prunus spinosa	0	1
Quercus spp.	14	2
Robinia pseudacacia	0	1
Salix spp.	1	4
Sambucus nigra	0	2
Ulmus laevis	5	0
Viburnum opulus	0	1
Number of *Vitis* specimens	31	27

Table 2

	March adult	juvenile	all	Danube adult	juvenile	all
Longidorus						
*L. attenuatus**	2	3	5	20	8	28
*L. elongatus**	0	0	0	3	1	4
L. intermedius	292	217	509	3	2	5
L. juvenilis	0	0	0	1	0	1
*L. macrosoma**	0	0	0	22	69	91
L. poessneckensis	29	47	76	12	18	30
L. sp.	0	0	0	4	3	7
L. sp.	0	0	0	6	7	13
Xiphinema						
*X. diversicaudatum**	132	71	203	45	36	81
X. histriae	0	0	0	18	10	28
X. pachtaicum	0	0	0	1	0	1
other Dorylaimids			496			440
Rhabditids			191			77
Tylenchids			15			261
∑			1495			1067
Samples			31			38

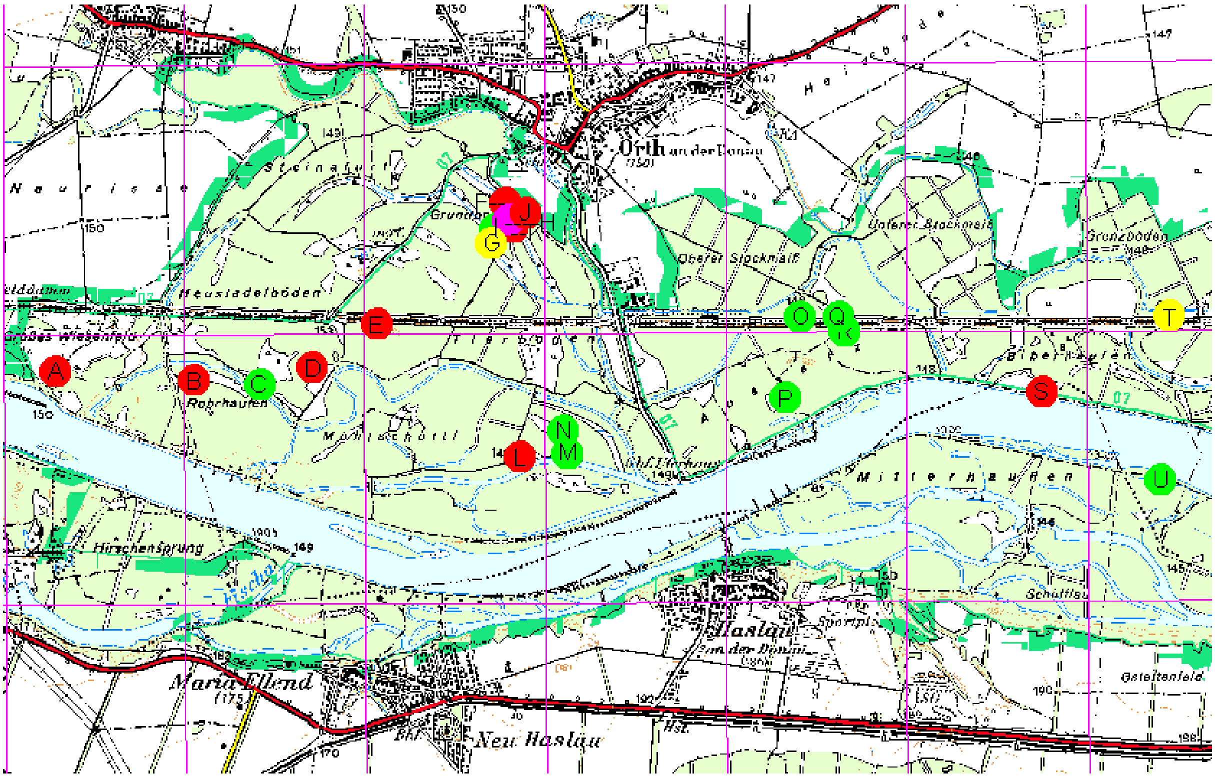
Table 3

	Donau			**March**	
Site	Virus	Longidoridae		Longidoridae	
		Xiphinema	*Longidorus*	*Xiphinema*	*Longidorus*
A			*L. intermedius*	*X. diversicaudatum*	*L. intermedius*
B			*L. elongatus*		*L. intermedius*, *L. poessneckensis*
C		*X. diversicaudatum*, *X. histriae*	*L. attenuatus*, *L. macrosoma*		*L. intermedius*
D		*X. diversicaudatum*, *X. pachtaicum*	*L. macrosoma*, *L. poessneckensis*		
E	GLRaV I	*X. diversicaudatum*	*L. poessneckensis*	*X. diversicaudatum*	*L. intermedius*
F	GLRaV I		*L. macrosoma*	*X. diversicaudatum*	*L. intermedius*, *L. poessneckensis*
G		*X. diversicaudatum*		*X. diversicaudatum*	*L. poessneckensis*
H					*L. intermedius*
I	GLRaV I				
J			*L. intermedius*, *L. macrosoma*		*L. intermedius*, *L. poessneckensis*
K		*X. diversicaudatum*		*X. diversicaudatum*	*L. poessneckensis*
L			*L. attenuatus*, *L. elongatus*	*X. diversicaudatum*	*L. intermedius*, *L. poessneckensis*
M	GLRaV I	*X. diversicaudatum*			*L. intermedius*, *L. poessneckensis*
N		*X. diversicaudatum*		*X. diversicaudatum*	
O			*L. poessneckensis*	*X. diversicaudatum*	*L. intermedius*
P		*X. histriae*	*L. attenuatus*		*L. intermedius*
Q	GLRaV I		*L. attenuatus*, *L. poessneckensis*		*L. intermedius*
R			*L. poessneckensis*		*L. intermedius*
S	SLRV	*X. diversicaudatum*	*L. attenuatus*		*L. intermedius*
T		*X. diversicaudatum*	*L. attenuatus*, *L. macrosoma*		*L. intermedius*, *L. poessneckensis*
U		*X. diversicaudatum*	*L. juvenilis*		*L. intermedius*
V					*L. intermedius*
W					*L. intermedius*
X				*X. diversicaudatum*	
Y				*X. diversicaudatum*	*L. intermedius*
Z				*X. diversicaudatum*	*L. intermedius*, *L. poessneckensis*
α				*X. diversicaudatum*	*L. poessneckensis*
β				*X. diversicaudatum*	*L. poessneckensis*
χ					*L. intermedius*
δ					*L. attenuatus*
ε					*L. intermedius*, *L. poessneckensis*

Table 4

Site **Donau**	**VVS 2**	**VVMD 5**	**VVMD 7**	**VVMD 27**	**VRZAG 62**	**VRZAG 79**
A	133/152	228/232	241/265	192	195	252
B	128/133	228/232	241	189	195	252
B’	133/157	228/232	241	189/191	195	252
C	128/132	232/240	241/243	179/189	181/197	252/256
D	128/133	226/236	239/263	189	195	252
E	146/152	232	241	190/1	195	252/256
F	128/152	232	241	192	195	252
G	128/133	232	239/263	189	195	252
H	133/142	232	259/263	189	195	246/252
I	133/152	232	241/263	191	195	252
J	133/152	232	243/265	189	195	252
K	142/153	232	263	189	195	252
L	133/146	232	241	189/211	193	252/256
M	133/146	232/266	251/261	189/211	193/195	256/260
M’	133/146	232/266	253/261	189/211	193/195	256/260
N	133/146	232/266	239/253	189/211	193/195	256/260
O	132	232	241	191	195	252
P	132/152	228/232	241	185	197	252
Q	147/152	226/228	241/251	165	181/197	252
R	133/152	232	241	189/191	195/197	252/256
S	128/133	232/240	241	192	195/197	252
T	133/152	232/238	241	189/191	195	252
March	**VVS 2**	**VVMD 5**	**VVMD 7**	**VVMD 27**	**VRZAG 62**	**VRZAG 79**
E	132	228/232	241/257	125	193/196	252
G	132	228/232	241	189	196	252
H	132	228/240	263	191	193/195	252/256
J	132/152	228	241/259	189	194/198	252
N	132	232	257/263	189	193/195	252
O	139	228/232	241/263	189	195	252
P	152	228/232	241/251	189	195	252
S	132	232	241	189	195	252
W	132	228	241	189	195	252
X	132	228	251/263	189	193/195	252
α	132/152	228/232	241	189/191	193/196	252/256
χ	152	228/232	241/257	181/189	196	244/252

Figure 1:


A
B
C
D
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G
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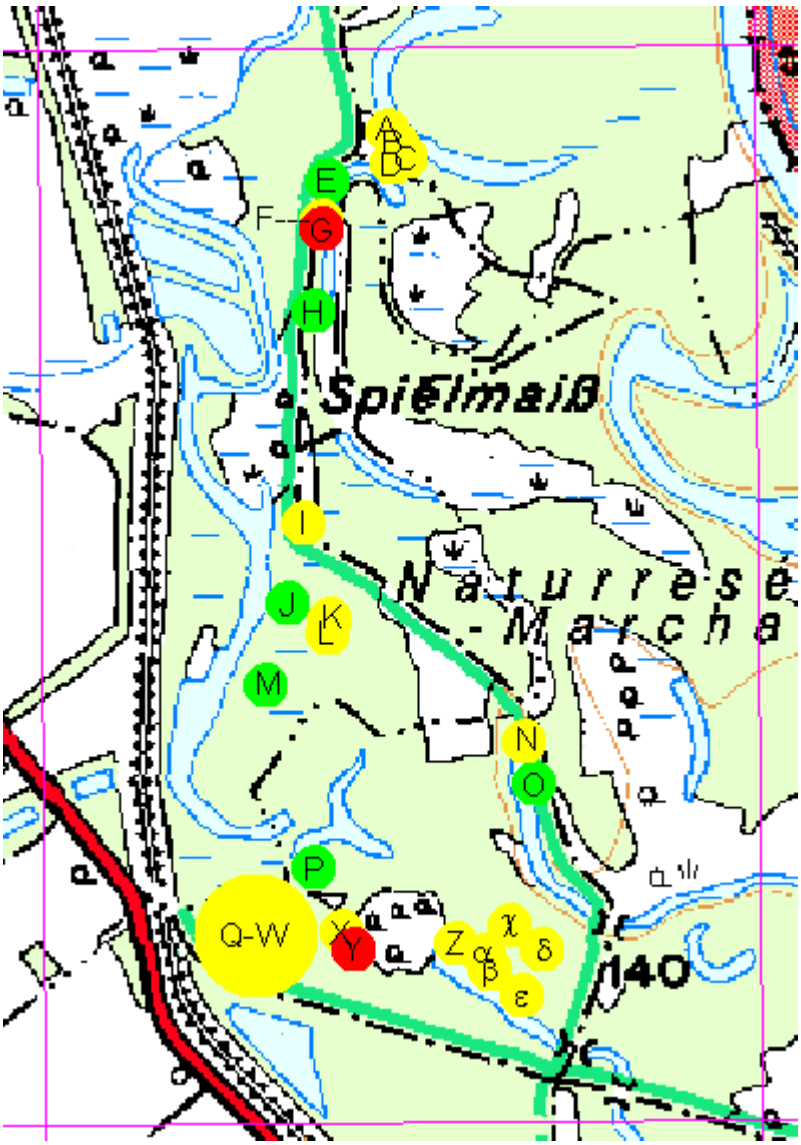
U
Orth an der Donau
Steinafurt
Heustadelböden
Großes Wiesenfeld
Rohrhaufen
Oberer Stockmaiß
Unterer Stockmaiß
Grenzböden
Biberhaufen
Mitterhaufen
Hirschensprung
Haslau an der Donau
Sportpl.
Maria Ellend
Neu Haslau
Schüttlau

Figure 2: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Naturreser
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140

Figure 3: