

## Four species of long-eared bats (*Plecotus*, Geoffroy, 1818; Mammalia, Vespertilionidae) in Croatia: field identification and distribution

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**A b s t r a c t.** The variability of the external characters of four sibling *Plecotus* species in Croatia was analysed. For the recently discovered species *P. macrobullaris* and *P. kolombatovici* we used specimens identified by mitochondrial DNA sequences as key specimens. Living individuals of *P. kolombatovici* can be distinguished from *P. macrobullaris* and *P. auritus* by means of shorter thumb and hind foot, more clearly than distinguishing *P. austriacus* from *P. auritus*. Using the triangular pad on the lower lip it is easy to distinguish *P. macrobullaris* from all other species in the field. Sympatric distribution is confirmed for *P. macrobullaris* and *P. auritus* as well as for *P. auritus* and *P. austriacus*. In Istria, *P. kolombatovici* was found only at one site sympatric with *P. austriacus* and at another site inhabiting the same church attic with *P. macrobullaris*.

**Key words:** *Plecotus*, long-eared bats, external characters, variability, habitat, Croatia

### Introduction

The process of identification of a bundle of morphologically very similar species of the genus *Plecotus* Geoffroy, 1818 started with the recognition of differences in bacula shape (Topál 1958), external characters (such as thumb length and snout shape), penis shape, body size and fur colouration as well as skull shape and measurements (Bauer 1960, Lanza 1960, Hanák 1962, Đulić 1980, Spitzemberger et al. 2001), and was carried on by using molecular techniques (Mayer & Helversen 2001, Spitzemberger et al. 2001, Kiefer & Veith 2002, Spitzemberger et al. 2002, Kiefer et al. 2002, Spitzemberger et al. 2003, Juste et al. 2004, Benda et al. 2004). Although it is easy to assign specimens unambiguously to the various taxa by molecular methods, morphological differences also need to be established to allow identification in the field. For studying the external morphology of sibling species, areas with sympatric distribution are of special interest. For our investigation we chose the western Balkan (including parts of Croatia), where like in Greece (Juste et al. 2004) four species of the genus *Plecotus* occur.

Until 25 years ago published records of *Plecotus* species in Croatia, like in many other countries, were questionable. Until this time (Wagner 1846, Kolombatović 1884, von Mojsisovics 1884, Jurinac 1884, Matisz 1896, Depoli 1899, Kraljević 1903, Boškay 1926, Karanam 1929, Topál 1954, Marjanac 1957, Đulić 1958, 1959) only one species had been recognized, mostly under the name *Plecotus auritus* (Linnaeus, 1758). Martino & Martino (1940) were the first to describe a new *Plecotus* taxon from the western part of former Yugoslavia: *P. auritus meridionalis* from St. Miklavž near Ormož (Slovenia). Bauer (1960) found the same taxon in Austria, realized

that it was an independent species and gave it the oldest available name, *P. austriacus* (Fischer, 1829). Mirić (1960) found this species in Petrovaradin fortress in Novi Sad (Serbia) too. Dulić (1970) and Dulić & Vrtković (1970) published records of *Plecotus* specimens from the Adriatic islands under the name *P. auritus* because of their brownish back and smaller dimensions in comparison with *P. austriacus*.

After the first critical comments on the taxonomy of long-eared bats in the Mediterranean area (Lanza 1960, Koch 1974, Dulić & Vrtković 1979), Đulić (1980) described a new subspecies, *P. austriacus kolombatovici*, from Adriatic islands and the neighbouring coast. Taxonomically ambiguous specimens from the Dinaric karst area (Ogulin, Senj, Smiljan, Ličko Petrovo Selo in Croatia and Donji Vakuf in Bosnia and Herzegovina) were classified as supposed hybrids between *P. a. austriacus* and *P. a. kolombatovici*. Specimens with external characters intermediate between *auritus* and *austriacus* have been found also in other regions: in Switzerland (Aellen 1961) and in Carinthia/Austria (Spitzemberger et al. 1993). These “intergrades” were later recognized as representing yet another independent species (Spitzemberger et al. 2001, Kiefer & Veith 2002), and Spitzemberger et al. (2003) showed that the oldest available name for this species is *P. macrobullaris* Kuzjakin, 1965. Bend้า et al. (2004) suggested a subspecific level of *P. kolombatovici* within the species *P. teneriffae*, but this problem is still unresolved (eg. different bacula shape etc.) and in this paper we used the name *P. kolombatovici*.

A few additional new findings for Croatia are mentioned in Červený & Krystufek (1988), Vrtković et al. (2001), and Spitzemberger et al. (2001, 2002, 2003). The latter two papers (Spitzemberger et al. 2002, 2003) reported Croatian specimens of the recently recognized species *P. kolombatovici* and *P. macrobullaris* whose identification was confirmed by molecular methods. Kiefer & Veith (2002) mentioned the occurrence of *P. macrobullaris* from Ogulin (identification genetically confirmed), and Kiefer et al. (2002) reported *P. kolombatovici* from Orebić and *P. auritus* from Zagreb.

Until 2002, 39 localities of *Plecotus* sp. from Croatia were published, most of them (33) represented by voucher specimens in the collections of the Croatian Natural History Museum, Zoological Department of Faculty of Science in Zagreb, Natural History Museums in Vienna and Budapest, and Senckenberg Museum in Frankfurt am Main.

In this paper we analyse the variation of external characters of *P. auritus*, *P. austriacus*, *P. kolombatovici* and especially of *P. macrobullaris*, and review the geographical distribution of these species in Croatia.

## Material and Methods

A total sample of 164 specimens from 57 localities in Croatia (Fig. 1) was examined morphologically. The major part (58 specimens) belongs to *Plecotus macrobullaris*. Most of the material (28 specimens of *macrobullaris*) was collected from 2001 to 2003 during intensive field work to study the distribution and identification of this species.

Specimens examined (\* = specimens examined also genetically):

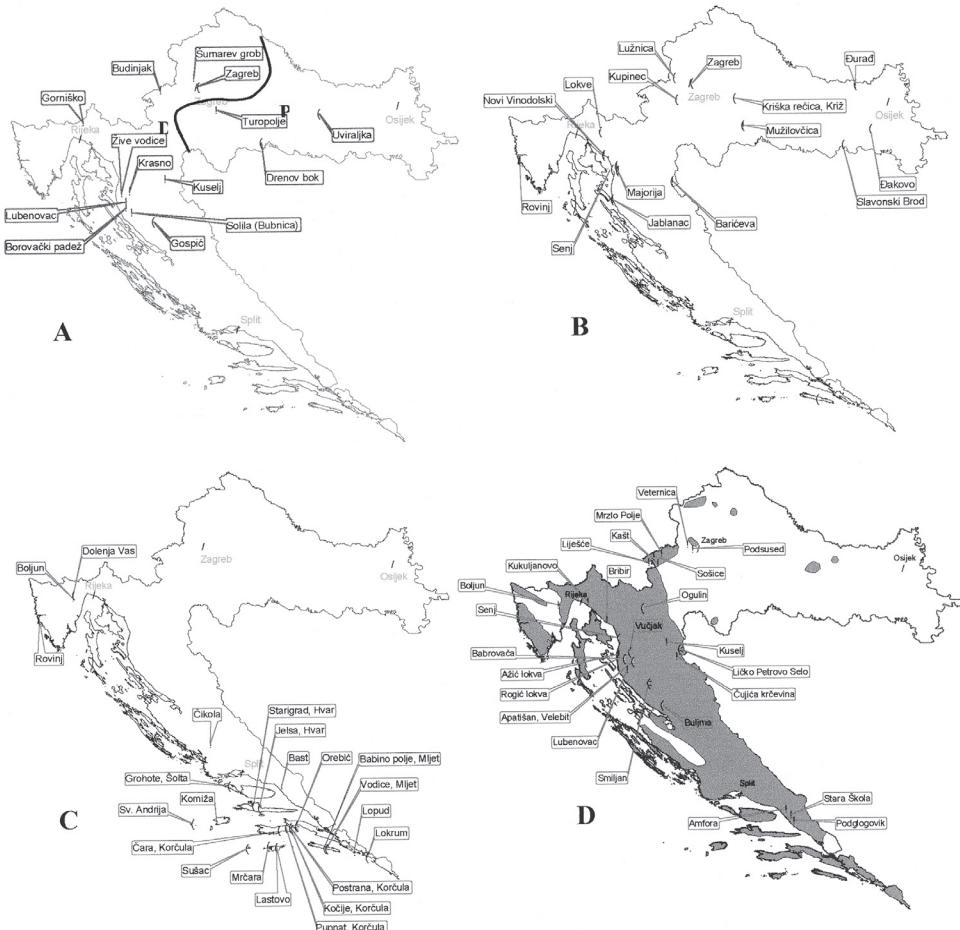
*Plecotus kolombatovici* (11 localities, 31 specimens): Rovinj (Rovigno), 1 male ZMB 17907; Boljun, 3 females (CNHM 6155\* /K5/, 6156, 6160), 12 females (released), from one additional released female\* wing tissue sample only (not measured); Čikola, 1 male (CNHM 6157); Dalmatia, 1 male (NMW 29860); Sv. Andrija island, 1 male (CNHM 6001\* /K2/);

Jelsa, Hvar island, 1 female and 1 male (ZDFSZ 2227, 2228); Sušac island, 1 male (CNHM 6010\* /K4/); Kočije, Korčula island, 1 male (ZDFSZ 3009); Mrčara island, 1 male (CNHM 6114\* /K3/); Lastovo island, 2 females (CNHM 6113, NMW 62004\* /K1/); Bast, 1 female (released); Vodice, Mljet island, 2 males (ZDFSZ 3089, 3090), 1 specimen sex? (ZDFSZ 3092); Lopud island, 1 male (ZIANP 1125);

**Plecotus macrobullaris** (22 localities, 58 specimens): Podsused-Stenjevec, 1 male (CNHM 222; in Đulić 1980 as *P. austriacus*); Mrzlo Polje, 4 females (CNHM 6040, 6041, 6042, 6043), 1 mumified specimen, sex? (CNHM 6151); Liješće, 1 female (released), Kašt, 2 females (released); Sošice, 1 mumified specimen, sex? (CNHM 6149); Boljun, 5 females (CNHM 6158, 6159, 6161, 6162, 6164), 7 females (released), Kukuljanovo, 1 male (CNHM 6169); Bribir (probably old village Bribir near Crikvenica), 1 female (NMW 28772); Sv.Vid, Senj, 1 male (CNHM 228; in Đulić 1980 as *P. kolombatovici*); Ažić lokva, 2 males (CNHM 6146, 6147); Rogić lokva, 3 males (released); Babrovača, 1 male (CNHM 6112\* /M3/); Vučjak, 1 male (CNHM 6098\* /M2/); Apatišan, 2 males (CNHM 6133\* /M4/, 6153), 1 male, 1 female (released); Lubenovac, 1 male (released); Buljma, 1 female (CNHM 5994\* /M1/); Smiljan, 2 males (CNHM 176, 209), 5 females (CNHM 177, 184–187); Lika region (without locality), 2 females (CNHM 56, 224); Ličko Petrovo Selo, 2 females (ZDFSZ 630, 631); Čujića krčevina, 1 female (CNHM 615), 1 female, 1 male (released); Kuselj, 1 male (CNHM 6163), 3 males (released); Podglogovnik, 2 males (CNHM 6123, 6124); Amfora, 1 male (CNHM 6126); Comparative material: Zgornje Jezersko (Slovenia), 1 male (NMW 55502\* /M8/); Postojnska jama (Cave Adelsberg, Slovenia), 1 female (NMW 29869); Travnik (Bosnia and Herzegovina), 1 female (NMW 29859), 1 male (NMW 29880); Livno (Bosnia and Herzegovina), 1 female (CNHM 6154\* only thumb preserved!);

**Plecotus austriacus** (17 localities, 26 specimens): Zagreb, 2 males (CNHM 986, 5983), 3 females (CNHM 175, 220, 6099\* /A1/); Kupinec, 1 male (CNHM 70); Lužnica, 1 female (CNHM 201); Kriška rečica, Križ, 1 female (CNHM 1070); N Mužilovčica (Lonjsko polje), 1 female (BM 68403); Brod (probably Slavonski Brod in Slavonia or Brod near Kupa river), 1 male (NMW 29858); Đurađ, 1 specimen (skull only) (CNHM 5971); Đakovo, 1 female (CNHM 942); Lokve, 1 female (CNHM 943); Barićeva cave (Ličko Petrovo Selo), 1 male (CNHM 6148); Rovinj (Rovigno), 1 male (ZMB 45291); Novi Vinodolski, 1 female (ZMB A 49.13); Francikovac, 1 female (ZMB A 49.13); Gaj, Senj (Zengg), 1 male (ZMB A 49.13 / AM02/88), 2 females (ZMB A 49.13 / AM 02/93, 02/100); Majorija (? = Medarija, Zengg), 1 male (ZMB A 49.13 / AM02/97); Jablanac, 1 female (ZMB 20482), 2 males (ZMB A 19.13 / AM02/94, 02/96); coast area between Rijeka and Obrovac (Cro.Lit.; without exact locality), 1 female (ZMB A 49.13); Dalmatia (without exact locality), 2 males (NMW 19424, 19434); Comparative material: Ašanja (Serbia), 1 male (CNHM 227); Šušara, Deliblatska peščara (Serbia), 1 female (CNHM 6102); Beograd (Serbia), 1 specimen, sex? (CNHM 6175);

**Plecotus auritus** (14 localities, 49 specimens): Zagreb, 1 female (CNHM 218); Šumarev grob, 2 males (released); Budinjak, 1 female (released); Turopolje, 1 male (released); Drenov Bok, 6 females (CNHM 930–934, 936), 1 male (CNHM 935); Uviraljka cave, 2 males (CNHM 6165, 6166), 1 female (released); Gorniško, 1 male (CNHM 5980); Žive vodice, 1 male, 3 females (released); Apatišan, 3 males, 3 females (released); Krasno, 1 female (released); Lubenovac, 3 males, 9 females (released); Borovački padež (Štirovača), 1 female (CNHM 6074), (other 15 females released without measurements); Solila (Bubnica), 1 male (CNHM 6073); Gospić, 1 female (CNHM 221); Lika (without exact locality), 1 male (CNHM 226); Kuselj, 1 male (CNHM 6122), 3 males, 3 females (released); Comparative material: Šušara (Serbia), 1 specimen sex? (CNHM 5984); Vermousa (Albania), 1 female (NMW 29881).



**Fig. 1.** Distribution of four *Plecotus* species in Croatia: A) *P. auritus*, B) *P. austriacus*, C) *P. kolombatovici*, D) *P. macrobullaris*. Grey area in D represents karst. Published data are marked with circles and new data with black dots. Published: A) Drenov bok, Gospic (Đulić 1980), Zagreb (Kiefer et al. 2002), Uvirajlka (Tvrtović et al. 2001); B) Zagreb, Kupinec, Lužnica, Kriška rečica, Slavonski Brod, Dakovo, Lokve, Senj, (Đulić 1980); C) Vodice (Mljet isl.), Kočije, Pupnat, Čara, Postrana (Korčula isl.), Jelsa (Hvar isl.), Grohote (Šolta isl.) (Đulić & Tvrtović 1979), Dolenja Vas, Rovinj, Lokrum (Đulić 1980), Starigrad (Červený & Kryštufek 1988), Lastovo, Mrčara, Sušac, St. Andrija (Spitzenberger et al. 2002), Orebić (Kiefer et al. 2002); D) Smiljan, Ličko Petrovo Selo (Đulić 1980), Ogulin (Đulić 1980, Kiefer & Veith 2002), Vučjak, Babrovača, Apatišan, Buljma (Spitzenberger et al. 2003).

## Morphological analysis

Forearm length (FA), claw length (CL), tragus length (TL) and tragus width (TW) were measured according to Stebbings (1967), ear length (E) according to Häussler & Braun (1991), thumb length (TH) according to Schober & Grimbacher (1998), and hind foot length (HF) without claws after Harrison (1964). Measurements were taken in the field using a calliper (FA, TH, CL, HF) and a specially scaled metal ruler (E, TL, TW) with a precision of 0.05 mm. In all preserved specimens the measurements were checked again with a Fowler digital calliper «Sylvac» with a precision of 0.01 mm.

We recorded the following qualitative morphological traits: presence or absence of long and robust foot hairs (von Helversen 1989), face shape (Strelkov 1988), presence or absence of triangle on the lower lip (Spitzemberger et al. 2002), and penis shape (Bauer 1960, Mucedda et al. 2002). Fur colour was determined for back, throat and venter.

Our sample contains 22 (14%) juveniles (mostly *P. austriacus*) and some subadults (at an age before the end of first hibernation). According to data of *Corynorhinus townsendii*, a member of a related genus, moulting of the juvenile pelage of *Plecotus* sp. finishes probably before the first autumn, but the subadult pelage is not changed before the following summer (Händley 1959). According to Stebbing (1967) the growth of the bones in *P. auritus* is not completed before the end of November of the first year. We compared the measurements of juvenile and subadult individuals with those of adults, because we expected different allometric growth. Hind foot, thumb and claw length are the first to reach adult values during the first year, one month later followed by forearm length (Howard 1995) and probably by tragus length and width. The ear length is the last trait to reach its full dimension. We therefore eliminated all measurements of juveniles from the biometrical analysis. Moreover, measurements of FA, TL, and TW were not included from subadult specimens found from July to March which had E values shorter than 34.5 mm in *P. austriacus*, *P. auritus* and *P. macrobullaris*, and shorter than 33.0 mm in *P. kolombatovici*. These values indicate probably the minimal ear lengths of adults. To show the influence of sexual dimorphism (Stebbing 1967), the measurements are reported for both sexes separately.

Basic statistic was computed using STATISTICA 6.0. Intraspecific sexual dimorphism was tested using the t-test. Differences between species and sexes for all variables were tested by one-way ANOVA and the Tukey test. From scatterplot diagrams total transitions (Sijarić 1980) are noted as measurement of identification efficiency. Identification of all museum specimens of *P. auritus*, *P. austriacus*, and *P. kolombatovici* / *macrobullaris* was reexamined (not presented) by PC analysis and biometrical plots of diagnostic skull and teeth measurements using standard methods (Spitzemberger et al. 2001, 2002, Bendau & Ivanova 2003).

#### Molecular genetic analysis

Key specimens for the morphological analysis were tested using a partial sequence of the mitochondrial control region (CR). With this sequence it is possible to assign unambiguously individuals to the respective taxa (Spitzemberger et al. 2001). From the recently described species (*P. kolombatovici*, *P. macrobullaris*) a set of key specimens (6 and 10 specimens respectively) was identified before morphometric analysis (see above, specimens marked with \*). In addition specimens of *P. macrobullaris* with published genetic and morphometric data were included in analysis (Figs 3 and 4): Ogulin, (Croatia) 1 male (SMF 44898 (Pind6 /M5/), Lienz (Austria) 1 male (NMW 34857 (Pleaus10 /M6/), Globasnitz (Austria) 1 male NMW 42564 (Pleaus9 /M7/) and Fischertratten (Austria) 1 female ZFMK 2001.327 (Pind3 /M9) (Kock 1974, Kiefer & Veith 2002, Kiefer et al. 2002, Spitzemberger et al. 2001, 2002). Moreover, one subadult female of *P. austriacus* was checked by mitochondrial DNA analysis too (CNHM 6099 /A1/).

DNA extraction, PCR amplification, cloning and sequence analysis were performed in the Laboratory of Molecular Systematics (NMW) in Vienna. All protocols are the same as described previously in Spitzemberger et al. (2002), sequencing (both strands) was performed by MWG-Biotech, Ebersberg, Germany.

## A b b r e v i a t i o n s

CNHM – Croatian Natural History Museum, Zagreb; ZDFSZ – Zoological Department, Faculty of Science, University of Zagreb; NMW – Museum of Natural History, Vienna; ZMB – Zoological Museum, Berlin; MNM – Zoological Museum, Budapest; SMF – Senckenberg Museum, Frankfurt am Main; ZIANP – Zoological Institute Academy of Sciences St. Petersburg; BMNH – Natural History Museum London.

m = mean value, s = standard deviation, n = number of specimens examined, p = level of significance.

## R e s u l t s

### V a r i a b i l i t y o f m e a s u r e m e n t s

External dimensions with basic statistics are presented in Table 1. In most specimens of *P. auritus* and *P. macrobullaris* the values of CL are about one third longer than in *P. austriacus* and *P. kolombatovici*, but the variability of this measurement in the former two species was extremely high and CL was therefore excluded from the series of good diagnostic characters and from statistical treatment. As in Austria (S p i t z e n b e r g e r et al. 2002), *P. macrobullaris* from Croatia shows in most characters a significant statistical difference ( $p < 0.01$ ) between males and females. In *P. kolombatovici* and *P. auritus* only one character differed significantly between the sexes, and in *P. austriacus* (small sample), contrary to the results from Austrian individuals, no differences were found in Croatia. Relatively high variability of HF and TH lengths within the *P. auritus* sample could be explained by geographical differences ( $p < 0.05$ ) between the subsamples from the Dinaric Alps plus Mt. Medvednica near Zagreb (*auritus* D; n = 17) and the Pannonian plain (*auritus* P; n = 11) (Fig. 2).

### U t i l i t y o f m e t r i c a n d q u a l i t a t i v e c h a r a c t e r s f o r i d e n t i f i c a t i o n

*P. macrobullaris* has significantly ( $p < 0.01$ ) longer FA, TH, HF, E and TL than *P. kolombatovici*, and *P. auritus* has significantly longer FA, TH, HF and E than *P. kolombatovici*. Between all other species significant differences were found only in two characters: *P. austriacus* has significantly longer FA and HF than *P. kolombatovici* and *P. auritus* has longer TH, but shorter TL than *P. macrobullaris*. Unfortunately, in most cases overlap is found in all external biometrical measurements. The scatterplot of TH / HF (Fig. 2) separates *P. auritus* from *P. austriacus*. The same combination of TH / HF (Fig. 3) and scatterplot TH / FA (Fig. 4) separates the cluster of specimens belonging to *P. macrobullaris* and *P. auritus* from *P. kolombatovici*. The total number of transitions between *P. kolombatovici* and the *P. auritus*-*P. macrobullaris* group in scatterplot TH / HF are 5 out of 85 specimens, by TH/FA only 4. When combining both scatterplots only 2 total transitions were found (2.4%). The identification of specimens within the *P. austriacus*-*P. kolombatovici* group and within the *P. auritus*-*P. macrobullaris* group, respectively, is not possible using external measurements.

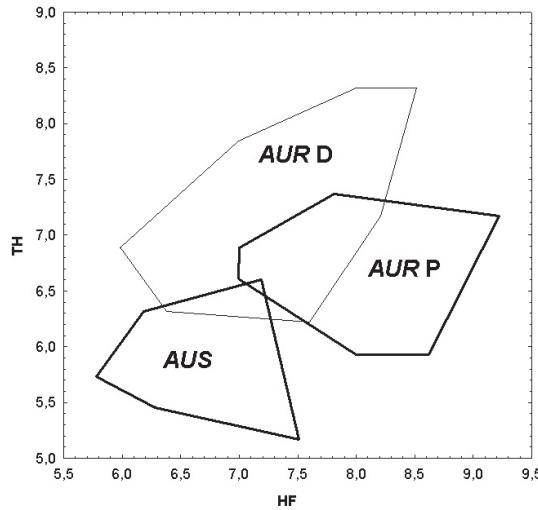
*P. kolombatovici* has on average the shortest forearm of all four species. Only in rare cases do adult specimens belonging to species other than *P. kolombatovici* have also very small forearms and thumbs, probably due to stressed situations during the growth period. We found, for example, one «dwarf winged» male of *P. auritus* (Kuselj near Plitvice, CNHM

**Table 1.** External measurements of all four *Plecotus* species from Croatia.

males						females					
mean	s	min	max	n	p	mean	s	min	max	n	
<i>P. macrobullaris</i>											
FA	39.95	1.57	37.30	42.50	25	**	41.22	1.26	39.00	43.50	30
TH	6.64	0.35	6.10	7.50	21	*	6.91	0.45	6.00	7.60	31
CL	2.35	0.55	0.90	3.50	22	*	2.73	0.49	1.50	3.50	31
HF	7.59	0.48	6.70	8.50	18	*	8.00	0.52	6.90	9.00	27
E	36.38	1.14	34.50	38.80	14	*	37.37	1.44	34.50	39.50	18
TL	16.21	0.68	15.00	17.50	18		15.85	0.76	14.50	17.30	16
TW	5.34	0.28	5.00	5.80	16	*	5.57	0.38	5.00	6.50	26
<i>P. kolombatovici</i>											
mean	s	min	max	n		mean	s	min	max	n	
FA	37.18	0.96	36.20	39.30	8	**	38.72	0.58	37.20	39.00	18
TH	5.64	0.29	5.30	6.10	7		5.63	0.25	5.14	6.20	18
CL	1.97	0.34	1.60	2.40	7		2.16	0.31	1.30	2.51	17
HF	6.20	0.34	5.80	6.70	5		6.26	0.54	5.26	7.00	16
E	34.57	1.22	33.00	36.50	7		35.63	1.74	33.80	38.30	6
TL	15.00	0.85	13.40	15.80	6		15.06	0.67	14.50	16.00	5
TW	5.08	0.43	4.50	5.50	4		5.26	0.25	5.00	5.50	5
<i>P. austriacus</i>											
mean	s	min	max	n		mean	s	min	max	n	
FA	39.10	1.42	37.50	40.20	3		40.10	0.87	39.10	41.80	7
TH	5.98	0.41	5.50	6.70	10		5.88	0.39	5.20	6.50	14
CL	1.89	0.25	1.50	2.20	10		2.06	0.34	1.20	2.40	13
HF	6.75	0.44	6.20	7.40	10		6.71	0.45	5.80	7.50	12
E	36.87	1.10	35.80	38.00	3		37.00	1.30	34.80	39.00	9
TL	15.60	0.53	15.00	16.00	3		15.91	0.93	14.10	17.00	8
TW	5.50	0.00	5.50	5.50	3		5.54	0.21	5.30	6.00	7
<i>P. auritus</i>											
mean	s	min	max	n		mean	s	min	max	n	
FA	39.63	1.39	37.50	42.00	12		40.28	1.15	37.60	42.00	26
TH	7.17	0.72	6.00	8.50	13		7.13	0.62	6.00	8.50	27
CL	2.43	0.68	1.20	3.00	9	*	2.83	0.34	2.00	3.50	22
HF	7.74	1.06	6.00	9.20	9		7.47	0.53	6.40	8.50	17
E	36.86	0.67	36.00	37.80	5		38.01	1.42	35.80	39.80	10
TL	14.58	0.58	14.00	15.50	6	**	15.52	0.64	14.50	16.80	15
TW	5.15	0.31	4.80	5.50	4		5.30	0.29	5.00	5.80	8

6122) with a forearm length of only 34.5 mm (Fig. 4). This bat was at least one year old (netted on June 4th), its other measurements (for example hind foot length – 8.5 mm) were completely within the species range.

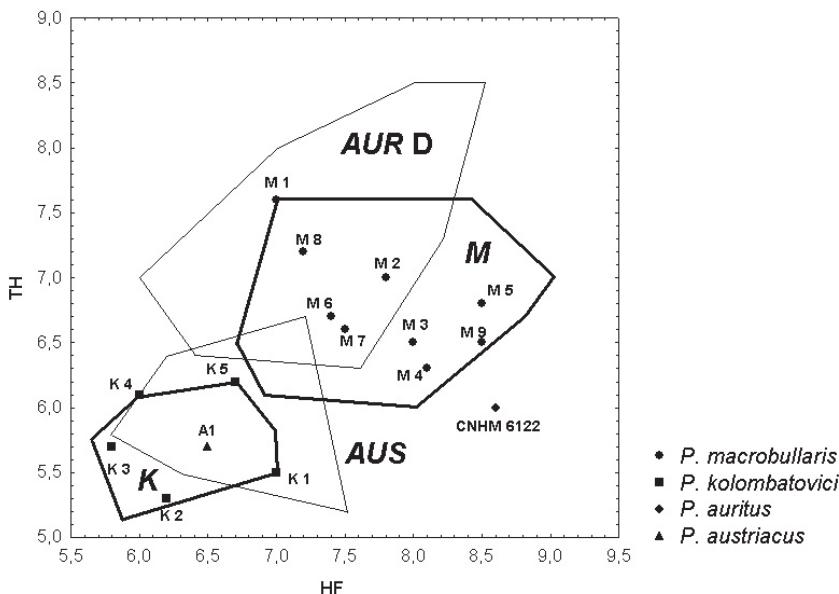
The presence or absence of longer hairs on the hind foot is not useful for identification. All species have visible hairs on their feet with differences resulting from different foot length. The shape of the snout is a good diagnostic feature for the field identification of *P. auritus* and *P. austriacus*, but specimens of *P. macrobullaris* have an intermediate shape (see Spitzer et al. 2002). Thus, this character was eliminated from the group of good diagnostic features in areas where *P. macrobullaris* occurs. In contrast to all other



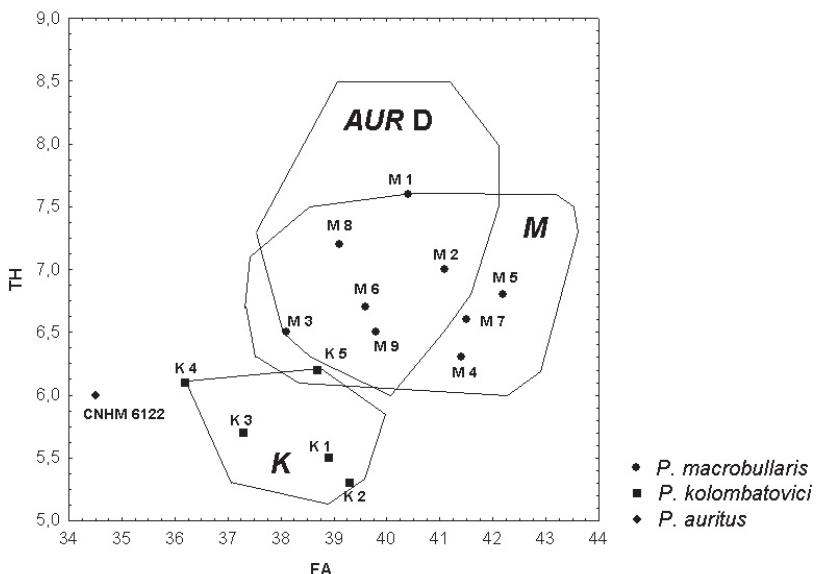
**Fig. 2.** Scatterplot of thumb (TH) / hind foot (HF) of *P. austriacus* (AUS, n = 22) and *P. auritus* from the Dinaric Alps (AUR D, n = 17) and the Pannonian Plain (AUR P, n = 11).

species *P. kolombatovici* has a visibly smaller snout, but this difference can be observed only by a *Plecotus* specialist.

The triangle on the lower lip is a constant character of Croatian *P. macrobullaris*. It was found in all specimens in the field and in well preserved bodies which were incubated in 75% ethyl alcohol prior to skull extraction (n = 49). In specimens preserved in alcohol of higher



**Fig. 3.** Scatterplot of thumb (TH) / hind foot (HF) of *P. auritus* (AUR D), *P. macrobullaris* (M), *P. austriacus* (AUS) and *P. kolombatovici* (K) from Dinaric Alps. Key specimens identified by mtDNA are marked by symbols. CNHM 6122 represents *P. auritus* with dwarf wings.



**Fig. 4.** Scatterplot of thumb (TH) / forearm (FA) of *P. auritus* (AUR D), *P. macrobullaris* (M) and *P. kolombatovici* (K) from the Dinaric Alps. Key specimens identified by mtDNA are marked by symbols. CNHM 6122 represents *P. auritus* with dwarf wings.

concentration, this feature showed a tendency to shrivel and become invisible. A somewhat different form of triangle was observed in a single *P. austriacus* male from Barićeva cave near Plitvice (CNHM 6148). Until now this character in its typical form was never observed in the other species.

Adults of *P. auritus* and *P. kolombatovici* all have typical brown to brown-grayish back fur, contra grey to greyish-brown back fur in *P. austriacus* and *P. macrobullaris*. Subadult specimens of *P. auritus* and *P. kolombatovici* had a greyish back too. Adult *P. kolombatovici* females from Boljun had a more greyish back than specimens from southern Adriatic islands. The ventral fur of most *P. macrobullaris* specimens examined was dense and pure white (especially on the throat) in contrast to topotypic *P. auritus*, whereas most *P. kolombatovici* specimens had a whitish throat and venter fur too. In both species we observed also some specimens with greyish ventral fur. One female *P. macrobullaris* from Boljun had a greyish venter, but her young had a clear white belly.

The penis shape is useful for differentiation between the *P. austriacus*-*P. kolombatovici* group (rounded or club-shaped), *P. auritus* (narrowing towards the end), and *P. macrobullaris* (intermediate; almost cylindrical).

For identification of the four Croatian *Plecotus* species we recommend the following protocol. First step – separation of *P. macrobullaris* by its triangle pad on the lower lip; second step – separation of *P. austriacus* by its greyish back fur and remarkable large and elongated face shape; third step – identification of *P. kolombatovici* and *P. auritus* by using TH and HF (see scatterplot diagram Fig. 3). In all cases checking of the other characters is useful. Adult males of the *P. austriacus*-*P. kolombatovici* group and of *P. auritus* and *P. macrobullaris* can be identified by different penis shapes too. Problems with identification of juveniles and subadults from July (first year) to spring (second year) remain, mostly because of the absence of distinctive adult fur colouration.

## Distribution

Our investigations added 10 new sites for *P. auritus*, 9 for *P. austriacus*, only 5 for *P. kolombatovici*, but 17 for *P. macrobullaris*. These new data reveal the general distribution pattern of the four *Plecotus* species in Croatia (Fig. 1). *P. macrobullaris* is restricted to karstic areas (Fig. 1.D). *P. austriacus* and *P. kolombatovici* were found to be allopatric except in Istria: *P. austriacus* is restricted to areas with continental influence (Fig. 1.B), *P. kolombatovici* only to Mediterranean habitats (Fig. 1.C).

Pannonian lowland and subpannonian hills. *P. austriacus* is dominant, and the sympatric *P. auritus* is present in colline and montane beech (*Fagus sylvatica*) forests and planar flooded common oak (*Quercus robur*) forests. In this area the northernmost subpopulation of *P. macrobullaris* in Croatia occurs, which inhabits a small isolated karst area on the eastern part of Mt. Medvednica near Zagreb city. In the old city of Zagreb *P. auritus* and *P. austriacus* occur syntopically.

Dinaric karst area. Dominant *P. auritus* occurs in this area in sympatry with *P. macrobullaris*. *P. austriacus* inhabits only river valleys and lowlands. *P. austriacus* and *P. macrobullaris* were found in the same locality only in a submontane karst beech forest of Ličko Petrovo Selo near Plitvice, but in different years. Syntopic occurrence of *P. macrobullaris* and *P. auritus* was recorded in three places: (1) Kuselj (Plitvice): in nets over a small pond near karst fir and beech forest; (2) Apatišan (Velebit Mt.): in nets of the same clearing of karst spruce, fir and beech forest; (3) Lubenovac (Velebit Mt.): in nets over a small pool near the edge of karst fir and beech forest. The southern edge of the distribution of *P. auritus* is the border of the continental vegetation, mostly maritime montane beech forest. *P. auritus* was most abundant in the extraazonal boreal spruce forest on Velebit Mts (Štirovača karst depression): on July 16<sup>th</sup> we netted 17 specimens during one night, mostly females in lactation, over a small pool.

Adriatic coast. In the mosaic of karst and flysch habitats of continental and Mediterranean forests area in Istria three species (*P. austriacus*, *P. macrobullaris*, and *P. kolombatovici*) were found. Only *P. macrobullaris* and *P. austriacus* occur on the foothills of the coastal mountains between Rijeka and Obrovac (N Dalmatia), but all *P. austriacus* findings came from the end of the 19th century only. In central and southern Dalmatia including islands only *P. kolombatovici* occurs and in the higher parts of the coastal karst (Mt. Biokovo) only *P. macrobullaris* was found. Besides scarce findings on the coast, one maternity colony of *P. kolombatovici* was found in Boljun (Istria). It shared the same church attic with a maternity colony of *P. macrobullaris*. A syntopic site of *P. kolombatovici* and *P. austriacus* is Rovinj (Istria): from there one *P. austriacus* (ZMB 45291) and two specimens of *P. kolombatovici* are preserved in the museums of Berlin and Frankfurt (ZMB 17907; SMF 32962 – Đulić 1980). Specimen SMF 32962 was later published as *P. macrobullaris* by Bend a & Ivanova (2003). All these records are from different years, as well as the findings of *P. austriacus* (Đulić 1980) and *P. macrobullaris* from Senj (redetermination of *P. kolombatovici* in Đulić 1980).

## Discussion

### Identification

The separation of Croatian *P. auritus* from *P. austriacus* with external measurements is only possible by the scatterplot TH/HF (Fig. 2), not with other character combinations

(S t e b b i n g s 1967) or using individual morphometric characters only (M u c e d d a et al. 1989). The same scatterplot (TH/HF) in combination with TH/FA should be used to distinguish *P. macrobullaris* from *P. kolombatovici*. This method has proved to be better than other bivariate plot combinations (S p i t z e n b e r g e r 2002, B e n d a & I v a n o v a 2003) and a multivariate (PC) analysis of skull measurements (S p i t z e n b e r g e r et al. 2001). Only scatterplots of the height of first upper incisor / the height of upper canine, proposed by S p i t z e n b e r g e r et al. (2002) yield the same useful result too, but only in specimens with low abrasion rate of crown tops of teeth (B e n d a & I v a n o v a 2003). When using only external measurements, the separation of *P. macrobullaris* from *P. austriacus* is in most cases problematic, and the separation of *P. macrobullaris* from *P. auritus* is impossible (Table 1, Fig. 3, Fig. 4).

Different measuring methodology of the tragus length (compare S t e b b i n g s 1967 and H ä u s s l e r & B r a u n 1991) prohibits the comparison of published data. The tragus width (S t e b b i n g s 1967, M u c e d d a et al. 2002) with a variation range of only 2.1 mm and a high overlap of values between different species is also not useful for identification. K i e f e r & V e i t h (2002) reported a relatively long ( $\geq 18$  mm) and wide ( $\geq 6$  mm) tragus as distinctive for *P. macrobullaris*, but in our sample both measurements vary within the range of *P. austriacus* values and it is therefore not usable for field identification. The thumb claw length is too dependent on age and intensity of use, especially in karst habitats. Already von H e l v e r s e n (1989) reported that *P. auritus* has shorter claws if it uses «Holzbeton» nestboxes.

Pure white throat colour and conspicuous density of longer throat fur (K i e f e r & V e i t h 2002) was a very good additional character for fast distinction of *P. macrobullaris* from *P. auritus* and *P. austriacus* in the continental area, but not always. S p i t z e n b e r g e r et al. (2002) reported that Austrian *P. macrobullaris* specimens preserved at the NMW ( $n = 16$ ) had a greyish throat and venter. This is also the case in some specimens from our sample. Also G a r i n et al. (2003) did not find the pure whitish ventral coloration of *P. macrobullaris* in the Basque region ( $n = 7$ ). The variability of this character needs further examination.

Identification of *P. macrobullaris* by presence of the triangular pad (Fig. 5) on the lower lip (S p i t z e n b e r g e r et al. 2002) is the best help in the field. It is the key character for distinguishing this species from similarly coloured *P. austriacus*. This character only separates sympatric living *P. auritus* from *P. macrobullaris* in the area of morphological similarity in other characters, like in the western Alps (A r l e t t a z et al. 1997). The method of preparing study specimens with a stitch to close the mouth of a skin proposed by H a l l (1962) damages the central part of the lower lip and this is the reason why this particular character cannot be observed in dry preserved museum material.

## D i s t r i b u t i o n

There are few records of *P. austriacus* in the area of Gorski kotar and Lika (narrowest part of Dinaric Alps), but an occasional connection of the Pannonian lowland population with the population in Istria probably does exist (Fig. 1.B). Istria is the western contact area between *P. austriacus* and *P. kolombatovici*. *P. kolombatovici* inhabits the Adriatic coast and has its western border of distribution in Istria. This may be due to the fact that *P. kolombatovici* meets the border of Mediterranean habitats in Istria, or because of the competition with the larger *P. austriacus*. In St. Andrija island and in the area near Čikola river (inland) *P. kolombatovici*



**Fig. 5.** Triangular pad on the lower lip as a distinctive character of *Plecotus macrobullaris* in Croatia.

foraging in the open, patrolling 5–8 m over bushes and forest clearings, were observed, thus indicating a similar foraging behaviour to *P. austriacus* (Flueckinger & Beck 1995). From the coastal part of N-Dalmatia east to Greece (Western Balkan) *P. austriacus* is absent and no evidence of a connection with the range of *P. kolombatovici* exists in this area. In Greece there is a second distributional contact between *P. austriacus* and *P. kolombatovici*: through the Vardar valley *P. austriacus* forms a deep break in Mediterranean Greece (Kryštufek et al. 1992, Hanák et al. 2001).

In the case of *P. auritus*, our findings change the previous impression of the abundance in the area east of the Adriatic (Howard 1995). It is a common species in continental forests probably in all of the Dinaric Alps. Sympatric occurrence with *P. austriacus* was expected, because of different foraging behaviours (*P. auritus* belonging to foliage gleaners – Swift & Racey 1983). Sympatric distribution and syntopic findings of *P. auritus* and *P. macrobullaris* indicate probable different foraging behaviour of *P. macrobullaris*, too. The discovery of morphometrically different *P. auritus* populations in biogeographically distinct areas (Dinaric, as part of Alpine area, and Pannonian) may be explained by the existence of two phylogenetic lineages of the brown long-eared bat reported for Austria (Spitzemberger et al. 2001). Larger samples from these populations should be tested morphometrically and genetically in the future.

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