

Evaluation of a facial feature to distinguish two sympatric Water Shrew species

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Short title

Neomys identification using facial line of demarcation

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Abstract

Species identification of the two sympatric Central European Water Shrews *Neomys fodiens* and *N. milleri* based on external features is tricky due to a relatively high variability of the traditionally used features (e.g., hair fringe on the hind feet and the tail), which in addition are often not readily visible on camera trap footage. For the Italian populations of these two species, it has relatively recently been suggested that the shape of the facial line of demarcation (line between the dark fur on the dorsal side and the lighter fur on the ventral side) can be used as an additional feature for species identification. In the current study, we evaluated this feature also in populations North of the Alps, focusing on Switzerland and – on a smaller scale – other parts of Europe. The examined, in part genetically identified specimens confirm that the facial line of demarcation shows a characteristic and mostly well discernible dichotomy between *N. fodiens* and *N. milleri*: in most of the examined *N. milleri* the dark facial mask involves the corners of the mouth and/or other parts of the mouth. In *N. fodiens*, however, there is usually an evenly spaced and relatively broad line of light fur between the mouth and the line of demarcation. This study corroborates that the shape of the facial line of demarcation is a helpful tool for the identification of these two *Neomys*-species and that this feature can be particularly relevant for studies using non-invasive monitoring methods such as camera traps.

Introduction

Two species of *Neomys* coexist in large parts of Central and Eastern Europe (Aulagnier et al. 2008, Igea et al. 2015): *N. fodiens* (Eurasian Water Shrew) and *N. milleri* (Mediterranean Water Shrew). Distinguishing features between the two species on the outside of the body include (Spitzenberger 1990a, b, Braun & Dieterlen 2005, Marchesi et al. 2008,): (1) a fringe of short, stiff hairs on the hind feet and the underside of the tail in *N. fodiens*, which are also present but less developed in *N. milleri*; (2) on average larger body and hindfoot size in *N. fodiens* compared to *N. milleri*. However, these features might be variable – also ontogenetically due to abrasion of the hair fringes – and are therefore notoriously difficult to discern, especially on camera trap footage. To date, the most reliable methods are genetic analysis and measurements of the skull (Müller & Dietrich 2021, Müller & Märki 2021).

Despite the effectiveness and recent developments of genetic methods to determine species and their occurrence, these methods are still relatively cost-intensive and sometimes limited in their accuracy (Ruedi et al. 2023). Moreover, genetic material and skulls are often not available, e.g., if museum specimens do not allow the extraction of tissue samples for DNA analysis and/or skulls, or if only photographic evidence is present. Particularly the latter point is relevant in light of recent advances in wildlife biology, where non-invasive and relatively cost and time efficient camera traps are used increasingly more often, including for the monitoring of small mammals (e.g., Croose & Carter 2019, Mos & Hofmeester 2020, Littlewood et al. 2021, McCleery et al. 2014, Soininen et al. 2015, Vinciguerra 2022). For such studies, reliable diagnostic external features to identify the target species are vital.

For the Italian populations of *N. fodiens* and *N. milleri*, one such diagnostic external feature has been newly suggested relatively recently (Lapini et al. 1995): the shape of the facial line of demarcation between the dark greyish fur on the dorsal side of the head, and the lighter coloured fur on the

ventral side (see Lapini et al. 1995, fig. 7 and Fig. 1 in this study). Specifically, *N. milleri* exhibits a convexity (i.e., a ‘notch’) in this line of demarcation, which is not present in *N. fodiens*.

To our knowledge, the shape of the facial line of demarcation as a feature to distinguish *N. fodiens* and *N. milleri* has so far only been mentioned regarding the Italian populations of these species (Lapini et al. 1995, Locatelli & Paolucci 1998, Amori et al. 2008). In the current study, we evaluated the reliability of this feature in *N. fodiens* and *N. milleri* from different parts of Europe, with a focus on areas in the North of the Alps, in particular Switzerland. This study thus contributes to our understanding of species determination according to external features in *Neomys* in a wide geographic area and might help inform monitoring programmes – especially studies using camera traps.

Materials and Methods

We included *N. fodiens* and *N. milleri* specimens from an as wide as possible geographical area within Switzerland and to a more limited degree across Europe (Fig. 2). For this, we sampled specimens from five Swiss museum collections, one British museum collection, and one private collection (alphabetical order of collection short name): Adrian Dietrich (AD, n=1); Bündner Naturmuseum, Chur, Switzerland (BNM, n=34); Muséum d'histoire naturelle, Genève, Switzerland (MHN, n=95); Naturhistorisches Museum Bern, Switzerland (NMBE, n=30); Naturmuseum St.Gallen, Switzerland (NMSG, n=27); Museum of Zoology, University of Cambridge, United Kingdom (UMZS, n=1); Naturmuseum Solothurn, Switzerland (ZMK, n=1).

Genetically determined specimens were preferred, but a restriction to only those would have greatly diminished the sample size. We thus decided to also include morphologically determined specimens (see below). We included different preparation types: whole specimens (fresh dead, frozen, or stored in ethanol), study skins (flat and round), and full mounts. Sex and age stage were not known for many specimens and thus not considered for the analyses. Extremely decayed and/or damaged

specimens were not considered. Detailed information on the here used specimens as provided by the different collection databases, including origin and species determination methods, are provided in the Supplementary Dataset.

We photographed all specimens and both authors evaluated the shape of the facial line of demarcation, if possible on both sides of the head. Further, we re-evaluated the established (traditional) species-specific set of diagnostic external features in every examined specimen, to double-check the species affiliation as provided by the respective collection databases. This evaluation included the examination of the extent of the hair fringes at the hind feet and the tail, relative hind feet size, as well as body size. If possible, tissue samples were extracted for genetic species determination by the Environmental Genomics and Systems Biology Research Group at the University of Applied Sciences (ZHAW) or the MHN. Genetic species determination was already available from the collection databases or was newly conducted during the current study for about a third of the here analysed specimens from Switzerland. In cases of ambiguous species determination via traditional morphological and genetic methods, we measured the height of the mandibular coronoid process with a digital calliper to the nearest 0.01mm (German: “Unterkieferasthöhe”, Bühler 1964).

In total, we examined 189 *Neomys*-specimens. Of these, we had to exclude 32 from the analyses because the line of demarcation was not discernible due to albinism, melanism, fading of the natural coloration, clotting of the fur due to liquids, or preparations that masque the facial line of demarcation. For example, flat hides were sometimes stretched around the cardboard on which they are mounted so that the facial line of demarcation came to lie on the rim of the cardboard and could not be discerned. From the remaining 157 specimens in which the facial line of demarcation could be evaluated, 117 were originally sampled across different areas within Switzerland (Fig. 2). Forty specimens stem from areas outside of Switzerland (n= 32, from Czech Republic, Finland, France, Italy, and Slovakia), or are of unknown origin (n=8).

Results

Of the 117 Swiss specimens featuring a visible facial line of demarcation, 3 showed a mismatch between results of genetic and morphological species determination using the height of the mandibular coronoid process (Bühler 1964). One additional specimen showed a mismatch between the species determination according to an established set of external features (see above) and the height of the mandibular coronoid process. These in total four specimens could not be unambiguously categorized as either *N. fodiens* or *N. milleri* in the first place and were excluded from further analyses. In sum, we could analyse 113 Swiss *Neomys*-specimens in the current study, 79 of which were determined as *N. fodiens* and 34 as *N. milleri*. These categorisations for every specimen are provided in the Supplementary Dataset.

In the here examined specimens, we could discern the following variation in the shape of the facial line of demarcation: In *N. fodiens*, the facial line of demarcation is running in a straight line from the rhinarium (i.e., most anterior part of the snout) towards the more caudal parts of the head near the pinna, without touching the labial angle, i.e., the corner of the mouth (Fig. 1a & b). There is usually a relatively broad and evenly spaced stripe of white fur on the lateral side of the rostrum, separating the facial line of demarcation from the upper lip of the mouth (Fig. 1a & b). In *N. milleri*, on the other hand, this line of demarcation is touching or almost touching the labial angle of the mouth (Fig. 1c – f). However, there is some variation in the shape of the facial line of demarcation in *N. milleri*. In some cases, there is a convexity in the line of demarcation just posterior to the labial angle, so that the darker dorsal fur is touching or almost touching (Fig. 1c) the corner of the mouth in lateral view. This convexity might be only faintly visible (Fig. 1d). In other cases, this convexity is less conspicuous, but the stripe of white fur on the rostrum, in between the line of demarcation and the upper lip of the mouth, is exceedingly dorsoventrally narrow and/or anteroposteriorly shortened (Fig. 1e & f) or totally absent (Fig. 1f), so that the line of demarcation touches large portions of the upper lip,

including the labial angle (Fig. 1e & f). In sum, the dark facial mask of *N. milleri* usually involves the corner of the mouth, while in *N. fodiens* it does not (Lapini et al. 1995).

Of the 113 analysed Swiss *Neomys*-specimens, 109 (96%) showed a match between species determination via genetic and/or traditional morphological methods and the above-described determination of the shape of the facial line of demarcation according to Lapini et al. (1995) (Fig. 1, Supplementary Dataset). Four of the Swiss specimens (4%) could not be correctly determined via the shape of the facial line of demarcation (Supplementary Dataset, Supplementary Fig. S1). Only one of those was genetically determined. In one of these cases, a *N. milleri* showed a facial line of demarcation as typical for *N. fodiens*; in three cases, *N. fodiens* showed a facial line of demarcation as typical for *N. milleri* (Supplementary Fig. S1). In the smaller international sample (n=32), featuring specimens from areas outside of Switzerland and across Europe, 30 specimens (94%) showed a match between the species determination via established methods and the shape of the facial line of demarcation. Two specimens (from France) were incorrectly determined (Supplementary Fig. S1).

Discussion

Our findings corroborate with what Lapini et al. (1995), Locatelli & Paolucci (1998), and Amori et al. 2008 suggested for Italian *Neomys fodiens* and *N. milleri* and suggest that the shape of the facial line of demarcation could be used as an additional tool for species identification also North of the Alps in Central European *Neomys*. This is useful if tissue or scat samples (for DNA-analysis) and mandibles are not applicable, e.g., in many museum specimens or if only photographs are available. Species identification via camera trap footage is generally difficult in eulipotyphlans (Kays et al. 2022), particularly in *Neomys*, where diagnostic features are usually hidden by other parts of the body and thus obscure. However, the facial line of demarcation is often readily visible in life animals and on camera trap footage and might therefore be a particularly useful tool for monitoring programs using camera traps (Fig. 3).

However, misidentification according to the shape of the facial line of demarcation still occurred in a few of the here examined specimens (Supplementary Dataset and Fig. S1). Reasons for this might be found in the variation of the character, especially in *N. milleri* (Fig. 1), which might hamper a dichotomous classification. Other reasons might be found in wrongly determined specimens in museum collections, which we could not further investigate and confirm in all the questionable cases. Also, hybridization between the two species might pose an issue for species identification, although this seems not likely due to their comparatively great genetic distance (Catzeflis 1984). In any event, due to the small number of misidentified specimens in the current study, we recommend that additional external features should – if possible – be used for species identification in sympatric *N. fodiens* and *N. milleri*. Whether the facial line of demarcation is a distinguishing feature also between the sympatric *N. anomalus* and *N. fodiens* on the Iberian Peninsula, remains to be tested.

Author contribution

LV introduced the idea for this study; both authors conceptualised the study and gathered the data; MG drafted the article and LV revised it critically.

Ethics statement

This work was based on specimens available in museum collections and no life animal was sacrificed.

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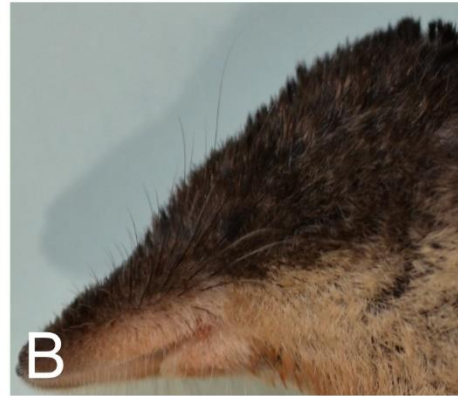
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Figure 1. Examples of the shape of the facial line of demarcation in *Neomys fodiens* (a, b) and *N. milleri* (c – f) in here examined museum specimens from Switzerland. The facial line of demarcation (arrow in a) marks the sharp transition between the dark greyish fur on the dorsal side of the head and the lighter coloured fur on the ventral side. In *N. milleri* (c – f) the dark facial mask usually involves the corner of the mouth, even exhibiting a convexity (arrow in c), while in *N. fodiens* (a, b) it does not. See text for detailed descriptions of the inter- and intraspecific variation of the shape of the facial line of demarcation. a) *N. fodiens*, BNM 404 (genetically determined, Grisons, photo mirrored); b) *N. fodiens*, NMSG-V-7992 (genetically determined, St.Gallen, photo mirrored); c) *N. milleri*, BMN 16840 (genetically determined, Grisons); d) *N. milleri*, BMN 17117 (genetically determined, Grisons); e) *N. milleri*, NMSG-V-8241 (determined via the height of the mandibular coronoid process, Grisons, photo mirrored); f) *N. milleri*, BMN 17112 (genetically determined, Grisons).

Neomys fodiens



Neomys milleri



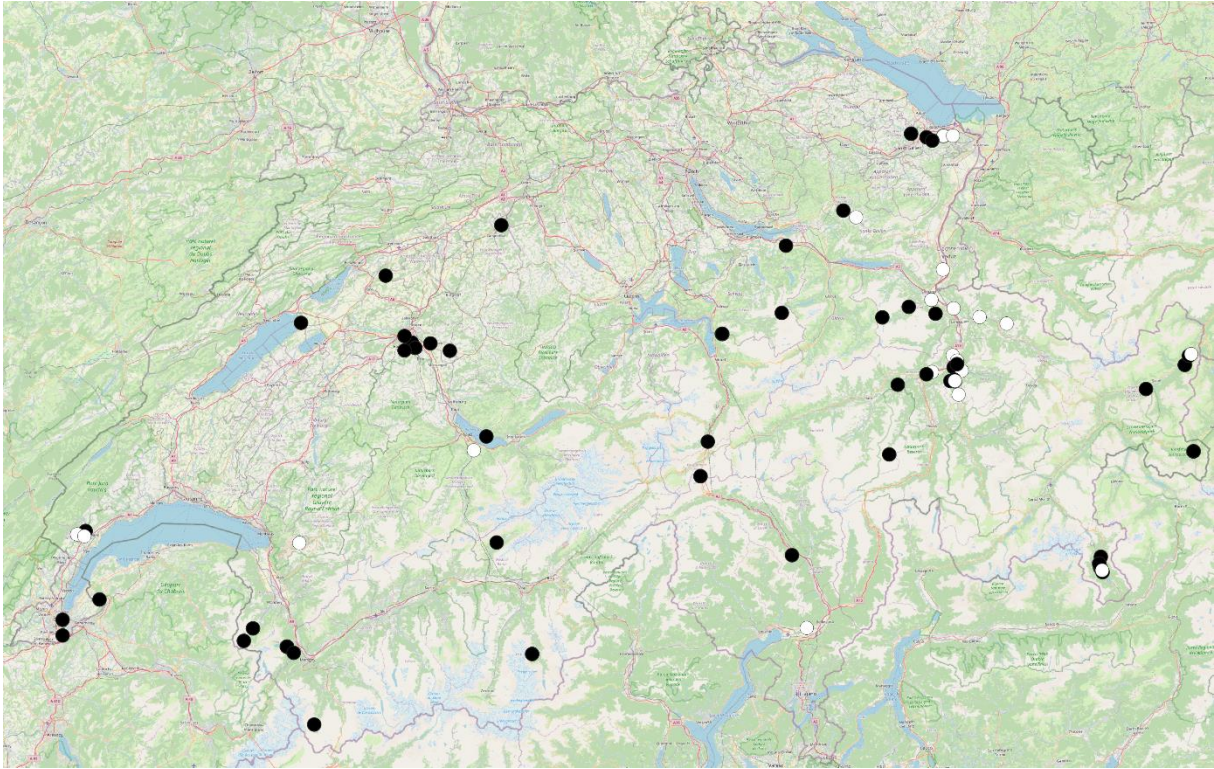


Figure 2. Geographical distribution of the here examined *Neomys*-specimens in Switzerland. Black = *N. fodiens*, white = *N. milleri*. Map data from OpenStreetMap (openstreetmap.org/copyright)

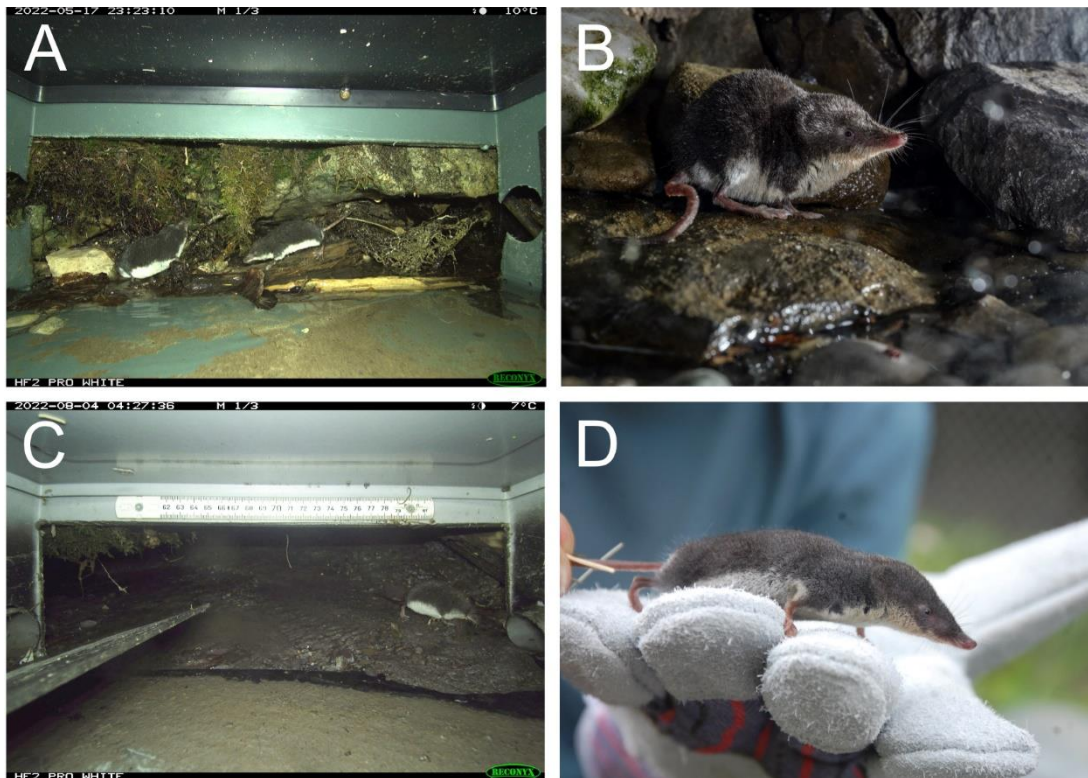


Figure 3. The shape of the facial line of demarcation in life *Neomys fodiens* and *N. milleri* is discernible on camera trap footage (a, c) and in captured animals (b, d). According to the species-specific diagnostic feature as proposed by Lapini et al. (1995) and further evaluated in the current study, these specimens could be determined as *N. fodiens* (a, b) and *N. milleri* (c, d), respectively. Camera trap footage in (a, c) was obtained using a Reconyx HyperFire2 Professional White Flash Small Mammal Camera built into a MiniMammalCamBox (Vinciguerra 2022) in the canton of St.Gallen (CH). The specimen in b) was captured in the canton of St.Gallen, subsequently genetically analysed, and determined as *N. fodiens*; photo: © René Güttinger, RGBlick. The specimen in d) was captured during a project conducted by UNA (unabern.ch) in the canton of Uri (CH), subsequently genetically analysed, and determined as *N. milleri*; photo: © Adrian Dietrich.

Supplementary Online Material

File 1 - Download source file (4.1 MB)

Supplementary Figure S1

File 2 - Download source file (34.24 kB)

Supplementary Dataset featuring specimen information.