

Genus	Supplement 14: 173-176	Wrocław, 15 XII 2007
-------	------------------------	----------------------

## A mitochondrial DNA control region phylogeny of the European woodpeckers Picidae\*

ROBERT RUTKOWSKI\*, PATRYCJA JAGÓŁKOWSKA, TOMASZ D. MAZGAJSKI & ŁUKASZ REJT

Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

\*Corresponding author: E-mail: robertrut@miiz.waw.pl

**ABSTRACT.** Hypervariable domain of mtDNA control region was analyzed in nine species of Picidae from Europe. In the majority of species, substantial polymorphism in the investigated sequence was found. Phylogenetic analysis remained in concordance with other studies of woodpeckers, applying mitochondrial as well as nuclear genes. However, using the hypervariable domain of control region to resolve the phylogeny of woodpeckers family showed that the Three-toed woodpecker (genus *Picoides*) has been grouped within a single monophyletic clade with members of genus *Dendrocopos*.

Key words: woodpeckers, Picidae, control region, mitochondrial DNA, phylogeny.

### INTRODUCTION

Mitochondrial DNA has been frequently used in studies of woodpeckers Picidae in order to resolve phylogeny of the family, particularly evolutionary relationship between Old and New World species (WEIBEL et MOORE 2002a, b; WEBB et MOORE 2005; WINKLER et al. 2005). The genes analysed were mainly mitochondrial: COI, cyt b and 12S rRNA, thus sequences presenting only a little variation on a species level. These studies did not clearly define evolutionary relation between woodpeckers from genus *Dendrocopos* and The three-toed Woodpecker (*Picoides tridactylus*). Moreover, according to our knowledge, none of the studies performed until now analysed the complete set of European species. Therefore, for example, systematic position of the Middle-spotted Woodpecker *Dendrocopos medius* is still unclear.

---

\*Proceedings of the 8th Conference of the Polish Taxonomical Society, Wiechlice 18-20 V 2007

The aim of the study was to apply a new molecular marker — a sequence of hypervariable domain of mtDNA and extended sample set of European species to reconstruct phylogeny of Picidae.

#### MATERIAL AND METHODS

Feathers, tissues and blood samples were collected from 108 individuals, representing 9 Picidae species from Europe (Table 1). Extraction of DNA, amplification of hypervariable domain of mitochondrial control region and sequencing of PCR product was performed according to methodology described elsewhere (RUTKOWSKI et al. Submitted).

For each species where more than one sample was collected and more than one haplotype was detected we estimated the number of haplotypes, nucleotide diversity  $\pi$  (NEI 1987) and haplotype diversity  $h$  (NEI 1987) using DNAsp (v. 3.51, ROZAS et al. 2003). For the total data set, we also estimated the mean number of differences among haplotypes ( $k$ ).

For obtained sequences of all analysed species, as well as for homological fragments of control region of three other woodpeckers: *Dryocopus pileatus*, *Sphyrapicus varius* and *Colaptes auratus* (accession numbers accordingly: NC008546; AF082045; AF082044) deposited in GenBank, we constructed a phylogenetic tree using the Neighbour-joining (NJ) method, as implemented in Mega software (KUMAR et al. 2004). Bootstrap values for each node were estimated with 1000 replications. As an out-group, sequence of mtDNA control region of *Pteroglossus azara* (Ramphastidae, Piciformes) (GenBank accession number: NC008549) was used.

Table 1. Number of samples ( $n$ ), number of haplotypes ( $H$ ), haplotype diversity ( $h$ ), nucleotide diversity ( $\pi$ ), number of identified base pairs (bp) number of parsimony informative sites (p.i.s.) in studied woodpecker's species.

Species	$n$	$H$	$h$	$\pi$	bp	p.i.s
<i>Dendrocopos major</i>	94	17	0.757	0.00390	379	9
<i>D. medius</i>	11	7	0.873	0.00794	379	2
<i>D. minor</i>	4	4	1.0	0.01350	380	2
<i>D. syriacus</i>	1	1	—	—	378	—
<i>D. leucotos</i>	2	1	—	—	379	—
<i>Picoides tridactylus</i>	4	1	—	—	378	—
<i>Picus viridis</i>	3	3	1.0	0.01980	381	0
<i>P. canus</i>	1	1	—	—	378	—
<i>Dryocopus martius</i>	6	6	1.0	0.00872	377	1

## RESULTS AND DISCUSSION

The analysis of PCR products in an automatic sequencer showed that species amplified fragments in the investigated species had 380 base pairs. Among them, from 305 to 321 base pairs were unambiguously identified, depending on species. Thus, comparisons between species were performed on a shareable fragment of 305 base pairs. Set we found 119 polymorphic sites and 29 haplotypes in the total data; nucleotide diversity among haplotypes  $\pi = 0.09603$ ; and mean number of nucleotide differences among  $k = 28.809$ . Results for each species are presented in Table 1. The neighbour-joining tree based on obtained sequences is presented in Fig. 1. Because haplotypes for each species were grouped together in a phylogenetic tree, we presented only results for single haplotype within the species.

Nearly all investigated species of which more than one sample was analysed presented a high level of haplotype diversity. We have not confirmed variability of the marker only in the case of *Picoides tridactylus* and *Dendrocopos leucotos*. However, samples of each of these two species were collected at one location, which could be considered as isolated population. Moreover, both of the species are thought to be declining in numbers and even endangered, at least in Poland (TOMIAŁOJĆ et STAWARCZYK 2003). Thus, low genetic variability could be a possible explanation for these results.

The topology of the phylogenetic tree remains in concordance with results from other authors, analysing mitochondrial (WEIBEL et MOORE 2002a; WEBB et MOORE 2005) as well as nuclear genes (WEIBEL et MOORE 2002b). The majority of discrepancies seems

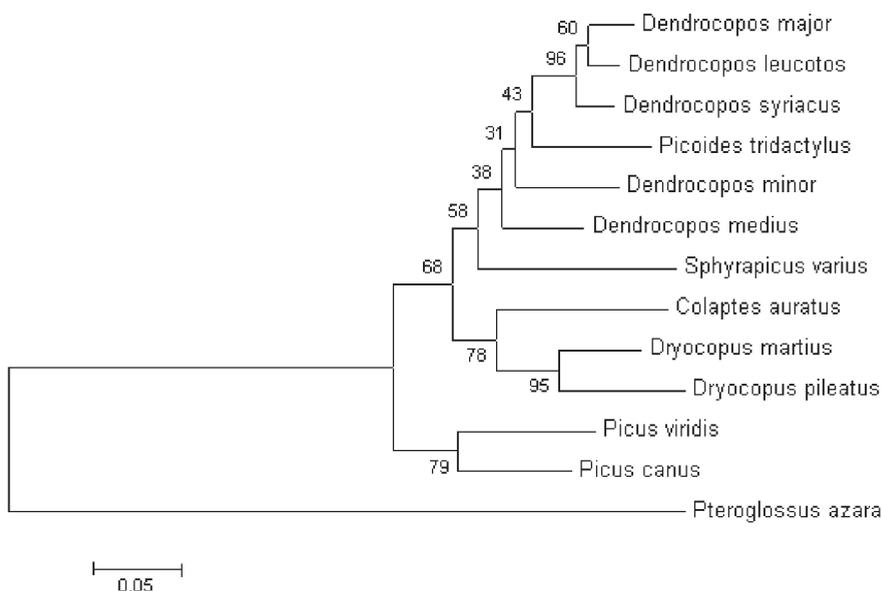


Fig 1. Phylogenetic tree based on sequence of hypervariable domain of mtDNA control region in studied woodpeckers, constructed with Neighbour-joining method. Bootstrap percentages are indicated above nodes

to be connected with interpretation of topology within the *Dendrocopos-Picoides* clade. WEIBEL et MOORE (2002a) separated *Picoides tridactylus* from the *Dendrocopos* clade, while WINKLER et al. (2005) grouped *P. tridactylus* together with other *Dendrocopos* species.

Interpretation of evolutionary relationship between genus *Dendrocopos* i *Picoides* has been frequently changed. PETERS (1948) described two genera: *Dendrocopos* with 31 species and *Picoides* with *P. tridactylus* and *P. arcticus*. Then, both genera were combined in one genus *Picoides* (SHORT 1982; WINKLER et al. 1995). However, recently „spotted” woodpeckers (*Dendrocopos*) were again separated from genus *Picoides* (Del HOYO et al. 2003). Our study suggests that the *Picoides tridactylus* is closely related to other *Dendrocopos* species, which supports the results of WINKLER et al. (2005).

#### ACKNOWLEDGEMENTS

This study was granted by Polish State Committee for Scientific Research (grant nr.: 3P04F 044 25). DOROTA ZAWADZKA, JERZY ZAWADZKI, PATRYK ROWIŃSKI, ZIEMOWIT KOSIŃSKI, ANDRZEJ WĘGRZYNOWICZ, PAWEŁ MIRSKI, TOMASZ TUMIEL and MAREK JĘDRA (Operation Carpatica) are gratefully acknowledge for samples collections. We also thank every other person who contributed in gathering material for genetic analysis.

#### REFERENCES

- Del HOYO, J., ELLIOTT, A. Y., CHRISTIE, D. (eds), 2003. Handbook of the birds of the world. Volume 8. 850 pp.
- KUMAR, S., TAMURA, K., NEI, M., 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics, **5**: 150–163.
- NEI, M. 1987. Molecular evolutionary genetics. New York: Columbia University Press. x + 512 pp.
- PETERS, J. L., 1948. Check-list of birds of the world. Vol. 6. Harvard University Press, Cambridge, xi+259.
- ROZAS, J., SANCHEZ-DELBARRIO, J. C., MESSEGUER, X., ROZAS, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics, **19**: 2496–2497.
- RUTKOWSKI, R., JAGÓŁKOWSKA, P., MAZGAJSKI, T., REJT, L., Submitted. Degenerate PCR primers for amplification of the mitochondrial control region hypervariable domain I of European Woodpeckers. Zoological Studies.
- SHORT, L. L., 1982. Woodpeckers of the World. Delaware Museum of Natural History, Greenville.
- TOMIAŁOJC, L., STAWARCZYK, T., 2003. The avifauna of Poland. Distribution, numbers and trends. Polskie Towarzystwo Przyjaciół Przyrody “pro Natura”. Wrocław.
- WEBB, D. M., MOORE, W. S., 2005. A phylogenetic analysis of woodpeckers and their allies using 12S, Cty b, and COI nucleotide sequence (class Aves; order Piciformes). Molecular Phylogenetic and Evolution, **36**: 233-248.
- WEIBEL, A. C., MOORE, W. S. A., 2002a. Test of a mitochondrial gene-based phylogeny of woodpeckers (Genus *Picoides*) using an independent nuclear gene,  $\beta$ -fibrinogen intron 7. Molecular Phylogenetic and Evolution, **22**: 247–257.
- , 2002b. Molecular phylogeny of a cosmopolitan group of woodpeckers (Genus *Picoides*) based on COI and cyt B mitochondrial gene sequences. Molecular Phylogenetic and Evolution, **22**: 65–75.
- WINKLER, H., CHRISTIE, D. A., NURNEY, D., 1995. Woodpeckers. Pico Press, Sussex, 406 pp.
- WINKLER, H., KOTAKA, N., GAMAUF, A., NITTINGER, F., HARING, E., 2005. On the phylogenetic position of the Okinawa woodpecker (*Sapheopipo noguchii*). Journal fur Ornithology, **146**: 103–110.