

# **Nebria (Nebriola) gostelia sp. nov. from the Penninian Alps near Biella, Piemonte, Italy (Coleoptera: Carabidae, Nebriinae)**

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# *Nebria (Nebriola) gosteliae* sp. nov. from the Penninian Alps near Biella, Piemonte, Italy (Coleoptera: Carabidae, Nebriinae)

Charles Huber, Alexander Szallies, Hannes Baur & Pier Mauro Giachino

## ABSTRACT

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The endemic species *Nebria (Nebriola) gosteliae* HUBER sp. nov. from the Penninian Alps near Biella, Piemonte, Italy, is described. Morphological, morphometrical and molecular methods were applied. A determination key is given.

Keywords: Coleoptera, Carabidae, *Nebria*, *Nebriola*, new species, morphometry, molecular analysis, determination key, Penninian Alps, Italy.

## Introduction

Specimens of the carabid genus *Nebria* LATREILLE, 1802 (subgenus *Nebriola* K. DANIEL, 1903) from the Biellese Alps (Penninian Alps, Piemonte, Italy), similar to *Nebria (Nebriola) laticollis* DEJEAN, 1826, were reported several times as a possible new taxon. Max Bänninger, specialist of the genus *Nebria* in the early 20<sup>th</sup> century, corresponded on the problem with the Italian entomologist Agostino Doderò who himself collected 1926–1928 several specimens of the enigmatic taxon near the monastery of Oropa (north of the city of Biella). Bänninger kept the sample and labeled it as a new taxon ("*Nebriola oropana* sp. n. (oder ssp. n. *pictiventris/laticollis*). Siehe Brief an Doderò 21. 10. 1928 und seine Antwort 30. 10. 1928. Beschreibungsentwurf vorhanden."). However, Bänninger did not publish it, and his manuscript draft is missing.

Some years later the Austrian coleopterologist Albert Winkler checked the sample of Doderò in the Bänninger collection (one specimen is labeled "A. Winkler vid. 12. 1941").

Even though Bänninger intended to describe the "Oropa" population, he did not discuss it in later publications (1951a, 1951b). Bänninger (1951a) announced the description of a taxon by Winkler from the M. Mucrone (near

Oropa) and M. Bo, but the specimens referred to the complex of *N. cordicollis/kochi*.

Dodero's "Oropa population" was forgotten. Magistretti (1965) did not mention it in his "Fauna d'Italia". Focarile (1987) subsumed the new taxon under *N. laticollis* although in the respective distribution map he curiously used a different symbol not explained in the legend for localities near Oropa.

Riccardo Sciaky rediscovered the Oropa population and labelled some specimens of the Dodero collection as a new species ("*Nebria debernadii* det. Sciaky, in lit."), but he did not publish it.

Casale & Vigna Taglianti (1992) remembered an unpublished species ("*specie inedita*") – sympatric but not syntopic with *N. cordicollis* ssp. *winkleri* BARI, 1971 – from the Monte Camino near the monastery of Oropa.

Bisio (1998) recognized *Nebriola* specimens from the Colle della Vecchia (Gaby, Valle Gressoney), from the Monte Camino and the Lago di Mucrone (Valle d'Oropa) as different from *N. laticollis*, in contrast to Focarile (1987). He assigned them to the unpublished species mentioned by Casale & Vigna Taglianti (1992). Ledoux & Roux (2005, p. 177) confirmed the specimens mentioned by Bisio as not identical to *N. laticollis*.

This mysterious "species" was always mentioned as related to *N. laticollis* due to its morphology and its ripicole natural history. A relationship to other ripicole species as *N. fontinalis* K. & J. DANIEL, 1890, *N. pictiventris* FAUVEL, 1888 and *N. morula* K. & J. DANIEL, 1891 was never discussed.

Because of the persisting uncertainty in the *Nebriola* specimens from the Oropa range we decided to investigate this old problem, making use of morphological, morphometrical and molecular methods. On account of our results we describe herewith a new *Nebria* species of the subgenus *Nebriola*.

## Material and Methods

Abbreviations used:

cAll	collection of G. Allegro, Moncalvo (Asti)
cBän	collection of M. Bänninger (in ETHZ)
cBin	collection of G. Binaghi (in MSNG)
cBis	collection of L. Bisio, Cuornè (Torino)
cCas	collection of A. Casale, Torino
cDod	collection of A. Dodero (in MSNG)
cGia	collection of P. M. Giachino, Torino
cMan	collection of C. Mancini (in MSNG)
cMon	collection of R. Monguzzi, Milano

cSci	collection of R. Sciaky, Milano
cSza	collection of A. Szallies, Reutlingen
cVig	collection of A. Vigna Taglianti, Roma (in MZUR)
ETHZ	Eidgenössische Technische Hochschule, Zürich
MC	Museo Civico di Storia Naturale di Carmagnola (Torino)
MSNG	Museo Civico di Storia Naturale di Genova
MZUR	Museo di Zoologia dell' Università di Roma
NMBE	Naturhistorisches Museum der Burgergemeinde Bern

Measurements: For the morphometric analysis 229 specimen of 6 ripicole *Nebriola* taxa were measured in addition to the "Oropa" population:

*Nebria fontinalis* K. & J. DANIEL, 1890. The nominal form and the ssp. *rhaetica* K. & J. DANIEL, 1890 were examined (Origin: Orobian Alps, Central Swiss Alps)

*N. laticollis* DEJEAN, 1826. The nominal form and the ssp. *pennina* JEANNEL, 1937 were examined (Western Alps)

*N. morula* K. & J. DANIEL, 1891 (Ligurian Alps)

*N. pictiventris* FAUVEL, 1888 (Western Alps)

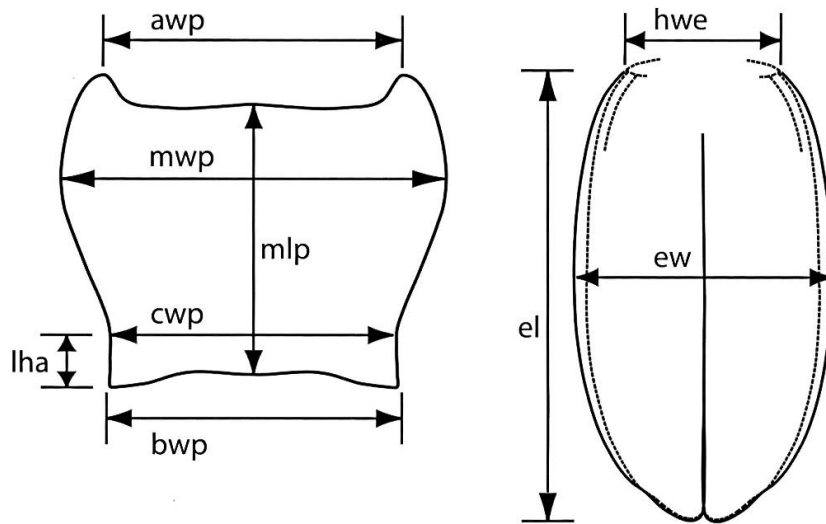
The *Nebriola* taxon *N. cordicollis* ssp. *kochi* SCHATZMAYR, 1939 (= ssp. *winkleri* BARI, 1971), which is sympatric but not syntopic with the "Oropa" population, was not included because of its obvious morphological and ecological differences (snowy soakage in scree slopes).

The following measurements on the head, on the pronotum and on the elytra were taken (Fig. 1):

- head width (measured between the eyes)
- anterior width of the pronotum (awp)
- maximal width of the pronotum (mwp)
- constriction width of the pronotum (cwp)
- basal width of the pronotum (bwp)
- length of the hind angle (lha)
- median length of the pronotum (mlp)
- humeral width of the elytra (hwe, measured between the humeral teeth)
- maximal width of the elytra width (ew)
- length of the elytra (el)

Morphometrics: For the analysis of the morphometric data principal component (PCA) and Fisher's linear discriminant analysis (LDA) were applied. Both are ordination methods but differ fundamentally in their application (see Pimentel (1979) for a readable account for biologists). For a LDA it is neces-





**Fig. 1: Measurements taken of the pronotum and of the elytra (symbolic shape); after Huber & Marggi (2008; modified). For the abbreviations see the text.**

sary to assign each specimen to a group (here to the different species) before the analysis; the LDA will then find the best separation of those groups. On the other hand, with PCA the whole dataset is considered as a single group. The results reveal the natural variation structure in the dataset. The method can be considered as a sort of test for groupings based on different sets of characters (e.g. molecular markers). In contrast to LDA, PCA is sensitive to the magnitude of the measurements. Hence, the raw data were transformed to natural logarithms. We furthermore applied a method known as "double centering", whereby the values of both, the column and row vector of the data matrix are centered, i.e. the column and row means are subtracted from each data value (Somers 1986, 1989). The procedure removes the isometric size from the analysis, i.e. specimens are differentiated according to their shape (proportions). This is convenient because the important taxonomic features for separating species of the subgenus *Nebriola* are related to shape, not size. All calculations and graphics were done with the R statistical software (R Development Core Team 2009). For the PCA, the function `prcomp()` was used, with the options `center=FALSE` and `scale=FALSE`. For LDA we applied the function `lda()` from the package MASS. Priors were set to 1/5 for all species. Further details for computation of the above analyses with R are given by Claude (2008). The 3 missing values present in the data set were replaced by medians.

**Molecular data:** To confirm the status of the new taxon as a previously unrecognized and distinct species we obtained and analysed mitochondrial DNA sequences from all known species of the subgenus *Nebriola*.

Two independent mitochondrial DNA fragments were amplified by polymerase chain reaction (PCR) from the DNA extracted from several *Nebriola* spe-

cies as template. Typically, 30–35 cycles with primers annealing at 55–61°C (TAG AAT TAG AAG ATC AAC CAG C and ACA TGA TCT GAG TTC AAA CCG G to amplify ND1 and CGT CGA TAC TCT GAT TAC CCN GAY GC and ATG ATT TGA TCC ACA AAT TTC TGW RCA YTG to amplify COII) were employed. The two amplicons were sequenced and in total rendered 1302–1303 bases of mitochondrial DNA. The ND1 amplicon of 508 (509 in *Nebria lariollei*) bases comprised part of the 16S rRNA gene, a tRNA<sup>Leu</sup> gene and part of the NADH dehydrogenase subunit 1 gene, ND1. The other COII amplicon spanned part of the gene of cytochrome oxidase subunit I, COI, a tRNA<sup>Leu</sup> gene and part of the gene of cytochrome oxidase subunit II, COII. All genetic sequences discussed here were submitted to the network of sequence databases and can be found by their respective accession numbers GU827699–GU827710 at <http://www.ncbi.nlm.nih.gov/nucleotide>.

The photographs were made with a Leica DFC 420 digital camera, with a Leica MZ 16A binocular and with the automated multifocus software imageAccess 8.3.

## Results

### Systematics

#### ***Nebria (Nebriola) gosteliae* HUBER sp. nov.** (Fig. 2)

Holotype ♂: I–Piemonte/Biella, Oropa, M. Mars, Lago Mucrone – Rifugio Coda, 2000 m, 21. 7. 2006, leg. C. Huber & M. Gosteli (NMBE).

Paratypes:

1 ♀, same data as holotype (NMBE).

1 ♂ 2 ♀ I–Piemonte/Biella, Oropa, Lago Mucrone, 1950 m, 21. 7. 2006, leg. C. Huber & M. Gosteli (NMBE).

1 ♂ 3 ♀ I–Piemonte/Biella, Oropa, Lago Mucrone, 2000 m, 27. 6. 2006, leg. A. Szallies (cSza).

1 ♂ 4 ♀ Sant. [= Santuario = Monastery] Oropa (Piemonte), 5. 1920, leg. C. Mancini. Handwritten label "*Nebria Liattii*" (cMan/MSNG).

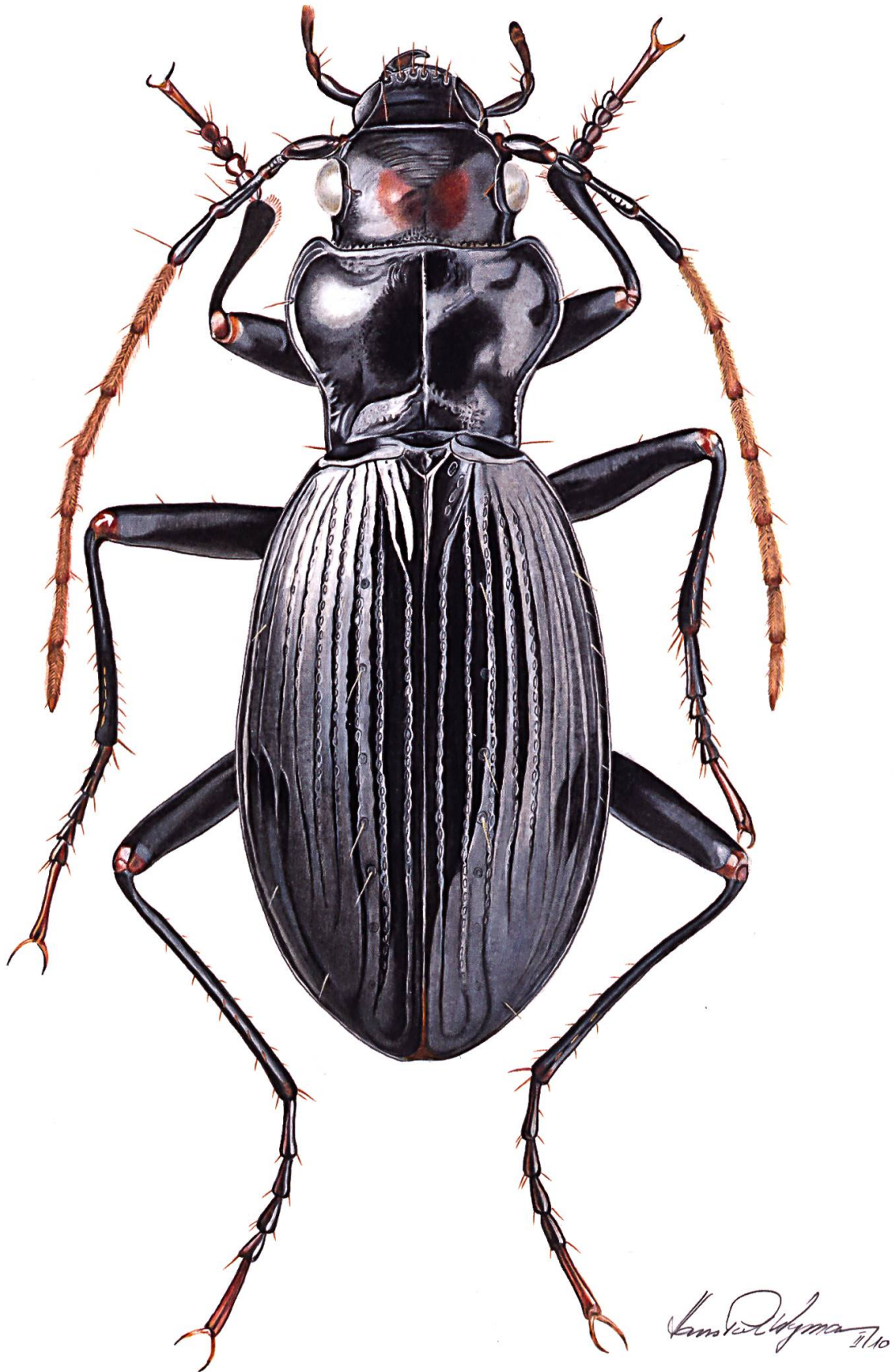
1 ♂ Biellese, Oropa, Lago Mucrone, 8. 9. 1931 [?], leg. Binaghi (cBin/MSNG).

1 ♂ 3 ♀ Sant. Oropa Biellese (Piem.), 8. 1926, leg. A. Doderer (cDod/MSNG).

8 ♂ 3 ♀ Sant. Oropa Biellese (Piem.), 6. 1928, leg. A. Doderer (cDod/MSNG).

1 ♀ same data (cBän/ETHZ).

- 3 ♂ Sant. Oropa, 7. 1928, leg. A. Dodero. Handwritten label "*Nebria oropana* sp. n." (cBän/ETHZ).
- 1 ♂ 1 ♀ Sant. Oropa Biellese (Piem.), 6. 1929, leg. A. Dodero. Handwritten label "*Nebria* n. sp." det. F. Capra [printed] and additional handwritten label "*debernardii* det. Sciaky, in lit." (MSNG).
- 3 ♂ 6 ♀ Sant. Oropa Biellese (Piem.), 6. 1929, leg. A. Dodero (cDod/MSNG).
- 2 ♂ 3 ♀ Sant. Oropa Biellese (Piem.), 6. 1929, leg. A. Dodero. Handwritten label "*Nebria (Nebriola) kochi* subsp. *winkleri* Bari 1971; Topotipi" [misidentification] and additional label "*Nebria (Nebriola) debernardii* n. sp. det. Sciaky 81" (cBin/MSNG).
- 1 ♂ 3 ♀ Sant. Oropa Biellese (Piem.), 6. 1928, leg. A. Dodero (cBin/MSNG).
- 2 ♂ 1 ♀ Sant. Oropa, 6. 1928, leg. A. Dodero. 1 specimen with a handwritten label "*Nebria pictiventris?* Fauv." (cBin/MSNG).
- 2 ♂ 1 ♀ M. Mucrone (VC), 2000 m, 10. 8. 1979, leg. R. Monguzzi (cMon).
- 1 ♂ M. Mucrone, Biella (VC), 10. 7. 1983, leg. R. Monguzzi (cMon).
- 1 ♂ M. Mucrone, Biella, 2000 m, 24. 8. 1993, leg. R. Monguzzi (cMon).
- 2 ♂ Oropa, M. Camino (Biella), 2200 m, 24. 8. 1996, leg. R. Monguzzi (cMon).
- 2 ♂ 10 ♀ M. Camino di Oropa (Biella), 2200 m, 18. 7. 1997, leg. R. Monguzzi (cMon).
- 7 ♂ 13 ♀ Prealpi Biellese, M. Rosso d'Oropa, 2200 m, 22. 7. 1980, leg. R. Monguzzi (cMon).
- 1 ♂ 1 ♀ same data (NMBE).
- 4 ♂ 3 ♀ Oropa, M. Rosso (VC), 2300 m, 26. 7. 1980, leg. R. Monguzzi (cMon).
- 2 ♂ Biellese (VC), Lago Mucrone, 14. 7. 1983, leg. Pescarolo (MC).
- 1 ♂ same data (cAll).
- 2 ♂ same data (cGia).
- 1 ♂ 3 ♀ Alpi Pennine, Oropa, M. Camino (s) 2200–2300 m, 13. VIII. 74, A. Vigna leg. Labelled "*Nebriola* n. sp., det. A. Vigna 1974" (MZUR)
- 1 ♂ 1 ♀ Italia, Piemonte, M. Camino (Oropa), 2100 m, 20. 6. 1994, leg. A. Casale (cCas). Handwritten red label "*Nebria* n. sp. A. Casale det."
- 1 ♂ 1 ♀ M. Camino, Oropa (Biella), 2250 m, 25. 6. 1995, leg. Ghittino (cCas).
- 4 ♂ Oropa (VC), L. Mucrone, 5. 8. 1978, leg. Sciaky. 1 specimen with a handwritten red label "*Nebriola nova?*" (cSci).
- 6 ♂ 9 ♀ Piemonte: Mt. Mucrone, Oropa (Biella VC), 21–2300 m, [undated], leg. Bocca (cSci).
- 1 ♂ 1 ♀ same data (cGia).
- 3 ♂ 8 ♀ Oropa (VC), M. Mucrone, 8. 1980, leg. Sciaky (cSci).
- 1 ♂ same data (cGia).
- 1 ♂ Mte. Mucrone, 7. 1982, leg. Dacatra (cGia).
- 1 ♂ 2 ♀ Colle della Vecchia, Gabi (AO), 2000 m, 8. 6. 1986, L. Bisio (cBis).



**Fig. 2:** *Nebria (Nebriola) gosteliae* HUBER sp. nov., male, habitus. Paratype: Lago Mucrone/Oropa/Biella, 27. 6. 2006, leg. A. Szallies. Body size: 9 mm. Illustration: Hans-Peter Wymann (Jegenstorf).



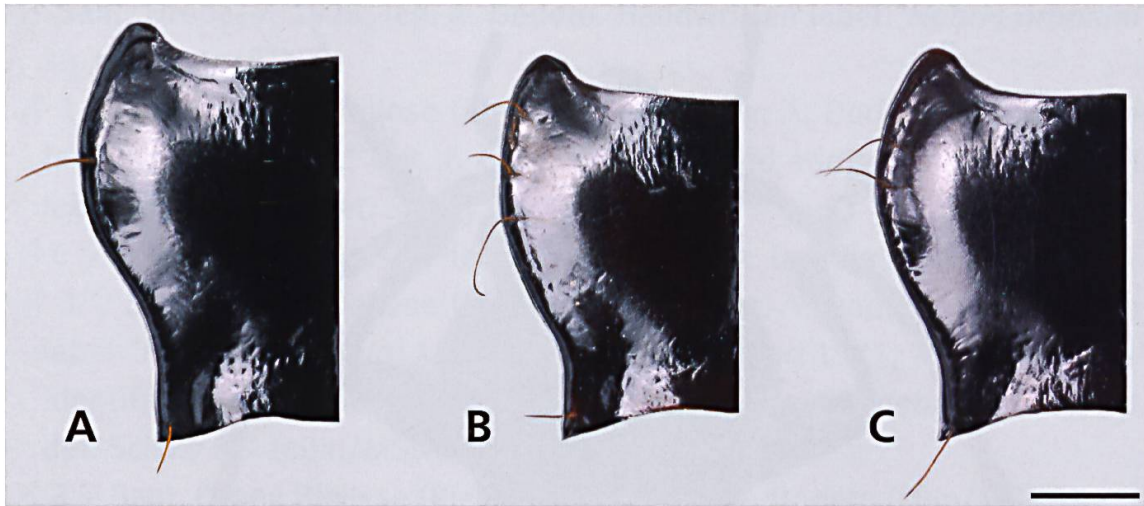


Fig. 3: Pronota, left half. – A: *Nebria gosteliae* HUBER sp. nov.; – B: *N. fontinalis* s. str. from Lago Piazzotti/Gerola Alta/Valtellina Italy, 2000 m; – C: *N. laticollis* ssp. *pennina* from Täschhütte/Wallis, Switzerland, 2700 m. Scale bar: 0.5 mm. Fotos: Hannes Baur (NMBE).

## Description

Colouration: Black. Head with two elongated brown spots of variable markedness. Tips of the mandibulae and the appendages of the head brownish or dark brown. Antennomeres 1–4 always darkened or even black, antennomeres 5–11 light brownish. Legs black, knees lightened brown, tarsi brown with the pretarsus lighter brown than the tarsomeres 1–4.

Body length: 8.5–9.5 mm (males), 9–10 mm (females).

Head: Labrum with anterior margin faintly convex, bearing a row of 6 setae. Apical margin of clypeus straight. Vertex impunctate. 1 supraorbital seta. Antennae long and slender extending to the middle of the elytra. Antennal scape subcylindrical, medially faintly thickened, basally narrowed, as long as the eye's diameter, with 1 dorsal seta. Maxillary stipes basolaterally with 6 robust setae. Penultimate labial palpomere trisetose. Mentum with a bifid medial tooth. Submentum with a row of 16 setae. Microsculpture of the head isodiametric.

Pronotum: Cordate (Figs. 2, 3A), transverse, widest at apical third, narrowed basally; ratio width/length of the pronotum =  $1.44 \pm 0.03$ . Lateral margin convex, strongly rounded to the anterior and posterior angles, concave distinctly before the posterior angles, posterior angles therefore with long parallel sides. Lateral groove narrow. Anterior angles rounded, slightly protruding. Posterior angles acute, moderately protruding backwards. Pronotal disc convex. Basal fovea deep, anterior and posterior transverse impressions and median longitudinal impression distinct. Basal fovea, the anterior and the

**Fig. 4: Aedeagi.**

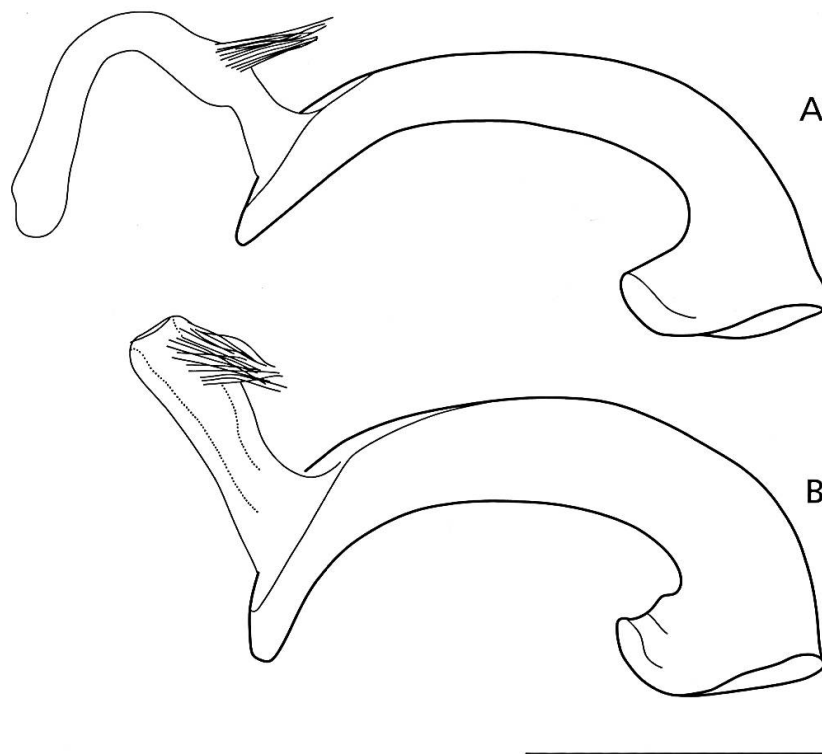
– A: *Nebria gosteliae*

HUBER sp. nov.;

– B: *N. laticollis* ssp.

*pennina* (internal sac partly everted). Scale

bar: 1 mm.



posterior transverse impressions punctate; the anterior transverse impression sometimes longitudinally wrinkled. Apical and basal margination absent. Basolateral seta present. 1 midlateral seta at the widest part of the pronotum. Microsculpture of the pronotum isodiametric, pronotum impunctate on disc. Proepisternum smooth. Prosternal process unmargined.

Elytra (Fig. 2): Elytral silhouette ovoid-elongate, sides evenly rounded, flattened, without humeral bulge, widest at middle. Humeri reduced, with a marked humeral tooth and a humeral carina. Basal margination towards the humeral tooth straight or slightly sinuate. Striae distinct; weakly punctate, reaching the apex. Intervals weakly convex. Interval 3 with 5–7 pores near striae 3. Marginal gutter narrow, interval 9 with a row of 10 setiferous pores. Apical carena developed. Scutellar pore absent. Microsculpture of the elytrae isodiametric. Hind wings brachypterous, present as a short, narrow, strap-like vestige of the dimension of 1–2 antennomeres. Mesepisterna faintly punctate. Metepisterna as wide as long, faintly punctate or even impunctate. Metacoxa with 1 basal and 1 apical setae.

Ventrites: Fourth to sixth visible abdominal sterna (sensu Ledoux & Roux 2005) each with (1)2–3 posterior paramedial setae. Anal sternum with 1 paramedial seta in the male and 2 in the female. All sterna with faint impressions laterally.

Legs: Slender. Hind tarsi conspicuously delicate. Pretarsus of the hind tarsi distinctly longer than tarsomeres 3–4.

variable	1. LDA	2. LDA
hw	0.149	<b>0.606</b>
awp	0.232	0.090
mwp	<b>-0.892</b>	0.062
cwp	<b>0.480</b>	-0.358
bwp	0.063	-0.103
mlp	-0.108	-0.033
lha	0.227	<b>1.344</b>
hwe	0.161	-0.154
ew	-0.210	-0.158
el	0.207	0.074

**Tab. 1: Coefficients of first and second linear discriminant function. The most significant values are in bold.**

Aedeagus (Fig. 4): Aedeagus enlarged at basal orifice. Tube slender, regularly curved to apex and base. Everted internal sac dorsally with a distinct tuft of long setae in the first third of the shaft, which is a subgeneric character of *Nebriola*.

#### Differential diagnosis:

*N. fontinalis* ssp. *rhaetica* differs from *N. gosteliae* HUBER sp. nov. by the reddish-brownish appendices, by the less rounded lateral margin of the pronotum and by the polysetose lateral margin of the pronotum (unisetose in *N. gosteliae* HUBER sp. nov.; Figs. 3A, 3B). The everted internal sac of the aedeagus in *N. fontinalis* ssp. *rhaetica* with a diminished tuft of short setae.

In *N. gosteliae* HUBER sp. nov. the lateral border of the pronotum is unisetose, whereas in *N. laticollis* there is bisetosity in ssp. *pennina* and unisetosity in the nominal form respectively. The pronotum in *N. laticollis* is distinctly more transverse (ratio width/length =  $1.54 \pm 0.03$ ; Fig. 3C) than in *N. gosteliae* HUBER sp. nov. Elytral humeri are protruding in *N. laticollis* (as in *N. pictiventris*), but are lacking in *N. gosteliae* HUBER sp. nov. The ventrites of *N. gosteliae* HUBER sp. nov. have a variable number of (1)2–3 paramedial setae, whereas *N. laticollis* is well characterized by the paramedially unisetose ventrites (Ledoux & Roux 2005). The tube of the aedeagus in *N. laticollis* is thicker and clearly apically curved in contrast to *N. gosteliae* HUBER sp. nov. (Fig. 4).

The sympatric (but not syntopic) *N. cordicollis* ssp. *kochi* differs from *N. gosteliae* HUBER sp. nov. by its less transverse pronotum ( $1.35 \pm 0.05$ ), by its short posterior angles, by its drop shaped elytra (widest behind the middle), and by its variable number of (up to 4) basal setae of the metacoxa.

classification						
taxon	<i>gosteliae</i> sp. nov.	<i>fontinalis</i>	<i>laticollis</i>	<i>morula</i>	<i>pictiventris</i>	total
<i>gosteliae</i> sp. nov.	45	1	0	0	0	46
<i>fontinalis</i>	1	34	2	0	0	37
<i>laticollis</i>	0	0	74	1	1	76
<i>morula</i>	0	0	0	25	0	25
<i>pictiventris</i>	0	1	0	0	44	45

classification in %						
taxon	<i>gosteliae</i> sp. nov.	<i>fontinalis</i>	<i>laticollis</i>	<i>morula</i>	<i>pictiventris</i>	total
<i>gosteliae</i> sp. nov.	97.8	2.2	0	0	0	100
<i>fontinalis</i>	2.7	91.9	5.4	0	0	100
<i>laticollis</i>	0	0	97.4	1.3	1.3	100
<i>morula</i>	0	0	0	100	0	100
<i>pictiventris</i>	0	2.2	0	0	97.8	100

**Tab. 2: Leave-one-out classification (crossvalidation) of specimens using linear discriminant analysis (LDA).**

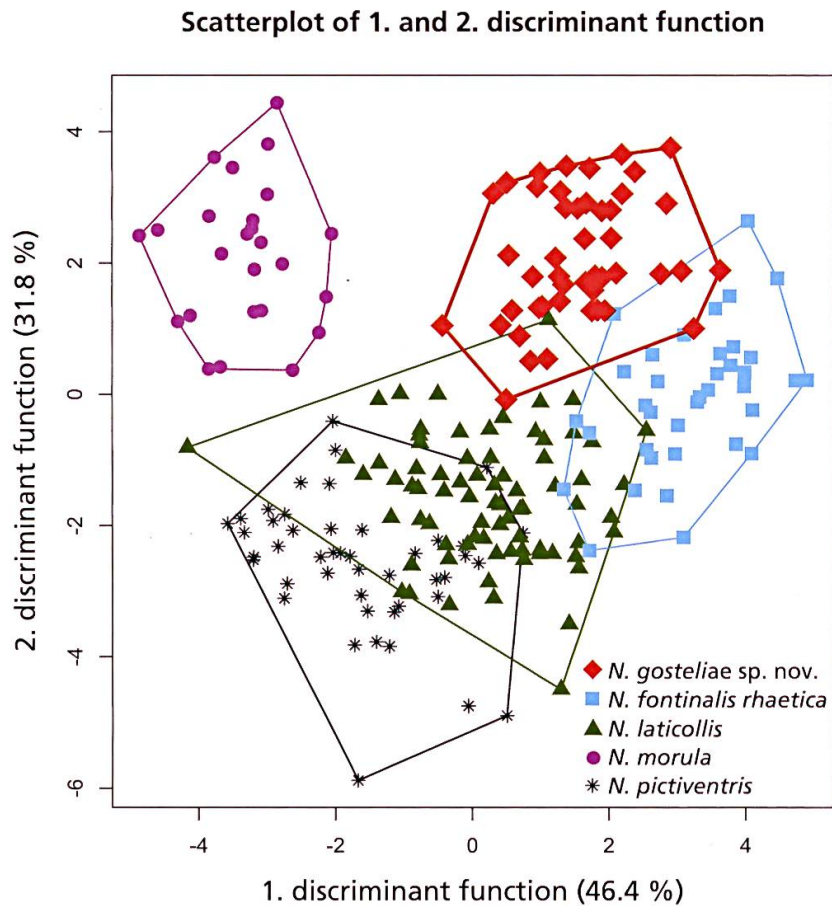
Main characters of *N. gosteliae* HUBER sp. nov.: The new species is characterized by the long sided hind angles of the pronotum (Fig. 3A), by the unisetose lateral margin of the pronotum, by the ovoid-elongate elytra, and by the delicate hind tarsi.

Natural history: *N. gosteliae* HUBER sp. nov. is a hydrophilic, ripicole and cold-stenothermic species. Specimens were found under stones in brooklets at alpine altitude. The larvae are unknown.

Geographical range (Fig. 7): *N. gosteliae* HUBER sp. nov. is restricted to the local range of the Lago di Mucrone near Oropa/Biella and to the mountain ridge to the Colle della Vecchia, 10 km north of the Lago di Mucrone.

Etymology: The new species is dedicated to the memory of the late Dr. Margret Gosteli Huber, the principal author's wife, who passed away in October 2008. Although a mollusc specialist herself, she enjoyed joining him on his carabid collecting trips. One of her last mountain trips was the one to the Lago di Mucrone near Biella where she helped to collect *Nebriola* specimens for this study.



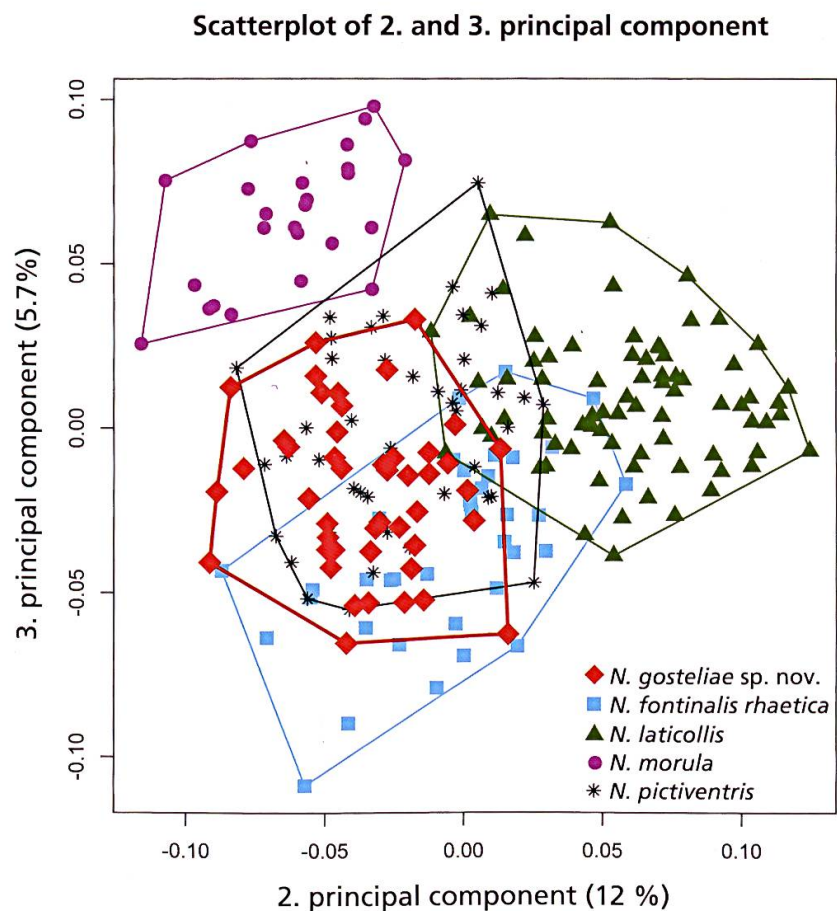
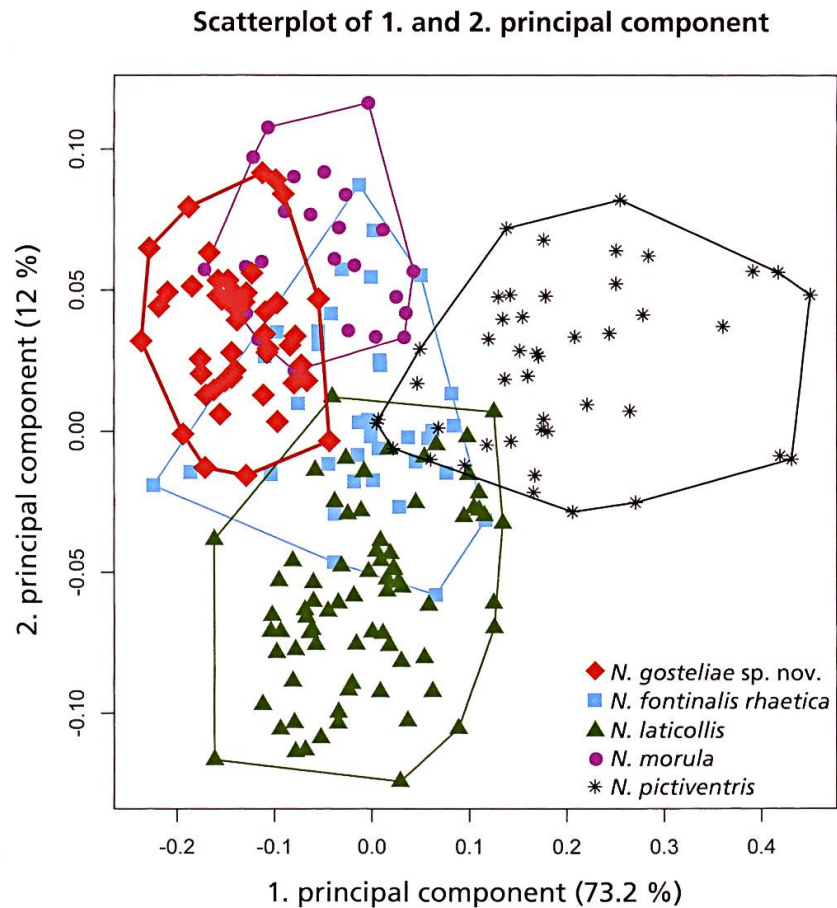


**Fig. 5:** Results of the linear discriminant analysis (LDA), based on raw data. The first two discriminant functions account for 78.2% of the variance in the data. In the scatterplot *N. gosteliae* HUBER sp. nov. overlaps somewhat with *N. fontinalis rhaetica* and *N. laticollis* ssp. *pennina* respectively but it is well separated from the remaining species (see also results from leave-one-out classification, Tab. 2).

## Morphometrics

Due to the morphometric data *Nebria gosteliae* HUBER sp. nov. can be distinguished to a high degree from the other *Nebriola* species. With linear discriminant analysis (LDA), the first and second discriminant function (comprising 78.2% of the total variation) separate the species clearly from the remaining ones (Tab. 1, Fig. 5). There is only a slight overlap with *N. fontinalis* ssp. *rhaetica* and *N. laticollis*. Using the leave-one-out classification 222 of 229 measured specimens were correctly classified (96.9%). Only a single specimen of *N. gosteliae* HUBER sp. nov. (2.2%; Tab. 2) was misclassified as *N. fontinalis* ssp. *rhaetica*. The posterior probability for classification in *N. fontinalis* ssp. *rhaetica* is  $p < 0.793$ , in *N. gosteliae* HUBER sp. nov.  $p < 0.206$ . Hence, the probability for the classification of the respective specimen in *N. gosteliae* sp. nov. is still distinctly above 5%. Principal component analysis (PCA) generally confirms the findings from LDA (Fig. 6). The first three components (accounting for 90.9% of the total variation) distinguish *N. gosteliae* HUBER sp. nov. from all species except from *N. fontinalis* ssp. *rhaetica* (Tab. 3). The latter overlaps with all the other species but can be recognized by several other morphological characters as well as by the genetic markers.

Fig. 6: Results of the principal component analysis (PCA) based on the size corrected log data (see material and methods section). The first three principal components explain almost 90.9% of the variance in the data and largely support the findings from the LDA. *Nebria gosteliae* HUBER sp. nov. is mostly distinguished from the other taxa except for *N. fontinalis* ssp. *rhaetica*. In the upper scatterplot the first component separates *N. gosteliae* HUBER sp. nov. from *N. pictiventris* and partly from *N. morula*, while the second component separates it largely from *N. laticollis*. In the lower scatterplot the third component distinguishes *N. gosteliae* HUBER sp. nov. from *N. morula*. *N. fontinalis* ssp. *rhaetica* almost entirely overlaps with the remaining taxa. However, this species can be recognized by several other morphological characters (see text).



variable	1. PC	2. PC	3. PC
hw	0.064	-0.372	0.193
awp	0.082	-0.006	0.168
mwp	0.162	-0.047	<b>0.569</b>
cwp	0.090	<b>0.469</b>	-0.039
bwp	0.077	<b>0.451</b>	-0.051
mlp	0.101	<b>-0.501</b>	0.145
lha	<b>-0.945</b>	0.003	0.012
hwe	0.137	0.322	0.006
ew	0.118	-0.034	-0.298
el	0.114	-0.285	<b>-0.705</b>

Tab. 3: Coefficients of principal components 1–3. Values over 0.4 are in bold. In the first component lha has by far the strongest influence, meaning that most of the variation in this component is due to this variable alone. In the second component cwp and bwp contrast with a high negative value in mlp. A specimen with high values for the second component thus has high values in cwp (and bwp) but low values for mlp. Hence, this axis reveals differences in shape. The same goes for the third component where mwp contrasts with el.

## Molecular data

A distance matrix with the percentage of base mismatches among pairs of species is given in Tab. 4. *N. lariollei* from the Pyrenees exhibits the most distant sequence, differing by 6.2–7.2% from the *Nebriola* species originating from the Alps. The sequences of *N. gosteliae* HUBER sp. nov. are about equally distant to all the other Alpine *Nebriola* sequences, thereby confirming the status of the new taxon as representing a phylogenetically sundered entity.

Phylogenetic analysis of shared base characters failed to give conclusive results as to clades or lineages among the *Nebriola* species examined here nor as to the sister clade of *N. gosteliae* HUBER sp. nov.

Shared transversions for instance are positions in purine bases (A and G) that are shared by at least two sequences that in another set of at least two sequences are substituted for pyrimidine bases (C and T). As the most unlikely spontaneous genetic mutation, the pyrimidine-purine transversion is thought to reflect distinct lines of descent best because of low frequency of occurrence. Transition of bases, that is interchange of a purine or pyrimidine, is thought to be a more frequent event. In all the 1303 base positions examined here, 17 transversions and 24 transitions could be found. Tab. 5 shows the number of transversions and transitions *N. gosteliae* HUBER sp. nov. shares with individual species of related *Nebriola*.



	<i>N. gosteliae</i>	<i>N. laticollis</i>	<i>N. pictiventris</i>	<i>N. morula</i>	<i>N. fontinalis</i>	<i>N. lariollei</i>
<i>N. gosteliae</i>	0					
<i>N. laticollis</i>	3.8	0				
<i>N. pictiventris</i>	3.5	2.8	0			
<i>N. morula</i>	3.5	2.6	3	0		
<i>N. fontinalis</i>	3.3	3.5	3.2	3.6	0	
<i>N. lariollei</i>	7.0	7.1	6.6	7.2	6.2	0

**Tab. 4: Uncorrected sequence distance (uncorrected "p"-distance or observed divergence) matrix of the *Nebriola* species studied. The plain percent difference in positions of two respective sequences is given.**

<i>N. gosteliae</i> versus	TV (15)	TI (28)
<i>N. laticollis</i>	6	11
<i>N. pictiventris</i>	5	17
<i>N. morlua</i>	7	15
<i>N. fontinalis</i>	9	14
<i>N. lariollei</i>	7	10

**Tab. 5: *Nebria gosteliae* HUBER sp. nov. compared to other *Nebriola* species. The number of shared transversions (TV) and transitions (TI) are shown in columns. The total of phylogenetically relevant transversions were 15 and of transitions 28. Phylogenetically relevant base changes are shared at least among two sequences.**

Sequences of *N. praegensis* HUBER & MOLENDI, 2004, *N. fontinalis* ssp. *rhaetica* and of various subspecies of *N. cordicollis* CHAUDOIR, 1837, being quite similar to each other and to the ones of *N. fontinalis* s. str. (up to 2% difference at the most), were also compared to the sequences presented here. These results are not shown (and the respective sequences are unpublished as of yet), because no additional phylogenetic information on the subgenus *Nebriola* could be drawn from them.

## Key

(based on Ledoux & Roux (2005) with changes, subspecies not considered)

- 1 Elytra short, broad-oval, evenly rounded..... 2
- Elytra elongate, long-oval or drop shaped (widest behind the middle)..... 6
- 2 Pronotum faintly narrowed to the base; base almost as wide as the anterior margin between the anterior angles. Species of the Pyrenees. ....  
..... ***lariollei* LE BÈGUE DE GERMINY, 1865**
- Pronotum distinctly narrowed to the base. Species of the Alps. .... 3
- 3 Sterna 4–6 paramedially unisetose. Western Alps.... ***laticollis* DEJEAN, 1826**
- Sterna 4–6 each with 2–3 paramedial setae. .... 4
- 4 Pronotum transverse, to the base deeply concave and the base distinctly narrower than the distance between the anterior angles. Maritime Alps, Ligurian Alps. .... ***morula* K. & J. DANIEL, 1891**
- Pronotum less transverse, distinctly narrowed to the base, but not deeply concave. .... 5
- 5 Posterior angles of the pronotum laterally short, often converging. Humeri of the elytra rounded. Antennae brownish, at most the 1<sup>st</sup> antennomere darkened. Savoy, Dauphiné, Isère, Maritime Alps. ....  
..... ***pictiventris* FAUVEL, 1888**
- Posterior angles of the pronotum laterally conspicuously long, parallel. Humeri of the elytra oblate. Antennae brownish, but the antennomeres 1–4 darkened or even black. Endemic species of the Biellese Alps. ....  
..... ***gosteliae* HUBER sp. nov.**
- 6 Labial palpi long and slender. Base of the metacoxa unisetose (occasionally bisetose or asymmetrically bisetose in the nominal form of the Orobian Alps North of Bergamo). Everted internal sac of the aedeagus with a row of short setae. Orobian Alps, Central Swiss Alps. .... ***fontinalis* K. & J. DANIEL, 1890**
- Labial palpi short. Base of the metacoxa with 2–4 setae (exceptionally unisetose in the ssp. *ticinensis* BÄNNINGER, 1951). Everted internal sac of the aedeagus with a tuft of long setae. .... 7
- 7 Antennomere 5 shorter or at most as long as antennomere 3. Swiss Alps, Swiss Jura. .... ***cordicollis* CHAUDOIR, 1837**
- Antennae very slender, antennomere 5 distinctly longer than antennomere 3. Endemic species of the Black Forest near Präg.....  
..... ***praegensis* HUBER & MOLEND, 2004**

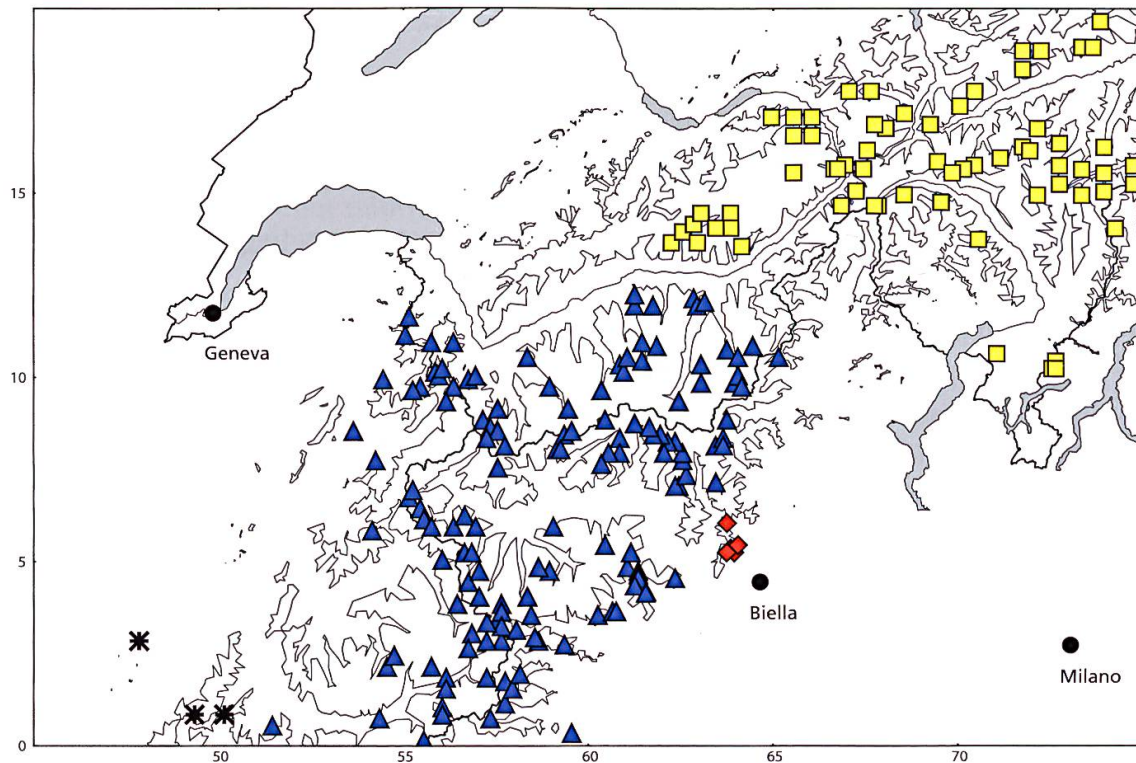


Fig. 7: Distribution map of *Nebria gosteliae* HUBER sp. nov. (red diamonds), *N. laticollis* ssp. *pennina* (blue triangles), *N. fontinalis* ssp. *rhaetica* (yellow squares) and *N. pictiventris* (asterisks). Mountains higher than 2000 m above sea level are in light grey. Axes: Swiss grid in kilometres. After Bisio (1998), Coulon & al. (2000) and Luka & al. (2009).

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