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MORPHOMETRIC DISCRIMINATION BETWEEN *MARTES MARTES* AND *MARTES FOINA* IN ITALY: THE USE OF THE *BACULUM*

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ABSTRACT - *Martes martes* and *Martes foina* are the only two Italian species of the genus *Martes*. As they are very similar in morphology and coloration, their taxonomic identification may represent a hard task. We aimed to assess the reliability of *baculum* length as a diagnostic tool for Italian martens. A sample of 53 adult males belonging to the genus *Martes* was collected. The identification of individuals as pine- or stone marten followed a 2-step procedure: i) morphometric analysis and ii) cross-checking with genetic analysis. According to the diagnostic ranges of *baculum* length reported in the available literature, 21 individuals were classified as stone marten and 15 as pine marten, while 17 specimens remained unidentified. Using a "*Baculum* Index", defined as: (Head Length + Body Length) / Total *Baculum* Length, the sample was split into two distinct groups: 21 individuals fell into the 6.24-8.10 range and 32 fell into the 9.52-11.17 range. Genetic analysis led to the same results. While *baculum* length is insufficient to identify Italian marten species with certainty, the *Baculum* Index showed to be a reliable diagnostic tool.

Key words: *Baculum* index, discrimination, morphometry, pine marten, stone marten

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INTRODUCTION

The genus *Martes* includes five morphologically very similar species: *M. americana*, *M. foina*, *M. martes*, *M. melampus* and *M. zibellina* (Macdonald 1984). Two of them, *M. foina* and *M. martes,* are the only representatives of this genus in Italy and, when sympatric, are usually very difficult to distinguish on the basis of qualitative external morphological characteristics (Anderson 1970, Labrid 1986, Reig 1992).

Pine and stone marten exhibit differences in habitat use; the former is strictly associated with forest habitats, whereas the latter is able to adapt to a wide range of natural, semi-natural, and even urban habitats (e.g. Mitchell-Jones et al. 1999, Proulx et al. 2004). However, recent research showed that pine marten can also occur in fragmented woodland and agricultural landscapes (Posluszny et al. 2007, Balestrieri *et al.* 2010, Mergey et al. 2011).

Furthermore, in several European regions, the two species share the same trophic niche (Goszczynski 1976, 1986; Marchesi and Mermod 1989; Marchesi et al. 1989, Bermejo and Guitian 2000). As this situation suggests the pos-

sibility of competition between the two species, the monitoring and conservation of the pine marten (listed in the Habitat Council Directive 92/43/CEE) requires the accurate identification of individuals.

These reasons, together with the great difficulties encountered in distinguishing between the pine marten and the stone marten on taxonomic grounds, have prompted researchers to seek genetic protocols that can unequivocally identify these species (Domingo-Roura 2002; Birks et al. 2004; Vercillo et al. 2004; Colli et al. 2005, Fernandez et al. 2007; Lucentini et al. 2007; Pilot et al. 2007; Rozhnov et al. 2008; Ruiz-González et al. 2008).

In the context of morphologic research, the total length of the *baculum* is considered diagnostic for species characterization (pine marten: 38.9-46.0 mm; stone marten: 51.0-70.5 mm; Didier 1947; Saint Girons 1973; Heptner and Naumov 1974; Waechter 1975; Hartung 1980; Labrid 1986; Libois and Waechter 1991; Stubbe 1993a, 1993b; Baryshnikov et al. 2003).

By combining the most recent techniques of molecular analysis with the traditional morphometric methods, we aimed to: i) test *baculum* length as a tool for discriminating Italian marten species and ii) validate a more effective diagnostic morphometric method.

MATERIALS AND METHODS

Out of 94 road-killed specimens of the genus *Martes* collected from 1999 to 2007 in six regions of Italy (*Tab. 1*), 64 were males: 7 *juveniles* and 57 adults, with teeth and reproductive apparatus fully developed. In 4 adult specimens, the *baculum* had been snapped by motor vehicles, therefore the final number of specimens utilized in the study was 53.

For all specimens, the weight (g) and standard somatic linear dimensions (head and body length, hind foot length, tail length, ear height, all in mm) were recorded.

For each specimen, the *baculum* was removed from its protective sheath by boiling, and its total length was measured by a Vernier caliper to an accuracy of 0.05 mm.

Preliminary species identification was carried out according to the total length of the *baculum* (*B*TL). Subsequently, assuming that *B*TL is influenced by the size of the individual, the following *Baculum* Index was calculated: *B*I = Head + Body Length (HBL) / *B*TL.

Finally, each individual was identified as pine or stone marten on the basis of genetic (PCR-RFLP) analysis (see Vercillo et al. 2004 for details). For each specimen, DNA was extracted from a tuft of hair and a small sample of muscle tissue.

Mean *B*I values were compared by a t-test.

Bag-plots (Rousseeuw et al. 1999) were used to represent the relationship between HBL and *B*TL graphically. These are bivariate box-and-whisker plots in which:

- a bag contains the inner 50% of data;
- a fence (magnifying the bag by a factor of 3), which is not plotted, is used to determine outliers;
- a loop contains the points that fall between the bag and the fence.

RESULTS AND DISCUSSION

The comparison of *B*TLs with reference ranges led to the identification of 21 stone marten and 15 pine marten. In this way, however, 17 individuals remained unidentified, since their *B*TL values fell into the non-

Table 1 - Location of recovery and values of *B*TL (Total Length of the *Baculum*) and *B*I (*Baculum* Index) for all analysed samples.

Identification Code	Region	*B*TL	*B*I
Martes 1	Abruzzo	41.45	11.17
Martes 2	Lazio	41.70	11.10
Martes 3	Lazio	42.30	11.06
Martes 4	Lazio	43.10	10.21
Martes 5	Lazio	43.25	10.52
Martes 6	Lazio	43.80	10.15
Martes 7	Umbria	43.90	10.43
Martes 8	Umbria	44.40	9.93
Martes 9	Lazio	44.45	10.64
Martes 10	Lazio	44.60	10.74
Martes 11	Toscana	45.00	9.84
Martes 12	Lazio	45.10	10.75
Martes 13	Lazio	45.25	10.63
Martes 14	Lazio	45.35	10.61
Martes 15	Lazio	45.90	10.09
Martes 16	Lazio	46.10	10.10
Martes 17	Lazio	46.10	10.00
Martes 18	Lazio	46.25	10.63
Martes 19	Umbria	46.30	9.61
Martes 20	Umbria	46.30	10.19
Martes 21	Lazio	46.40	10.62
Martes 22	Lazio	46.70	10.28
Martes 23	Lazio	47.00	10.40
Martes 24	Umbria	47.10	10.65
Martes 25	Toscana	47.11	10.19
Martes 26	Umbria	47.70	9.64
Martes 27	Umbria	47.80	10.02
Martes 28	Umbria	48.55	9.88
Martes 29	Toscana	48.60	9.73
Martes 30	Lazio	48.75	9.89
Martes 31	Toscana	48.80	9.52
Martes 32	Lazio	50.50	10.17
Martes 33	Lazio	51.00	7.84
Martes 34	Lazio	55.30	7.97
Martes 35	Basilicata	56.25	7.11

Table 1 - continues

Identification Code	Region	*B*TL	*B*I
Martes 36	Marche	56.65	8.10
Martes 37	Lazio	56.90	7.47
Martes 38	Umbria	57.00	7.38
Martes 39	Umbria	57.30	7.71
Martes 40	Lazio	58.20	7.59
Martes 41	Marche	58.30	7.56
Martes 42	Umbria	58.30	7.71
Martes 43	Umbria	58.60	7.74
Martes 44	Umbria	58.70	7.88
Martes 45	Basilicata	58.90	7.23
Martes 46	Umbria	59.70	7.15
Martes 47	Umbria	59.90	7.07
Martes 48	Marche	60.10	7.25
Martes 49	Marche	60.10	7.13
Martes 50	Umbria	61.20	7.27
Martes 51	Umbria	61.20	7.07
Martes 52	Umbria	61.52	7.15
Martes 53	Marche	69.50	6.44

diagnostic "grey area" (46.1 – 50.9 mm) between the two ranges (*Tab. 1*). The *Baculum* Index (*Tab. 1*), the second criterion of identification proposed, allowed us to split the sample into two clearly distinct groups (*Fig. 1A*): 21 animals in the range 6.44 - 8.10, which included all of the specimens classified as stone marten by the *B*TL technique; 32 animals in the range 9.52 - 11.17, which included all of the specimens classified as pine marten by the *B*TL, plus those unidentified by the former method.

Genetic analyses confirmed the classification obtained by the BI, as all specimens that fell within the "grey area" according to the *B*TL technique were genetically classified as pine marten.

Bag-plot analysis (Fig. 1B) showed that all specimens genetically classified as pine marten constituted a single group, while those classified as stone marten formed another group, with the exception of two specimens (red points) which constituted outliers (*B*TL = 51.0 and 69.5, respectively). The area of separation between the two groups was broad (t = 23.58, df = 52, P < 0.001; *Fig. 2*).

With regard to Italian marten species, the length of the *baculum* was shown not to be a reliable diagnostic tool, particularly for pine marten, while calculation of the *B*I proved to be a safe and reliable method, as demonstrated by genetic analysis.

In conclusion, species classification based on morphometric features can be very useful for identifying sibling species, as is the case of pine and stone

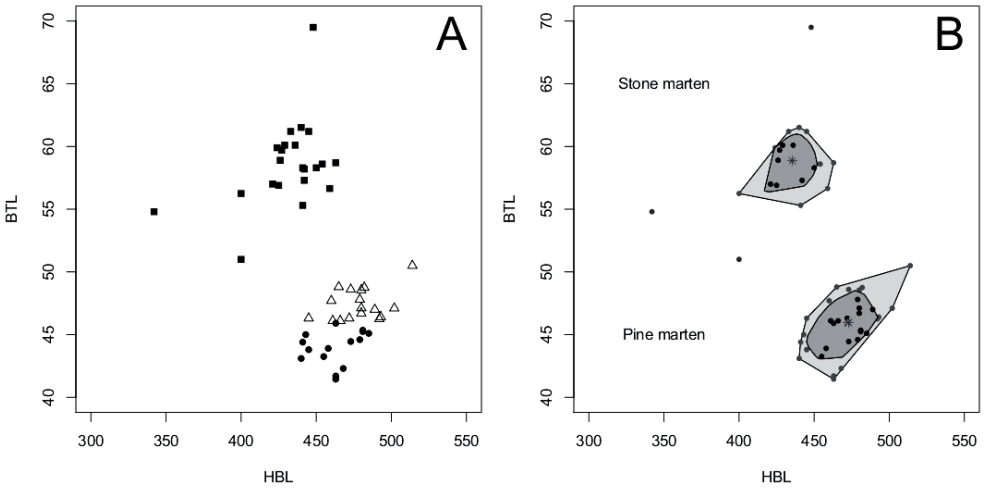

Figure 1 – A. Plot of HBL *vs*. *B*TL. *M. foina*: black squares; *M. martes*: black circles; unidentified specimens by *B*TL technique: white triangles; B. Bag-plot analysis: the dark grey polygon is the bag; the clear grey polygon is the loop; the star is the median; black points outside the polygon are outliers.

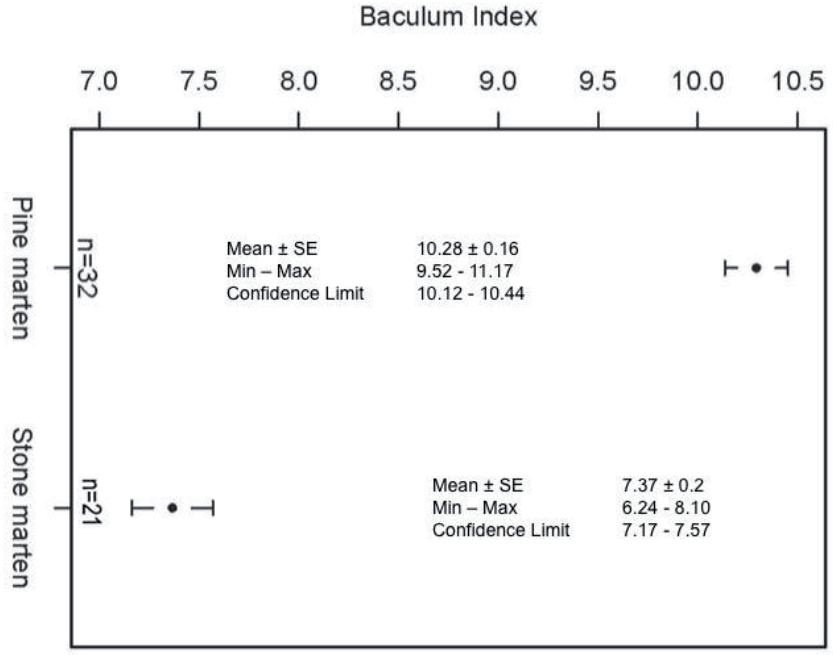

Figure 2 – Plot means and descriptive statistics of *B*I: black points represent the *B*I means of each species; the whiskers are 95% confidence intervals.

marten. It therefore seems important to go on searching for new and simple methods of morphological and morphometric diagnosis which, together with molecular techniques, can support zoological research.

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