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Feeding ecology of *Sabanejewia balcanica* and *Cobitis elongata* in Croatia

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A b s t r a c t . *Cobitis elongata* and *Sabanejewia balcanica* from the family Cobitidae were collected monthly from September 2004 to October 2005 from the Petrinjčica River in the Danube basin of Croatia. On each sampling date, samples of the macrozoobenthos were taken from three different substrates. The density of the macrozoobenthos was calculated and compared with the diet of the loaches. During all months the most dominant group in the macrozoobenthos and in the guts of the analysed loaches was insect larvae of the family Chironomidae. Ivlev's index was used as a measure of selectivity for various macroinvertebrate taxa in the fish rations and showed changing selectivity that indicate opportunistic feeding strategies and wide diet overlaps between the species.

Key words: macrozoobenthos, substratum, Ivlev selectivity index, diet overlap index

Introduction

Data on the biology and ecology of small and commercially uninteresting fish species such as the Cobitidae family are relatively scarce. A significant number of scientists have studied their distribution, morphology and taxonomy in Croatia (M r a k o v č i ć et al. 2000, S c h n e i d e r et al. 2000, D e l i ć et al. 2003a,b); however, papers discussing their ecological characteristics (e.g. feeding ecology) are almost non-existent.

In this paper we present the results of an investigation into the feeding ecology of the species *S. balcanica* (Karaman) and *C. elongata* Heckel & Kner collected in Croatia's Pannonian plain, from the Petrinjčica River. Both investigated species have been included under the Bern Convention and catalogued as vulnerable in the Red Book Freshwater Fish of Croatia (M r a k o v č i ć et al. 2006). Aside from feeding data, the objective was to define whether differences exist between the species *S. balcanica* and *C. elongata* regarding feeding types, feeding-related competition between the two species, and affinity for the substrate, i.e. food that is available in different habitats.

It is important to mention that there is a significant number of papers in Europe discussing the ecology and biology of the Cobitidae family, such as for the species *C. taenia* (S k ó r a 1966, R o b o t h a m 1977, B o r o ń & B o r o ń 1994, R i t t e r b u s c h & B o h l e n 2000), *C. paludica* and *C. calderoni* (V a l l a d o l i d & P r z y b y l s k i 2003).

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Material and Methods

The Petrinjčica River falls within the category of mid-sized streams (34 km in length). Hydrological and geographical data on the Petrinjčica are given in D e l i ć et al. (2003a,b). Specimens were sampled with a 2.5 kW electrofisher between September 2004 and October 2005, every second third of the month in the upper course of the Petrinjčica River, 20 km from the confluence with the Kupa River. In addition to sampling fish fauna, triplicate sampling of the macrozoobenthos was carried out at the same location, taking samples from diverse substrate types: larger stones (15–35 cm), pebbles (10 cm) and sand (1 mm). Sampling was carried out using a Surber sampler (30×30 cm, 250 mm mesh size). All sampled macrozoobenthos material was preserved in 96% EtOH in the field and in 70% EtOH in the laboratory. A detailed analysis of the macrozoobenthos was carried out in the laboratory and the density of all determined groups per square meter was calculated. Identification of invertebrates from the digestive tract and of macrozoobenthos samples was carried out according to N i l s s o n (1996, 1997).

Ivlev's index (I v l e v 1961) was used as a measure of selectivity (E) for various macroinvertebrate taxa in the fish rations: $E = (r_i - p_i)/(r_i + p_i)$ where r_i is the relative abundance of food category i in the digestive tract (as a proportion or percentage of all digestive tract contents) and p_i is the relative abundance of this prey in the environment. Values range from ± 1 to +1, with negative values indicating rejection or inaccessibility of the prey, zero indicates random feeding, and positive values represent active selection.
Diet overlap index is calculated as:

$$O=\Sigma P_{ik} \times P_{jk} / (\Sigma P_{ik}^2 \times \Sigma P_{jk}^2)^{1/2}$$

where P_{jk} signifies the amount in a number of samples of taxa *k* and individual *i*, P_{ik} signifies the same value only in individual *j*. Values range from 0 (no feeding overlap) to 1 (total feeding overlap) (K o k e š 1993).

Results and Discussion

A total of 141 specimens were collected for the analysis, with 78 *S. balcanica* and 63 *C. elongata*. The low number of specimens collected reflects their low abundance in the Petrinjčica stream. For this reason and due to the hydrological conditions, not a single loach specimen was collected in February, June, November and December. High water levels during February made macrozoobenthos sampling impossible.

Over the year, the presence of certain invertebrate groups varied due to their biological and ecological characteristics, and due to the different habitat and substratum types (Table 1, Table 2 and Table 3). Plecoptera, Ephemeroptera, Diptera and Oligochaeta were dominant throughout the year, while the groups with the lowest abundances were Crustacea and Odonata, explained by the absence of vegetation and the strong water current.

Only few groups of invertebrates, namely Gastropoda, Diptera, Plecoptera and Trichoptera, expressed differences according to the substrate. In both *S. balcanica* and *C. elongata,* macrozoobenthos dominates in the food composition, and sand was also found. The diets of *C. elongata* and *S. balcanica* are composed of a large number of macrozoobenthos taxa (Tables 4 and 5). Both species share a number of taxa in the food: Nematoda, Gastropoda, Bivalvia, Crustacea, Odonata, Plecoptera, Ephemeroptera, Diptera and Coleoptera, while Oligochaeta, Odonata and Heteroptera were present only in *C.*

Table 1. Analysis of the macrozoobenthos collected on the stone substrate in the Petrinjčica River expressed as specimens per square meter.

Taxa/month	2004		2005							
	Sept.	Oct.	Jan.	Mar.	Apr.	May	June	July	Aug.	Oct.
NEMATODA					40					10
GASTROPODA	880	240		290		60			100	10
BIVALVIA	10									
OLIGOCHAETA	800	350		70	220	820		50	10	1300
HIRUDINEA	20	10		10					10	
ARANEA						90		20		10
HYDRACHNELLAE					30					60
CRUSTACEA	30				10	80				
Gammarus sp.	30				10					
Ostracoda						80				
PLECOPTERA	10	20	110	340	790	2950	500	2340	10	130
EPHEMEROPTERA	230	10		170	2930	8180	280	1110	380	90
ODONATA					40					
DIPTERA	270	300	34190	1360	5520	10550	180	260	240	930
Athericidae	20						10		10	
Chironomidae	120	160	17130	850	5450	10040	170	150	200	890
Limonidae	130	130	20		40	20		100	20	20
Simuliidae		10	17040	500	360	10		10	10	
Pupae					370	480				20
TRICHOPTERA	560	220	50	160	620	5520	280	470	330	110
COLEOPTERA	880	220	10	300	340	1540	150		140	
Imago	90			250	240	1110	80		110	
Elmis sp.	70			200	160	1050	50	790	30	
Larvae	790	220	10	50	100	430	70	100	30	70
Esolus sp.	600	160	10	20	70	330	30	40	30	
Elmis sp.	60			10	20	10	10	20		
Riolus sp.	100	40		10	10	70	20			
Stenelmis sp.	30	10		10						
Larvae non det.										70
HETEROPTERA	10							30		

elongata. *C*. *elongata* had the most diverse diet in July, and only Diptera was noted in their diet during March, April, May and October. In contrast, *S*. *balcanica* had a more diverse diet, especially in May, July, August, September and October. The largest number of macrozoobenthos specimens per specimen of *C*. *elongata* was noted in September (96 food specimens, mainly Diptera larvae), while *S*. *balcanica* showed the highest number in May (276 food specimens, nearly exclusively Diptera larvae) (Tables 4 and 5).

In general, Chironomidae was the most common food item in the diet of *S*. *balcanica* and *C*. *elongata,* and was most prominent in the macrozoobenthos community. Chironomidae and other macroinvertebrates are preferred prey items for other *Cobitis* species (Boroń & Boroń 1994, Soriguer et al. 2000, Valladolid & Przybylski 2003, Marszal et al. 2003). On the other hand, some taxa were observed more often in one of the fish species. In particular, Odonata, Plecoptera, Ephemeroptera, and Trichoptera appear more frequently in the food of *S*. *balcanica* than of *C*. *elongata*.

Table 2. Analysis of the macrozoobenthos collected on the sandy substrate in the Petrinjčica River expressed as specimens per square meter.

Taxa/month	2004				2005							
	Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	June	July	Aug.	Oct.
NEMATODA										20		
GASTROPODA	400	90		90	560	130	90		50	240		30
OLIGOCHAETA	300	550			140	50	340	960	500	1190	80	130
HIRUDINEA									20			
ARANEA				10	70	200	50	10	220	100		
HYDRACHNELLAE											20	
CRUSTACEA			10	10								
Gammarus sp.			10	10								
PLECOPTERA				30						50	90	70
EPHEMEROPTERA	170	10	50			50	120	70	920	30	60	30
ODONATA			10								20	10
DIPTERA	200	560	50	40	190	110	1830	2500	4740	2210	450	510
Ceratopogonidae		20					10	20			20	
Chironomidae	130	190	40	10	180	110	1780	2450	4500	2190	410	510
Limonidae		320					10		80			
Psychodidae					1 (10)							
Simuliidae		10		30					10			
Stratiomyidae		10										
Tabanidae										10	10	
Pupae	20							30	150	10	10	
Diptera non det.	50											
TRICHOPTERA	150	10	30	20			20		320	30	60	
COLEOPTERA	90	30	20	10	30	40	110	130	1930	190	190	10
Imago						10			11350	30	60	10
Elmis sp.						10			1300	10	40	10
Larvae	90	30	20	10	30	30	110	130	580	160	130	
Esolus sp.	30	30	20			20	80	130	490	130	90	
Elmis sp.											20	
Riolus sp.	60			10		10	30		50	30		
Stenelmis sp.					30							
HETEROPTERA	10					10	90		30	10	20	

Gastropoda were present in the diet of both fish species in July only; interestingly as a minor food item in *C. elongata,* but as a dominant fraction of the diet in *S. balcanica*. The dominance of gastropods in the diet of *S. balcanica* in one particular month is not the result of an affinity of Gastropoda for a particular substrate, as Gastropoda is well represented in all substrates. They different proportion of Diptera in the food of the two loach species is most likely caused by the different abundance in different substrates.

In the guts of *C. elongata,* crustaceans of the order Isopoda were found but were not found in the collected macrozoobenthos; most likely the specimens had been feeding elsewhere.

The Ivlev selectivity index showed a positive selection for Diptera throughout the entire research period for *C. elongata* from all available substrates, while other taxa were selected only during certain sampling periods (Table 6). Hydrachnellae and Isopoda were not found in any of the available substrates, therefore the high positive selection for these taxa in July can be considered

Table 3. Analysis of the macrozoobenthos collected on the pebble substrate in the Petrinjčica River expressed as specimens per square meter.

Taxa/Month	2004				2005							
	Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May	June	Jul.	Aug.	Oct.
NEMATODA	10							30				
GASTROPODA	20	70	10	70	80	40	40	20	180	220	10	
BIVALVIA												
OLIGOCHAETA	30	1890	90		630	120	1040	490		30	80	160
ARANEA	20			10			210	10	40	20		430
HYDRACHNELLAE								60			60	
CRUSTACEA				10						70		10
Gammarus sp.				10						70		10
COLLEMBOLA							10					50
PLECOPTERA			30	30	100		960	510	430	700	130	
EPHEMEROPTERA	150				40	140	2410	1200	900	560	380	580
ODONATA											10	
DIPTERA	450	600	30	60	6680	660	5380	980	1270	460	570	1430
Ceratopogonidae		30										
Chironomidae	400		10	10	6500	550	5130	880	1000	340	510	1370
Limonidae		430	10			10		30	230	100		20
Simuliidae				50	170	100					30	10
Stratiomyidae	10											
Tabanidae			10									
Pupae	30				10		10	70	30	10	20	30
Diptera non det.	10	10					240					
TRICHOPTERA	360		10	20	20	20	440	340	1200	540	640	100
COLEOPTERA		60	20	20	170	220	1240	500	190	1330	290	290
Imago					10	120	560	230	170	1260	110	210
Elmis sp.						110	520	190	100	770	90	150
Larvae	120	60	20	20)	160	100	680	270	20	70	180	80
Esolus sp.	20	40		20	130	80	570	180	20	40	120	40
Elmis sp.					10		10			20	50	
Riolus sp.	10	20	20		10	20	90	50			10	
Stenelmis sp.					10		10	30		10		10
HETEROPTERA	10								30	20		

an artefact. Like *C. elongata*, *S. balcanica* showed a positive selection for Diptera during the entire year (Table 7). In contrast to *C. elongata*, *S. balcanica* also included Copepoda and Ostracoda in its diet and readily consumed Trichoptera. They often feed on Coleoptera, equally on larvae, where present, and adult stages. During the summer months, a positive selection was seen for Hydrachnellae, Gastropoda and Bivalvia. During the fall and winter months, a positive preference was seen for Ephemeroptera, which is less consumed in the summer. Periodically they are highly selective about Nematoda, which are found only on pebble substrates.

Apart from macrozoobenthos, phyto-components have also been found in the diet of different Cobitidae species (S k ó r a 1966, V a l l a d o l i d & P r z y b y l s k i 2003). In this study, no phyto-components were found in the diets of *S. balcanica* and *C. elongata*. The same result was obtained by B o r o ń & B o r o ń (1994) for *C. taenia* in Poland, while a small number of single algae for same species in Germany were found by R i t t e r b u s c h & B o h l e n (2000).

Table 4. Digestive tract contents of the species *Cobitis elongata* given as average number of items per analysed fish specimen.

Taxa/month	2004		2005					
	Sept.	Oct.	Mar.	Apr.	May	July	Aug.	Oct.
Number of fish	6	2	4	5	6	25	6	9
NEMATODA	0.3							0.1
GASTROPODA						1.9		
HYDRACHNELLAE						0.8		
CRUSTACEA						0.1		
Isopoda						0.1		
PLECOPTERA						0.1	0.2	0.1
EPHEMEROPTERA						0.2	0.7	
ODONATA							0.2	
DIPTERA	93.2	162	10	5	10.3	55.9	42.8	1.2
Ceratopogonidae		1					0.2	
Chironomidae	86.2	160.5	9.5	4.8	8.5	52.9	36.2	1.2
Limonidae		0.5				0.1		
Psychodidae					0.3			
Tabanidae			0.3					
Pupae	7		0.3	0.2	1.5	2.9	1.6	
Diptera non det.								
TRICHOPTERA	0.3					0.2		
COLEOPTERA	2					8.1	5.5	
Imago						0.0		
Imago non det.						0.0		
Larvae	2					8.08	5.5	
Esolus sp.	1.7					8.08	4.2	
Riolus sp.	0.3						0.7	
Stenelmis sp.							0.3	
Larvae non det.							0.3	
HETEROPTERA							0.3	

We attempted to identify the preferred feeding habitat of the two loach species, based on a comparison of consumed food and available food. However, this was not possible due to the similarity in macrozoobenthos composition on all three analysed substrates.

The food compositions of both species were very similar for all investigated periods. The dietary overlap index ranged from 0.82 in October 2004 to 1.00 in March and July 2005 (Table 8). High values in dietary overlap resulted from the high frequency of Diptera, mostly Chironomidae in the stomach content of both species (Tables 4 and 5).

Our results and results on the feeding ecology of species *C. paludica* and *C. calderoni* from the Lozoya River are similar in abundance of certain groups of macroinvertebrates (Valladolid & Przybylski 2003), and they differ from results on the feeding ecology of *C. taenia* from the Zegrzynski Dam Reservoir (Boroń & Boroń 1994) and Lake Müggelsee in Germany (Ritterbusch & Bohlen 2000) and *Cobitis* sp. from the Polish Lake Lucien (Marszal et al. 2003), respectively. Cladocera, Copepoda and Ostracoda are preferred prey items of *C. taenia* (Boroń & Boroń 1994, Ritterbusch & Bohlen 2000), while in addition to Chironomidae, the dominant groups in the diet of *Cobitis* sp. are Cladocera and Copepoda (Marszal et al. 2003). The

Table 5. Digestion tract contents of the species *Sabanejewia balcanica* given as average number of items per analysed fish specimen.

Taxa/month	2004		2005					
	Sept.	Oct.	Jan.	Mar.	May	July	Aug.	Oct.
Number of fish	7	7	6	4	15	9	6	24
NEMATODA	0.4				0.1		0.1	
GASTROPODA						51.3		
BIVALVIA		0.1				0.1		
HYDRACHNELLAE		0.5				0.9	0.1	
CRUSTACEA	2				0.5		0.8	
COPEPODA	0.1							
OSTRACODA	1.9				0.5			
Crustacea non det.							0.8	
PLECOPTERA			1.3			0.4		
EPHEMEROPTERA		0.1	0.6		0.1	1.9		0.1
DIPTERA	41	5.7	1.6	0.5	274.1	18.8	4.6	1.2
Athericidae		0.3						
Ceratopogonidae								0.0
Chironomidae	33.6	4.7	1.5	0.3	273	14	3.5	0.9
Limonidae		0.3						0.1
Simuliidae				0.3				
Tabanidae		0.1						
Pupae	7.4	0.3	0.2		0.5	4.9	1.2	
Diptera non det.								0.0
TRICHOPTERA	0.3	0.5	0.2			2	0.3	0.1
COLEOPTERA	1.1				0.2	7.1	0.1	0.3
Imago					0.1	0.2		
Elmis sp.					0.1	0.1		
Imago non det.						0.1		
Larvae	1.1				0.1	6.9	0.1	0.3
Elmis sp.								
Esolus sp.	0.6					5.7	0.1	0.2
Riolus sp.						1.1		
Stenelmis sp.					0.1	0.1		
Larvae non det.	0.6							0.1

abovementioned groups, other than Chironomidae, are less abundant as prey items in the feeding of *C. paludica* and *C. calderoni* (Valladolid & Przybylski 2003), while they are not present in the diet of *C. elongata* and *S. balcanica*.

These differences are consequence of different habitat types (streams or lakes) in which these species live and their biological characteristics as effect of mouth morphology (Bohlen 2003, Robotham 1982), reproductive biology features (Bohlen 2003) and feeding behaviour (Vallad olid 1996). *C. paludica* and *C. calderoni* in Spain (Vallad olid & Przybylski 2003) and *S. balcanica* (Delić et al. 2003a) and *C. elongata* (unpublished data) in Croatia prefer upper and middle courses of the river with two substrate types, pebbles and finely stones, while *C. taenia* in Poland prefer a habitat type with a dominance of sand and mud, with the exception of typical mountain streams (Boroń & Boroń 1994).

Table 6. Ivlev selectivity index (E) for *Cobitis elongata* on three investigated substrates. Positive values indicate a selection of the item, negative values indicate avoidance of the item and zero values indicate neutrality. The three substrate types are stones (st), sand (sa) and pebbles (pe).

Taxa/substratum	2004						2005																	
	September			October			March			April			May			July			August			October		
	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe
NEMATODA	**1**	**1**	-0.4							-1					-1		-1					0.8	1	1
GASTROPODA	-1	-1	-1	-1	-1	-1	-1	-1	-1		-1	-1	-1		-1	**1**	-0.3	-0.3	-1		-1	-1	-1	
BIVALVIA	-1																							
OLIGOCHAETA	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
HIRUDINEA	-1			-1			-1												-1					
ARANEA			-1					-1			-1	-1	-1	-1	-1	-1	-1	-1				-1		-1
HYDRACHNELLAE										-1					-1	1	1	1		-1	-1	-1		
CRUSTACEA																								
Isopoda																1	1	1						
Amphipoda	-1																	-1						-1
non det.													-1											
COLLEMBOLA												-1										-1	-1	-1
PLECOPTERA	-1			-1			-1			-1		-1	-1		-1	-0.9	-0.8	-0.9	-0.4	-0.9	-0.9	**0**	**0**	1
EPHEMEROPTERA	-1	-1	-1	-1	-1		-1	-1	-1	-1	-1	-1	-1	-1	-1	-0.9	-0.3	-0.9	-0.9	-0.6	-0.8	-1	-1	-1
ODONATA										-1									**1**	-0.7	-0.1		-1	
DIPTERA	0.8	0.7	0.4	0.6	0.4	0.6	0.3	0.7	0.3	0.3	0.2	0.4	0.5	0.2	0.6	0.8	0.2	0.7	0.6	0.3	0.5	0.2	0.1	0.3
TRICHOPTERA	-0.9	-0.9	-0.9	-1	-1		-1		-1	-1	-1	-1	-1		-1	-0.9	-0.4	-0.9	-1	-1	-1	-1		-1
COLEOPTERA																								
Imago	-1						-1	-1	-1	-1		-1	-1		-1	**1**	-0.8	-0.9	-1	-1	-1		-1	-1
Larvae	-0.8	-0.5	-0.6	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	**0.7**	**0.5**	**0.7**	**0.6**	-0.1	**0.1**	-1		-1
HETEROPTERA	-1	-1	-1					-1			-1					-1	-1	-1	**1**	-0.5	1			

Table 7. Ivlev selectivity index (E) for *Sabanejewia balcanica* on three investigated substrates. Positive values indicate a selection of the item, negative values indicate avoidance of the item and zero values indicate neutrality. The three substrate types are stones (st), sand (sa) and pebbles (pe).

Taxa/substratum	2004									2005															
	September			October			January			March			May			July			August			October			
	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	st	sa	pe	
NEMATODA	1	1	0										1	1	-0.9		-1		1	1	1	-1			
GASTROPODA	-1	-1	-1		-1	-1		-1	-1	-1	-1	-1	-1		-1	1	0.8	0.8	-1		-1	-1	-1		
BIVALVIA	-1			1	1	1										1	1	1							
OLIGOCHAETA	-1	-1	-1	-1	-1	-1		-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
HIRUDINEA	-1			-1						-1									-1						
ARANEA			-1					-1			-1		-1	-1	-1	-1	-1	-1				-1			-1
HYDRACHNELLAE				1	1	1									-1	1	1	1	1	0.1	0	-1			
CRUSTACEA																									
Copepoda	1	1	1																						
Ostracoda	1	1	1										1	1	1										
Isopoda																									
Amphipoda	-1																	-1							-1
non det.													-1						1	1	1				
COLLEMBOLA																						-1	-1	-1	
PLECOPTERA	-1			-1			0.9	1	0.9	-1			-1		-1	-0.9	-0.4	-0.9	-1	-1	-1	-1	-1		
EPHEMEROPTERA	-1	-1	-1	0.5	0.4	1	1	1	0.9	-1	-1	-1	-0.9	-0.9	-0.9	-0.8	0.5	-0.7	-1	-1	-1	0.1	0.3	-0.4	
ODONATA																				-1	-1		-1		
DIPTERA	0.9	0.7	0.4	0.6	0.3	0.5	-0.4	0.4	-0.3	0.3	0.7	0.3	0.5	0.2	0.6	0.5	-0.4	0.3	0.5	0.2	0.4	0.1	0	0.2	
TRICHOPTERA	-0.9	-0.9	-0.9	-0.3	0.8	1	0.9	1	0.9	-1		-1	-1		-1	-0.6	0.5	-0.7	-0.6	0	-0.7	-0.1	1	0.2	
COLEOPTERA																									
Imago	-1								-1	-1	-1	-1	-0.9	1	-0.9	1	-0.4	-0.9	-1	-1	-1		-1	-1	
Larvae	-0.8	-0.4	-0.6	-1	-1	-1	-1	-1	-1	-1	-1	-1	-0.9	-0.9	-0.9	0.5	0.3	0.6	0	-0.6	-0.5	0.5	1	0.7	
HETEROPTERA	-1	-1	-1								-1					-1	-1	-1		-1					

Table 8. Diet overlapping between species *S. balcanica* and *C. elongata* throughout the research period.

Date			*Cobitis elongata*						
			2004		2005				
			IX	X	III	V	VII	VIII	X
Sabanejewia balcanica	2004	IX	0.94						
		X		0.82					
	2005	I							
		III			1.00				
		IV							
		V				0.99			
		VII					1.00		
		VIII						0.88	
		X							0.83

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