

Great spotted woodpeckers *Dendrocopos major* exert multiple forms of phenotypic selection on Scots pine *Pinus sylvestris*

Lukasz Myczko and Craig W. Benkman

L. Myczko, Inst. of Zoology, Poznań Univ. of Life Sciences, Wojska Polskiego 71C, PL-60-625 Poznań, Poland. – C. W. Benkman (cbenkman@uwoyo.edu), Dept of Zoology and Physiology, Univ. of Wyoming, Laramie, WY 82071, USA.

Relatively few animal species extract seeds from closed conifer cones because of the forces required to spread apart or penetrate the woody scales. Those species that forage on seeds in closed cones tend to forage selectively, and therefore act as selective agents on cone structure. However, little is known about the foraging preferences and thus phenotypic selection that is exerted on conifers by many species that forage extensively on seeds in closed cones, including especially woodpeckers (Picidae). Great spotted woodpeckers *Dendrocopos major* are one of the main predators of seeds in closed cones of Scots pine *Pinus sylvestris* in central and eastern Europe. To estimate the cone preferences of these woodpeckers foraging on Scots pine, we contrasted traits of cones that were and were not foraged on by woodpeckers. Woodpeckers preferred to forage on shorter cones when scales were thin (smaller apophyses) but preferred cones of intermediate length when scales were thicker, providing evidence for correlational selection. The preference for intermediate-sized cones indicates that woodpeckers exert disruptive selection on cone length when cones have thicker scales, but the overall selection on cone length across all scale types indicates directional favoring the evolution of longer cones. Woodpeckers avoided cones with thicker scales, which would lead to directional selection favoring the evolution of thicker scales. Preferences for intermediate-sized cones have been found in tree squirrels and directional selection favoring the evolution of cones with thicker scales may be a common outcome of the foraging behavior of birds.

Species interactions structure ecological communities, and natural selection experienced during these interactions drives much of adaptive evolution (Thompson 2005). Consequently, evolutionary ecologists have invested a tremendous amount of effort characterizing natural selection (Brodie et al. 1995, Kingsolver et al. 2001, Siepielski et al. 2009). Studies of phenotypic selection exerted by conifer-seed-eating animals provide one such set of studies. These studies have demonstrated that several seed predators, namely red or common crossbills (*Loxia curvirostris* complex), Clark's nutcrackers *Nucifraga columbiana*, tree squirrels (*Sciurus* spp. and *Tamiasciurus* spp.), and cone borer moths *Eucosma recisoriana*, have had considerable impacts on conifer cone evolution (Benkman et al. 2001, 2003, 2010, Siepielski and Benkman 2004, 2007, Benkman and Parchman 2009, Mezquida and Benkman 2010). Although these studies show that variation in selection exerted by these animals can account for geographic variation in cone structure in a number of conifer species, many other seed predators forage extensively on seeds in the closed cones and may further alter the evolution of conifer cones. One Eurasian species, the great spotted woodpecker *Dendrocopos major*, is well known for foraging on seeds in closed conifer cones. However, the extent to

which woodpeckers exert selection on conifer cone structure is unknown. Such selection should result in evolution because various cone traits, including those measured in our study, are heritable in various species of conifers (references in Benkman et al. 2010).

During winter, great spotted woodpeckers forage mainly on conifer seeds in central and eastern Europe (Hogstad 1971, Osiejuk 1998, Kędra and Mazgajski 2001). A single great spotted woodpecker removes seeds from up to 50 Scots pine *Pinus sylvestris* cones per day (Pulliainen 1963, Winkler and Christie 2002). In comparison, the average annual production of cones by a Scots pine tree varies from 40 to a maximum of 650 cones per tree (Tyszkiewicz 1952). Woodpeckers therefore have the potential to consume a large fraction of the standing seed crop. Although some studies have analyzed the sizes of cones foraged on by these woodpeckers (Hordowski 1995, Kędra and Mazgajski 2001), the structure of cones available in the environment was not quantified. Thus, these studies do not allow us to estimate cone preferences and the form of selection exerted by woodpeckers. Here, we estimate the cone use by great spotted woodpeckers, and infer the form of selection they exert on Scots pine, the most widely distributed pine in the World (Critchfield and Little 1966), and discuss the

potential implications for cone evolution. The assumption is that the patterns of seed predation are related to the selection exerted by woodpeckers on cone structure.

Material and methods

The study area included Scots pine plantations with scattered trees of other species (native birch *Betula*, oak *Quercus*, and introduced locust *Robinia*) within Puszcza Zielonka Landscape Park, Wielkopolska, Poland (52°30'N, 17°2'E). We have no reason to expect that the variation in cone traits among trees in plantations would differ substantially from those in natural stands. Regardless, even if they did differ, this should not affect the patterns of cone use in relation to cone traits. In the first winter (2007–2008), pine stands were searched in order to locate woodpecker anvils. Woodpeckers use sites called anvils to extract seeds from cones; great spotted woodpecker was the only species of woodpecker foraging on conifer cones in the study area. Typically, anvils are crevices in trees in which the woodpeckers wedge cones to process them (Fig. 1, Winkler and Christie 2002, Bondo et al. 2008). Every bird uses many anvils, but only a few main anvils are used often (Kędra and Mazgajski 2001, Winkler and Christie 2002). We searched for anvils in stands that were > 50 yr old, because younger stands did not attract many woodpeckers. The most frequently used anvils in a given territory were chosen for study. In order to avoid taking cones from more than one anvil used by the same bird, all chosen anvils were at least 250 m apart and woodpeckers were not observed flying between these anvils. A given anvil was attributed to a woodpecker gender if only same-sex birds were recorded in the territory on at least three occasions.

During the next year, foraged on cones were collected from underneath 24 anvils from September 2008 until March 2009. Closed cones in trees were green from September to October and brown afterwards. Subsequently, 300 cones were randomly chosen for measurement from those gathered from each anvil. In September 2009, after cones had fallen from trees, a random sample of 300 unforaged on cones from 10 to 25 pines was gathered from a 10-m radius from each anvil and measured (only 262 and 292 cones were measured from two anvils). We chose a 10-m radius for sampling because woodpeckers collect most of their cones from within 10 m of the anvil (Pulliainen 1963, see also Kędra and Mazgajski 2001). Cones were put in containers, marked, and transported to the laboratory. Cones were kept in water for 24 h, and then cone lengths, which are a representative measure of cone size (Benkman et al. 2003), were measured to the nearest mm and the form of the apophyses (the part of the scale visible when cone scales are closed, Fig. 1) were recorded. The continuous variation in apophysis size was divided into three commonly used categories (Fig. 2, Staszkiwicz 1993): plana (form Christ.) had flat or slightly protruding scales; gibba (form Christ.) had pyramidal shaped apophyses that projected less than half the width of the scales; and reflexa (form Heer.) had apophyses with heights that were equal to or greater than half the width of the scales.

We used generalized linear mixed models (GLMMs) with a logit link function and binomial errors to test for the effects of sex, cone length, [cone length]², apophysis category, and an interaction between cone length and apophysis category on the probability of seed predation by woodpeckers. We included anvil as a random variable in all GLMM models and used Akaike information criterion (AIC) to compare models with differing fixed effects. Cones



Figure 1. An anvil with Scots pine cones (a 43 mm long plana form on the left and a 54 mm long reflexa form on the right), to illustrate how a woodpecker secures cones in anvils and how variation the size of both the apophyses and cones could influence the extent to which the cones can be wedged securely into the crevice of the anvil.

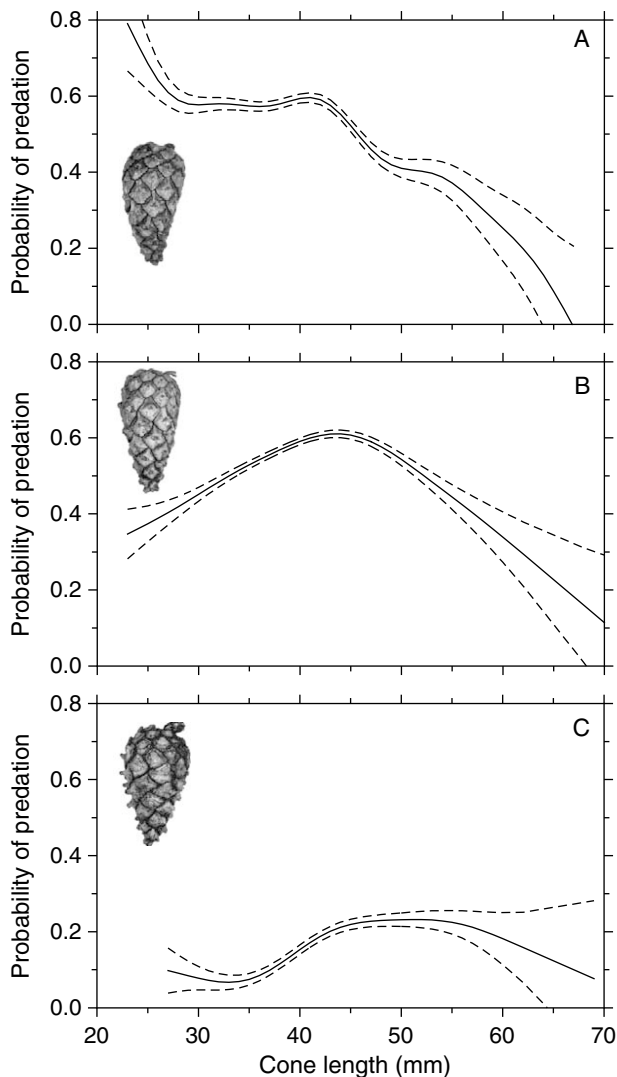


Figure 2. The probability of seed predation by great-spotted woodpeckers in relation to cone length for each of the three categories of apophysis: cones with thinner scales, plana (A); intermediate-sized scales, gibba (B); and thick-scales, reflexa (C). The solid curves are based on cubic splines and the dashed lines represent plus or minus one standard error (A: $n = 5429$ cones; B: $n = 6720$ cones; C: $n = 2205$ cones). Representative cones of each apophysis category are shown.

that were foraged on by woodpeckers were assigned a value of one (most of the seeds were eaten by woodpeckers, Hordowski 1995), whereas cones that were not foraged on were assigned a value of zero. The sex of the woodpecker was determined for 19 of the 24 anvils; eight were used by females and 11 by males. Initially, we used data from only the 19 anvils for which the sex of the woodpecker was identified. The fully parameterized model excluding sex was marginally better than the full model including sex ($\Delta AIC = 3.06$), and the effect of sex was not significant ($p = 0.36$); therefore we excluded sex from further analysis and used data from all 24 anvils. We used Helmert contrasts to test whether the probability of predation differed between apophysis categories; these orthogonal contrasts compared the first two categories to each other and the mean of the first two categories to the third category. We

used cubic splines to visualize the probability of predation in relationship to cone length for each category of apophysis and for all categories combined (Schluter 1988). All GLMM models were run in R using package lme4; other analyses including the cubic splines were run in R (R Development Core Team 2009, Bates and Maechler 2010).

Results

The full model including cone length, $[\text{cone length}]^2$, apophysis category, and an interaction between cone length and apophysis category had the strongest support (all other models excluding one or more of the variables had $\Delta AIC > 80$). Cone length, $[\text{cone length}]^2$, apophysis category, and an interaction between cone length and apophysis category were all significant ($p < 0.001$). The graphical analyses using cubic splines (Fig. 2) help visualize how the probability of predation varied with cone length and apophysis category. Woodpeckers preferred shorter cones when apophyses were small (scales were thin, Fig. 2A), and their preferred cone length increased with increasing scale thickness (Fig. 2). This shift in cone length preferences with increasing scale thickness presumably caused the significant statistical interaction between the two ($p < 0.001$). A result is that selection exerted by woodpeckers favors different trait combinations, which represents correlational selection. The pattern of predation in relation to cone length for cones with scales of intermediate thickness (Fig. 2B) indicates that selection exerted by woodpeckers on such cones was disruptive (short and long cones tended to be avoided by woodpeckers and thus favored by selection). The preferred cone length for this apophysis category was 44 mm, which approximated the average length of Scots pine cones foraged on in another study in Poland (43 mm, estimated from Table 2 in Kędra and Mazgajski 2001). This length represents about 20% of the total length of the woodpecker (20–24 cm, Winkler and Christie 2002). Overall, predation was higher on shorter and intermediate-sized cones than on longer cones (Fig. 3). Predation was also higher on cones with thinner scales (plana) than on cones with intermediate-sized scales (gibba), and higher on these two apophysis categories than on cones with the

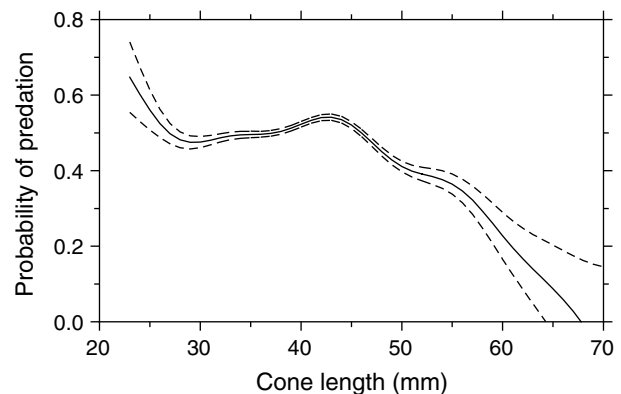


Figure 3. The overall probability of seed predation by great spotted woodpeckers tends to be highest for the smallest and intermediate-sized Scots pine cones and declines rapidly for the largest sized cones ($n = 14354$ cones). The curves are as in Fig. 2.

thickest scales (reflexa, GLMM Helmert contrasts: $p < 0.001$). This indicates that selection exerted by woodpeckers favors an increase in cone length (size) and scale thickness.

Discussion

As in previous studies on animals foraging on seeds in closed conifer cones (Benkman et al. 2001, 2003, 2010, Siepielski and Benkman 2004, 2007, Benkman and Parchman 2009, Mezquida and Benkman 2010), we found that great spotted woodpeckers have preferences for cones with certain traits leading to phenotypic selection on cone structure. In particular, woodpeckers avoided cones with enlarged apophyses (Fig. 2, reflexa form) causing directional selection favoring their evolution. Several studies have shown that cones with enlarged apophyses (thicker scales and longer spines) also deter foraging crossbills (Coffey et al. 1999, Mezquida and Benkman 2010). These results imply that thicker cone scales act to deter woodpeckers and crossbills, and we suspect deter other seed predators that shred or pry apart scales from their distal ends to access seeds; the enlarged apophyses block access to seeds whether the scales are closed or have small gaps between them. In the case of woodpeckers, cones with larger apophyses may also be more difficult to secure in the crevice of the anvil (Fig. 1). In contrast, tree squirrels forage by starting at the base of cones, biting off successive scales at the base well away from the apophyses. Enlarged apophyses, except near the base of the cone, therefore, are more likely to have evolved as defenses to deter birds than tree squirrels (Coffey et al. 1999).

Combining all apophysis categories, woodpeckers avoided the longest cones (Fig. 3). This should result in overall selection favoring trees producing the longest cones. However, there is also evidence that woodpeckers prefer foraging on intermediate-sized cones (Fig. 3) especially when cones had thicker cone scales (Fig. 2B, C) causing disruptive selection on cone length for the two largest apophysis categories. We cannot determine whether cone length is the target of selection or correlated with another trait (e.g. cone width or mass) under selection without including additional traits in the analyses (Lande and Arnold 1983). Regardless, disruptive selection has been detected infrequently (Endler 1986, Kingsolver et al. 2001), and has not been detected in studies of other conifer-seed-eating animals. However, studies on tree squirrels, like ours on great spotted woodpeckers, have found that tree squirrels have a preferred cone length (Mezquida and Benkman 2005, Parchman and Benkman 2008, Benkman et al. 2010). Consequently, tree squirrels exert selection for smaller cone sizes when cones average smaller than the preferred length, and they exert selection for larger cone sizes when cones average larger than the preferred length. Tree squirrels should exert disruptive selection if the preferred cone size approximates the average cone length. An earlier study on Scots pine in Scotland (Summers and Proctor 1999) showed that crossbills preferred foraging in trees having smaller Scots pine cones, whereas red squirrels *Sciurus vulgaris* preferentially foraged in trees having larger cones. However, the overall form of selection experienced by Scots pine cones is unknown as it will depend upon the

form and relative strength of selection exerted by the various seed predators (Benkman et al. 2001, Siepielski and Benkman 2004).

The form of selection woodpeckers exert on other conifers will likely depend on their cone sizes. Although we found no evidence of woodpeckers foraging on cones of other conifers in this study, great spotted woodpeckers feed on cones from a variety of conifers including the relatively small cones of European larch *Larix decidua* and the much larger cones of European black pine *Pinus nigra* and Norway spruce *Picea abies* (Winkler and Christie 2002). We expect that woodpeckers exert directional selection favoring the evolution of smaller cones in larch and favor the evolution of larger cones in black pine and spruce.

We also found evidence of correlational selection, which arises when selection acts on the covariance between traits (Brodie 1992). Correlational selection has not been reported in previous studies of conifer-seed-eating animals (Benkman et al. 2003, Mezquida and Benkman 2010). Similarly, correlational selection is reported much less often in the literature than other forms of selection (Kingsolver et al. 2001, Siepielski et al. 2009). Nevertheless, we have not tested for correlational selection in an exhaustive manner in our previous studies for two reasons. First, we lacked plausible mechanistic explanations for correlational selection. Second, we did not detect correlational selection when we had tested for it. We are uncertain why the preferred length of cones by woodpeckers increases with increases in apophysis size (Fig. 2). Cones are wedged lengthwise in anvils (Winkler and Christie 2002), so that cones of a particular width are likely to fit most securely (Fig. 1). This could explain the preference for an intermediate-sized cone. If the tips of the apophyses on cones with enlarged apophyses also are broken off as the cone is wedged into the anvil groove (Fig. 1), then the widths of cones with largest apophyses are likely to be reduced to the greatest extent by foraging woodpeckers. This could make it difficult for woodpeckers to secure cones with enlarged apophyses, and might account for the preference for longer (larger) cones with increases in apophysis size.

In sum, we have documented how foraging preferences of great spotted woodpeckers are related to two cone traits. One was apophysis size, which is a trait comparable to scale thickness measured in other studies, and the other was cone length, which is a representative measure of cone size. Previous studies have often detected selection exerted on scale thickness and cone size (Benkman et al. 2003, Siepielski and Benkman 2007, Mezquida and Benkman 2010). Great spotted woodpeckers, like crossbills, avoid cones with the thickest scales causing directional selection favoring trees producing cones with larger apophyses (thicker scales). Woodpeckers preferred shorter cones when cones had small apophyses (plana) and when all three categories of apophyses were combined implying directional selection favoring the evolution of longer cones. However, the cone length preferences shifted with apophysis category, with intermediate sizes of cones preferred for the two larger apophysis categories. Future studies on conifer-seed-eating animals should test for correlational selection more consistently, and detailed observations of foraging great spotted woodpeckers are needed for developing and testing mechanistic hypotheses for the correlational selection we

detected. The use of Scots pine by great spotted woodpeckers also varies geographically (Hogstad 1971). Whether this variation is related to cone structure, or whether variation in the intensity of seed predation by woodpeckers causes variation in cone structure or both is unknown but worth investigating.

Acknowledgements – We would like to thank M. Tomaszewska for support during the investigation, W. Hochachka, D. Janzen, T. Sparks, A. Siepielski, R. Summers, E. Svensson, and P. Tryjanowski for useful comments on this paper, and M. Talluto for help with some of the analyses.

References

- Bates, D. and Maechler, M. 2010. lme4: linear mixed-effects models using Eigen and Eigen. – R package ver. 0.999375-37, <<http://CRAN.R-project.org/package=lme4>>.
- Benkman, C. W. and Parchman, T. L. 2009. Coevolution between crossbills and black pine: the importance of competitors, forest area, and resource stability. – *J. Evol. Biol.* 22: 942–953.
- Benkman, C. W., Holimon, W. C. and Smith, J. W. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. – *Evolution* 55: 282–294.
- Benkman, C. W., Parchman, T. L., Favis, A. and Siepielski, A. M. 2003. Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. – *Am. Nat.* 162: 182–194.
- Benkman, C. W., Parchman, T. L. and Mezquida, E. T. 2010. Patterns of coevolution in the adaptive radiation of crossbills. – *Ann. N. Y. Acad. Sci.* 1206: 1–16.
- Bondo, K. J., Gilson, L. N. and Bowman, R. 2008. Anvil use by the red-cockaded woodpecker. – *Wilson J. Ornithol.* 120: 217–221.
- Brodie, E. D. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. – *Evolution* 46: 1284–1298.
- Brodie, E. D., Moore, A. J. and Janzen, F. J. 1995. Visualizing and quantifying natural selection. – *Trends Ecol. Evol.* 10: 313–318.
- Coffey, K., Benkman, C. W. and Milligan, B. G. 1999. The adaptive significance of spines on pine cones. – *Ecology* 80: 1221–1229.
- Critchfield, W. B. and Little, E. L. Jr 1966. Geographic distribution of the pines of the World. – USDA Forest Service, WA.
- Endler, J. A. 1986. Natural selection in the wild. – Princeton Univ. Press.
- Hogstad, O. 1971. Notes on the winter food of the great spotted woodpecker, *Dendrocopos major*. – *Sterna* 10: 233–241, in Norwegian with English summary.
- Hordowski, J. 1995. Characteristics of the great spotted woodpecker *Dendrocopos major* anvils in mixed forests of the Pogórze Przemyskie plateau. – *Not. Orn.* 36: 374–377, in Polish with English summary.
- Kędra, A. H. and Mazgajski, T. D. 2001. Factors affecting anvils utilization by great spotted woodpecker *Dendrocopos major*. – *Pol. J. Ecol.* 49: 79–86.
- Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P. and Beerli, P. 2001. The strength of phenotypic selection in natural populations. – *Am. Nat.* 157: 245–261.
- Lande, R. and Arnold, S. J. 1983. The measurement of selection on correlated characters. – *Evolution* 37: 1210–1226.
- Mezquida, E. T. and Benkman, C. W. 2005. The geographic selection mosaic for squirrels, crossbills and Aleppo pine. – *J. Evol. Biol.* 18: 348–357.
- Mezquida, E. T. and Benkman, C. W. 2010. Habitat area and structure affect the impact of seed predators and the potential for coevolutionary arms races. – *Ecology* 91: 802–814.
- Osiejuk, T. S. 1998. Study on the intersexual differentiation of foraging niche in relation to abundance of winter food in great spotted woodpecker *Dendrocopos major*. – *Acta Ornithol.* 33: 137–141.
- Parchman, T. L. and Benkman, C. W. 2008. The geographic selection mosaic for ponderosa pine and crossbills: a tale of two squirrels. – *Evolution* 62: 348–360.
- Pulliaainen, E. 1963. Observations on the autumnal territorial behaviour of the great spotted woodpecker, *Dendrocopos major* (L.). – *Ornis Fenn.* 40: 132–139.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. – *Evolution* 42: 849–861.
- Siepielski, A. M. and Benkman, C. W. 2004. Interactions among moths, crossbills, squirrels and lodgepole pine in a geographic selection mosaic. – *Evolution* 58: 95–101.
- Siepielski, A. M. and Benkman, C. W. 2007. Convergent patterns in the selection mosaic for two North American bird-dispersed pines. – *Ecol. Monogr.* 77: 203–220.
- Siepielski, A. M., DiBattista, J. D. and Carlson, S. M. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. – *Ecol. Lett.* 12: 1261–1276.
- Staszkiwicz, J. 1993. Zmienność szyszek. Variability of cones. – In: Białobok, S., Boratynski, A. and Bugala, W. (eds), *Biologia sosny zwyczajnej. The biology of Scots pine*. – Sorus, pp. 36–38, in Polish.
- Summers, R.W. and Proctor, R. 1999. Tree and cone selection by crossbills *Loxia* sp. and red squirrels *Sciurus vulgaris* at Abernethy forests, Strathspey. – *For. Ecol. Manage.* 118: 173–182.
- Thompson, J. N. 2005. The geographic mosaic of coevolution. – Univ. of Chicago Press.
- Tyszkiewicz, S. 1952. Nasiennictwo leśne z zarysem selekcji drzew. Forest tree seed production with basic tree selection. – PWRiL, in Polish.
- Winkler, H. and Christie, D. A. 2002. Family Picidae (woodpeckers). – In: Hoyo, J., Elliott, A. and Sargatal, J. (eds), *Handbook of the birds of the World. Vol. 7. Jacamars to woodpeckers*. Lynx Edicions, pp. 296–558.