

# DNA barcoding of the potter wasp genus *Eumenes* Latreille, 1802 (Hymenoptera : Vespidae : Eumeninae) in Switzerland

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## DNA barcoding of the potter wasp genus *Eumenes* Latreille, 1802 (Hymenoptera: Vespidae: Eumeninae) in Switzerland

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We sequenced the mitochondrial marker COX1 for all eight species (*E. coarctatus* (Linnaeus, 1758), *E. coronatus* (Panzer, 1799), *E. mediterraneus* Kriechbaumer, 1879, *E. papillarius* (Christ, 1791), *E. pedunculatus* (Panzer, 1799), *E. pomiformis* (Fabricius, 1781), *E. sareptanus* André, 1884 and *E. subpomiformis* Blüthgen, 1938) of *Eumenes* Latreille, 1802 occurring in Switzerland. We examined 37 individuals, 18 of them belonging to the very similar species *E. coarctatus* (11) and *E. pedunculatus* (7). In the morphologically variable *E. coarctatus*, we examined the status of two previously recognized morphological forms, corresponding to the former subspecies *coarctatus* (7) and *lunulatus* (4). The genetic analyses are congruent with current species delimitation and reveal a clear barcoding gap: the smallest interspecific distance was more than three times larger than the largest intraspecific distance. All species represented by more than one individual formed a monophyletic group. Within *coarctatus*, the two morphs did not form monophyletic groups in our genetic analyses. Rather, geographically close populations, which partially included both morphs, were genetically more similar than distantly located populations. In light of the genetic results, we evaluated diagnostic characters for the separation of all eight species. Moreover, we examined the available types for all these species. An important open question in the taxonomy of Swiss *Eumenes* is the status of the two closely related taxa *E. sareptanus* and *E. dubius* de Saussure, 1852.

Keywords: COX1, DNA barcoding, Heath potter wasp, Switzerland

### INTRODUCTION

Potter wasps (Hymenoptera: Vespidae: Eumeninae) of the genus *Eumenes* Latreille, 1802 are solitary wasps occurring in all zoogeographical regions except the Pacific (van der Vecht & Carpenter 1990: 22). In southern and central Europe they are rather common. Their Greek scientific name (*Eumenes*) means «benevolent» and hints at the fact that they are harmless even if they may look dangerous to some people. Using mud mixed with salivary secretions the females of European species build urn-shaped cells for their larvae, which are provided with paralyzed lepidopteran larvae (Blüthgen 1961: 202; Witt 2009: 236). Of the twelve species of the potter wasp genus *Eumenes* known in Europe (Gusenleitner 1999), eight are supposed to occur in Switzerland (Neumeyer 2014): *Eumenes coarctatus* (Linnaeus, 1758), *E. coronatus* (Panzer, 1799), *E. mediterraneus* Kriechbaumer, 1879, *E. papillarius* (Christ, 1791), *E. pedunculatus* (Panzer, 1799), *E. pomiformis* (Fabricius, 1781), *E. sareptanus* André, 1884, and *E. subpomiformis* Blüthgen, 1938. *Eumenes* species are mainly identified by diagnostic features such as pilosity and integument color (Gusenleitner 1999). Both of these morphological traits are variable within vespid wasps; in particular, temperature and humidity have been shown to have an important influence on phenotypes (MacLean *et al.* 1978).

Tab. 1. Identity numbers and locality information for individuals used in this study. Abbreviations of cantons are explained in Fig. 1.

name	sex	Specimen ID number		date	canton	municipality	site	coordinates						height	leg.	coll.
		RN	GBIFCH					deg	min	sec	deg	min	sec			
genus	species			[dd.mm.yyyy]				deg	min	sec	deg	min	sec	[m]		
Delta	♂	RN0302	GBIFCH0055913	14.06.2012	AG	Möniken-Wildegg	Möniken	47	24	47.0	08	10	56.3	385	Rainer Neumeyer	Rainer Neumeyer
Delta	♂	RN0341	GBIFCH0054895	17.07.2013	TI	Losone	Gerre	46	10	52.7	08	44	33.7	230	Rainer Neumeyer	Rainer Neumeyer
Katamenes	arbusculum	RN0421	GBIFCH0057507	13.06.2013	VS	Savièse	Le Ferradze	46	14	29.4	07	19	36.5	760	Sonia Gerber	Sonia Gerber
Katamenes	arbustum	RN0427	GBIFCH0057694	02.07.2013	VS	Betten	Zwischen-Achtu	46	22	45.1	08	04	37.8	1310	Sonia Gerber	Sonia Gerber
Eumenes	coarctatus	RN0262	GBIFCH0055898	14.06.2012	TG	Neunforn	Farhof: Gile	47	35	40.2	08	45	47.7	375	Holger Martz	ETHZ
Eumenes	coarctatus	RN0260	GBIFCH0055919	15.06.2012	SH	Neunkirch	Widen	47	41	53.8	08	30	45.8	450	Andreas Müller	ETHZ
Eumenes	coarctatus	RN0264	GBIFCH0055899	28.05.2012	TG	Neunforn	Farhof	47	35	40.3	08	45	35.8	375	Holger Martz	ETHZ
Eumenes	coarctatus	RN0237	GBIFCH0055923	14.06.2012	TG	Neunforn	Farhof: Gile	47	35	40.2	08	45	47.7	375	Holger Martz	ETHZ
Eumenes	coarctatus	RN0245	GBIFCH0055914	15.08.2012	AG	Möniken-Wildegg	Roosmatt	47	24	38.9	08	10	56.2	370	Rainer Neumeyer	Rainer Neumeyer
Eumenes	coarctatus	RN0273	GBIFCH0055776	08.08.2012	VS	Sierre	Finges: Compensation A9	46	17	13.2	07	33	10.0	530	Sonia Gerber	Sonia Gerber
Eumenes	coarctatus	RN0271	GBIFCH0055789	28.05.2012	VS	Leuk	Finges: Compensation A9	46	18	24.7	07	37	33.4	650	Sonia Gerber	Sonia Gerber
Eumenes	coarctatus	RN0315	GBIFCH0055904	22.08.2011	VS	Leuk	Brenfjong: bei Satellitenstation	46	19	06.7	07	38	36.6	919	Rainer Neumeyer	Rainer Neumeyer
Eumenes	coarctatus	RN0425	GBIFCH0057674	01.07.2013	VS	Hohlehn	Ladu	46	19	27.4	07	46	16.9	1175	Sonia Gerber	Sonia Gerber
Eumenes	coarctatus	RN0424	GBIFCH0057456	13.06.2013	VS	Savièse	Chervignine	46	14	53.7	07	19	36.5	585	Sonia Gerber	Sonia Gerber
Eumenes	coarctatus	RN0307	GBIFCH0055905	11.08.2013	VS	Leuk	Susten: Pflanzwald	46	18	44.6	07	36	34.1	570	Sonia Gerber	Sonia Gerber
Eumenes	coronatus	RN0269	GBIFCH0058189	09.07.2012	VS	Leuk	Pflanz (612/128)	46	18	32.1	07	36	20.8	570	Sonia Gerber	Sonia Gerber
Eumenes	coronatus	RN0299	GBIFCH0055900	09.08.2012	TI	Malvaglia	Bolla	46	23	02.6	08	58	39.0	355	Rainer Neumeyer	ETHZ
Eumenes	coronatus	RN0263	GBIFCH0055922	16.06.2012	TG	Neunforn	Farhof: Schärffäuli	47	35	34.4	08	45	59.6	372	Mike Herrmann	ETHZ
Eumenes	coronatus	RN0304	GBIFCH0055902	10.07.2012	AG	Müthlau	Unter Schoren	47	14	33.8	08	24	28.0	387	Rainer Neumeyer	ETHZ
Eumenes	mediterraneus	RN0416	GBIFCH0063996	02.09.2004	VS	Sion	Valère	46	14	04.8	07	22	02.4	593	Paul Marchesi	MHNN
Eumenes	papillarius	RN0259	GBIFCH0055920	09.07.2012	TG	Neunforn	Farhof	47	35	40.5	08	45	23.8	380	Holger Martz	ETHZ
Eumenes	papillarius	RN0301	GBIFCH0055901	09.08.2012	TI	Malvaglia	Bolla	46	23	10.7	08	58	39.2	355	Rainer Neumeyer	ETHZ
Eumenes	papillarius	RN0316	GBIFCH0055906	04.07.2012	ZH	Bauma	Fischbach: Joggelbüel	47	23	00.7	08	50	48.3	660	Rainer Neumeyer	Rainer Neumeyer
Eumenes	papillarius	RN0442	GBIFCH0051811	12.08.2013	AG	Eiken	Wuerhüsli	47	32	24.1	07	59	29.4	311	Mare Halder	ETHZ
Eumenes	pedunculatus	RN0317	GBIFCH0055907	13.05.2011	ZH	Weisach	Mühle unt.	47	33	14.7	08	26	42.3	425	Rainer Neumeyer	Rainer Neumeyer
Eumenes	pedunculatus	RN0443	GBIFCH0053598	10.08.2013	GR	Rothenbrunnen	Mühle	46	45	49.9	09	26	00.7	680	Mike Herrmann	ETHZ
Eumenes	pedunculatus	RN0422	GBIFCH0055918	18.06.2013	GR	Samedan	Ariefa	46	31	50.1	09	51	42.3	1780	Hansueli Tinner	BNM
Eumenes	pedunculatus	RN0303	GBIFCH0055903	20.08.2012	AG	Müthlau	Schachen	47	14	09.6	08	24	15.7	389	Rainer Neumeyer	ETHZ
Eumenes	pedunculatus	RN0281	GBIFCH0055921	24.07.2012	TG	Neunforn	Farhof: Becke	47	35	56.4	08	45	48.1	445	Mike Herrmann	ETHZ
Eumenes	pedunculatus	RN0429	GBIFCH0055915	28.08.2012	AG	Bözen	Hessenberg	47	29	53.3	08	06	00.8	495	Georg Artmann	Georg Artmann
Eumenes	pedunculatus	RN0430	GBIFCH0055916	14.08.2009	BE	Schwarzhäuser	Kiesgrube Wynau	47	15	19.8	07	46	49.7	416	Georg Artmann	Georg Artmann
Eumenes	poriformis	RN0342	GBIFCH0054894	16.07.2013	TI	Meride	zona est	45	53	24.0	08	57	25.1	565	Rainer Neumeyer	Rainer Neumeyer
Eumenes	poriformis	RN0312	GBIFCH0055908	21.09.2011	VS	Sion	Tous tes Saints	46	14	07.8	07	21	52.9	570	Rainer Neumeyer	Rainer Neumeyer
Eumenes	poriformis	RN0270	GBIFCH0058190	09.08.2012	VS	Leuk	Pflanz (612/128)	46	18	32.1	07	35	57.4	565	Sonia Gerber	Sonia Gerber
Eumenes	poriformis	RN0308	GBIFCH0055909	10.08.2013	VS	Gampel	Jeizibärg	46	19	17.9	07	44	06.7	1155	Rainer Neumeyer	Rainer Neumeyer
Eumenes	sarepilanus	RN0428	GBIFCH0055917	31.05.2009	BS	Basel	Badischer Bahnhof	47	34	42.0	07	36	00.9	260	Georg Artmann	Georg Artmann
Eumenes	subpomiformis	RN0305	GBIFCH0055910	10.08.2013	VS	Gampel	Jeizinen: Underi Matte	46	19	31.2	07	43	20.0	1450	Rainer Neumeyer	Rainer Neumeyer
Eumenes	subpomiformis	RN0274	GBIFCH0058188	29.05.2012	VS	Vex	L'Aiore	46	12	36.3	07	24	40.0	740	Sonia Gerber	Sonia Gerber
Eumenes	subpomiformis	RN0418	GBIFCH0055467	02.07.2010	VS	Gampel	Jeizinen	46	19	38.4	07	43	18.2	1525	Paul Marchesi	MHNN
Eumenes	subpomiformis	RN0309	GBIFCH0055911	10.08.2013	VS	Gampel	Jeizibärg	46	19	12.0	07	43	49.4	1045	Rainer Neumeyer	Rainer Neumeyer
Eumenes	subpomiformis	RN0310	GBIFCH0055912	10.08.2013	VS	Gampel	Jeizibärg	46	19	12.0	07	43	49.4	1045	Rainer Neumeyer	Rainer Neumeyer

Particularly problematic is the highly variable species *Eumenes coarctatus*. In Switzerland and elsewhere, two taxa (*coarctatus*, *lunulatus*) were long considered to be two distinct species, until Gusenleitner (1998: 160) declared them as subspecies. Recently, Castro & Sanza (2009) suggested that even the subspecies rank was not appropriate for these two taxa and thus the taxon *E. lunulatus* Fabricius, 1804 is currently regarded as a synonym (Castro & Sanza 2009: 265) of *coarctatus*. We further examine this problem with the help of DNA barcodes. In particular, we explore how many species are hidden under the name *coarctatus* and how many species of *Eumenes* actually occur in Switzerland. We also use specimens identified using molecular barcodes to re-examine the morphology of all *Eumenes* species present in Switzerland.

#### MATERIAL AND METHODS

Thirty-seven ingroup specimens of the genus *Eumenes* were included in the molecular analysis, representing eight presumed species (Tab. 1). The focus was on the eleven specimens of the Heath potter wasp (*Eumenes coarctatus*), seven of them belonging to the morph «*coarctatus*» (identical to the former subspecies *Eumenes coarctatus coarctatus*), four to the morph «*lunulatus*» (identical to the former subspecies *Eumenes coarctatus lunulatus*). In addition, two specimens each of *Delta unguiculatum* (Villers, 1789) and *Katamenes arbustorum* (Panzer, 1799) were used as outgroup taxa to root the tree. Most specimens were collected in 80 % ethanol in the field, but we also included some specimens that were killed with ethyl acetate. DNA was extracted from one single leg to preserve a nearly intact specimen. All specimens were collected in Switzerland. All DNA extractions are deposited in the DNA bank of the Swiss Barcode of Life initiative (Swissbol; [www.swissbol.ch](http://www.swissbol.ch)), and all sequences are deposited on BOLD ([www.boldsystems.org](http://www.boldsystems.org)).

Full lab protocols can be found in Praz *et al.* (2008) and Neumeyer *et al.* (2014). DNA was isolated using the Nucleospin Tissue 96 kit; PCR reactions were performed with GoTaq Polymerase (Promega) in a Biometra T1 thermocycler. PCR products were purified enzymatically using a mix of the enzymes Exonuclease I (Fermentas) and FastAP Thermosensitive Alkaline Phosphatase (Fermentas) and sequenced in both directions with the primers used in the original amplification using BigDye terminator technology (Applied Biosystems). Big Dye products were purified with Sephadex (GE Healthcare Life Sciences) and analyzed on a ABI-3130 DNA sequencer at the Genetic Diversity Center, ETH Zürich

As a marker we sequenced the 658 bp fragment of the mitochondrial gene cytochrome oxidase I (COX1), used as a universal barcode (Hebert *et al.* 2003). As primers we used LepF and LepR (Hebert *et al.* 2004) with the following conditions: an initial denaturation of 1 min at 94 °C, then six cycles of 1 min at 94 °C, 1.5 min at 45 °C, and 1.25 min at 72 °C, followed by 36 cycles of 1 min at 94 °C, 1.5 min at 51 °C, and 1.25 min at 72 °C, with a final step of 5 min at 72 °C. Chromatograms were edited in Geneious R6 (Kearse *et al.* 2012); the nucleotide matrix was converted into amino acid to ensure that no stop codon was found. We used maximum likelihood analyses in RAxML 7.2.8 (Stamatakis *et al.* 2005) to reconstruct phylogenetic trees, separating the dataset in three partitions corresponding to the three codon positions and applying a GTR + G model to each partition. Bootstrap support values were calculated based on 1000 bootstrap replicates. Trees were rooted

Tab. 2. Abbreviations of depositories (museums and private collections) and other institutions mentioned.

abbreviation	full name
museums and other institutions	
AM	American Museum of Natural History, New York, USA
BNM	Bündner Naturmuseum, Chur
ETHZ	Eidgenössische Technische Hochschule, Zürich
ILNB	Institut für Landschaftsökologie und Naturschutz in Bühl, Germany
LSL	Linnean Society of London, GB
MCHNS	Musée cantonal d'histoire naturelle, Sion
MFNB	Museum für Naturkunde, Berlin, Germany
MHNS	Muséum d'histoire naturelle de Neuchâtel
MNHN	Muséum national d'histoire naturelle, Paris, France
MZL	Musée cantonal de zoologie, Lausanne
NHML	Natural History Museum, London, GB
NMBE	Naturhistorisches Museum der Burgergemeinde Bern
ZMUC	Zoologisk Museum, Københavns Universitet, Denmark
ZSM	Zoologische Staatssammlung München, Germany
private collections	
GA	Georg Artmann-Graf, Olten (SO)
RN	Rainer Neumeyer, Zürich
SG	Sonja Gerber, Sion

with the sequences of *Delta unguiculatum*. Genetic distances were calculated in Paup 4.0 (Swofford 2002), applying a GTR model of sequence evolution.

For taxonomy and classification we followed Gusenleitner (1999) with updates by Castro & Sanza (2009). Abbreviations used for specimen depositories and other institutions or private collections cited in this study are given in Tab. 2. We also studied the available type material and read the original descriptions (André 1884, Blüthgen 1938, Christ 1791, Fabricius 1781, 1804; Kriechbaumer 1879, Linnaeus 1758, Müller 1923, Panzer 1799) of the species and (former) subspecies of *Eumenes* occurring in Switzerland. Stack-photographs of mounted specimens were taken with a Keyence VHX-2000 digital microscope at the NMBE.

## RESULTS

### *Barcoding*

We obtained high quality sequences of the mitochondrial gene COX1 for 37 individuals of *Eumenes*, two individuals of *Katamenes arbustorum* and two of *Delta unguiculatum* (Fig. 1). All sequences are 658 bp long, except one (RN269, 636 bp). Interspecific genetic distances within the genus *Eumenes* were comprised between 6.26 (between *E. subpomiformis* and *E. papillarius*) and 13.06 (*E. sareptanus* and *E. mediterraneus*). Intraspecific distances were comprised between 0 and 1.86: they were equal to 0.0 within *E. pedunculatus*, *E. pomiformis*, *E. papillarius*; 0.61 within *E. subpomiformis*; 0.63 within *E. coronatus*; and up to 1.86 within *E. coarctatus* sensu lato. Thus, a clear barcoding gap was observed in our dataset, as the minimal interspecific distance (6.26) was substantially larger than the maximal intraspecific distance (1.86).

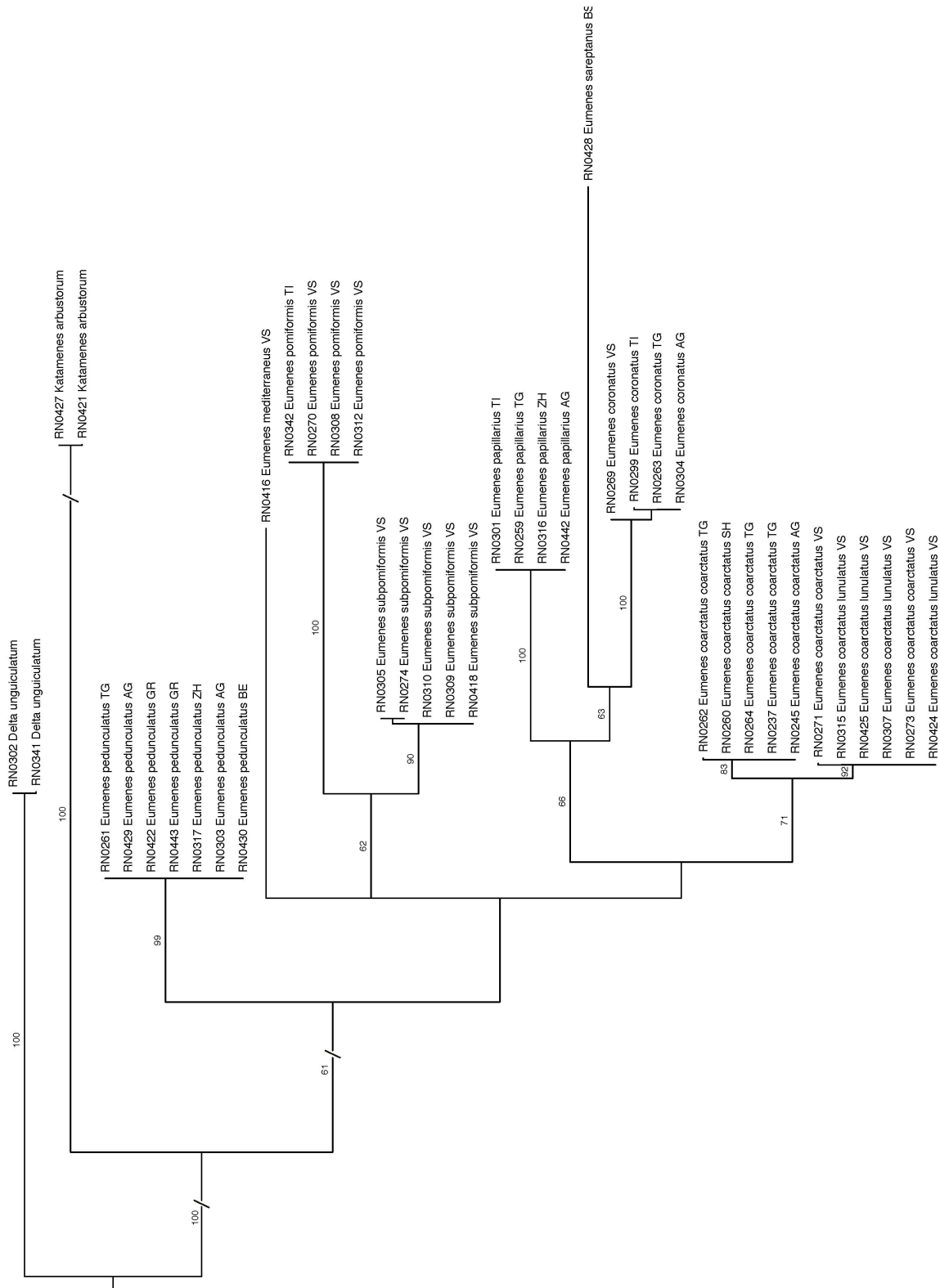


Fig. 1. Phylogenetic tree based on maximum likelihood analyses of sequences of the mitochondrial marker cytochrome oxidase 1 (COX1). Numbers shown at nodes are bootstrap values based on 1000 bootstrap replicates performed in RAxML v.7.0.4 (only values  $\geq 50$  are shown). Branches interrupted by an oblique line have been shortened for better graphic representation. The abbreviations after the species names indicate the canton where an individual was found: AG = Argovia, BE = Berne, BS = Basel-City, GR = Grisons, SH = Schaffhausen, TG = Turgovia, TI = Ticino, VS = Valais, ZH = Zurich.

The phylogenetic analysis of COX1 (Fig. 1) reveals two clades within the taxon *Eumenes coarctatus*, but their genetic distance (up to 1.86) appears small compared to other interspecific distances; consequently, we consider it unlikely that two species should be recognized within the taxon *coarctatus* s. l.. Moreover, these two clades of *coarctatus* do not group the specimens according to the morphs «*coarctatus*» and «*lunulatus*» but rather according to the geographic origin of the specimens (Fig. 1). Indeed, the six individuals from the canton of Valais (VS) in southwestern Switzerland form one clade although two individuals (RN273, RN271) belong to the morph «*coarctatus*» and four individuals (RN315, RN425, RN424, RN307) to the morph «*lunulatus*». The other clade includes five individuals (RN262, RN260, RN264, RN237, RN245) from northern Switzerland, all of them representing the morph «*coarctatus*». Thus, our analysis gives no reason to consider the two morphs («*coarctatus*», «*lunulatus*») of *E. coarctatus* as subspecies, but rather confirms the opinion of Castro & Sanza (2009) mentioned in the introduction.

Regarding the other species, our analysis (Fig. 1) is congruent with traditional taxonomy and confirms the recognition of eight species (*coarctatus*, *coronatus*, *mediterraneus*, *papillarius*, *pedunculatus*, *pomiformis*, *sareptanus*, *subpomiformis*) of *Eumenes* occurring in Switzerland (Neumeyer 2014). Only two individuals (RN418, RN443) originally appeared in the wrong clade but a re-examination showed that they were misidentified. RN418 (*E. subpomiformis*) is a male and was confused with *E. pedunculatus* due to the unusually bright and sparsely punctate tergite 2. The hairs on the clypeus are however short, as typical for *E. subpomiformis* (Gusenleitner 1999: 566). RN443 (*E. pedunculatus*) on the other hand is a female and was misidentified as *E. coarctatus*, which is sometimes quite similar in the female sex, but a reexamination using the good diagnostic traits of Gusenleitner (1972: 81) confirmed that it was *E. pedunculatus*.

#### *Type study*

We examined the available type specimens of all Swiss species (Neumeyer 2014) to verify their identities. In particular, all type specimens were examined, except for those destroyed or lost.

#### ***Eumenes coarctatus* (Linnaeus, 1758)**

*Vespa coarctata* Linnaeus, 1758: 573; ♀ (LINN 2796: <http://linnean-online.org/16751/>, LINN 2797: <http://linnean-online.org/16752/>), holotype; Europa; LSL coll.

*Eumenes lunulata* Fabricius, 1804: 290; ♀, holotype (lectotype designation in Gusenleitner 1970 unnecessary, given that no other types are known according to van der Vecht & Fischer 1972: 129); Österreich [Austria]; ZMUC coll. (Sammlung Kiel).

The Linnean type is broken into two pieces (LINN 2796, LINN 2797) and is not available for loan, but we examined it at NHML. The clypeus is entirely black (<http://linnean-online.org/16752/>) and the second tergite is very bright with only a few, widely spaced points (<http://linnean-online.org/16751/>). Such a morph in *E. coarctatus* is unknown from Switzerland, where all specimens have a large yellow spot at the base of the clypeus (Fig. 2) and more closely spaced punctuation on the second tergite, which is also less bright (Fig. 3). On the other hand, the Linnean type has no erect hairs on the second sternite (<http://linnean-online.org/16751/>), excluding any confusion with *E. coronatus*. Moreover, the interantennal yellow spot



Fig. 2. Frontal view of the head of a female of *Eumenes coarctatus* (individual RN0262) (morph «*coarctatus*») from Neunforn (canton of Turgovia) in northern Switzerland.



Fig. 3. Dorsal view of the tergite 2 of a female of *Eumenes coarctatus* (individual RN0262) (morph «*coarctatus*») from Neunforn (canton of Turgovia) in northern Switzerland.





Fig. 4. Dorsal view of the gaster of the female holotype of *Eumenes lunulata* Fabricius, 1804 from Austria (ZMUC coll.).



Fig. 5. Dorsal view of the tergite 2 of a female of *Eumenes coarctatus* (individual RN0306) (morph «*lunulatus*») from Leuk in the canton of Valais.

does not reach the clypeus (<http://linnean-online.org/16752/>), making confusion with *E. pedunculatus* unlikely. Hence, the Linnean type certainly represents what is currently understood as *E. coarctatus* but probably represents a northern European morph.

The type of *Eumenes lunulata* Fabricius is a badly preserved specimen with the head missing. However, the second tergite is densely punctate (Fig. 4), matching our morph «*lunulatus*». (Fig. 5).

### ***Eumenes coronatus* (Panzer, 1799)**

*Vespa coronata* Panzer, 1799: 64, 12; ♂, type destroyed (van der Vecht & Fischer 1972: 126); «Habitat Norimbergae in floribus. Dn. [Dominus] Sturm.» [Lives in flowers of Nuremberg. Mr Sturm].

*Eumenes atricornis* Fabricius, 1804: 289; holotype; Österreich [Austria]; ZMUC coll. (Sammlung Kiel).

The locality (Nuremberg = Nürnberg) of the Panzer type is the well known city in Middle Franconia of Bavaria (Germany).

Since the extremity of the gaster and head are missing, it is hard to conclude whether the Fabricius type is a female as stated by Blüthgen (1961: 205) or a male, as written on the label. In any case it has erect hairs on the second sternite, which is diagnostic for *E. coronatus* (Gusenleitner 1999: 566).

### ***Eumenes dubius* de Saussure, 1852**

*Eumenes dubia* de Saussure, 1852; 3 ♀♀, 1 ♂, cotypes; «Le midi de la France» [«The south of France»]; MNHN coll.

This species is not thought to occur in Switzerland (Neumeyer 2014). However, in contrast to Gusenleitner (1972, 1999, 2008), Castro (1992: 30 ff.) considers *Eumenes sareptanus* André, 1884 as a synonym of *E. dubius*. The examined cotypes of *E. dubius* are very bright (e.g. with clypeus entirely yellow); their pilosity is overall very short on the entire body and the ventral side of the flagellum is yellow, also in the female sex. Additionally, the terminal lamella of the second tergite is transparent as in *E. mediterraneus*. In contrast, all Swiss specimens of *E. sareptanus* are less bright (e.g. with clypeus partially black) and with longer pubescence on both vertex and mesoscutum. Moreover, the flagellum is almost entirely black, even in the males, and the second tergite always has an opaque terminal lamella. Based on these morphological differences, we consider the taxa *E. dubius* and *E. sareptanus* as distinct species for now, the former being absent from Switzerland.

### ***Eumenes mediterraneus* Kriechbaumer, 1879**

*Eumenis* [sic!] *mediterranea* Kriechbaumer, 1879; ♂, lectotype (designated in Blüthgen 1943: 301); Dalmatien [Dalmatia]; ZSM coll.

The lectotype of this distinctive species, unlikely to be confused with any other Swiss *Eumenes*, is in good condition.

### ***Eumenes papillarius* (Christ, 1791)**

*Sphex papillaria* Christ, 1791; ♂, type destroyed (Blüthgen 1961: 206); no type locality mentioned, but Blüthgen (1961: 206) mentions «Kronberg (Taunus) [Germany]».

Christ (1791: 325–326 + Tab. 32) describes several traits but none of them can be considered as diagnostic. Neither the characteristic long hairs on the second tergite nor the striking dark longitudinal keel on the last male flagellomere are mentioned. Even the figure shown (Christ 1791: Pl. 32, Fig. 10) does not permit the identification of any European species of *Eumenes*. Therefore we encourage the designation of a neotype for *E. papillarius* by using a sequenced specimen from the Taunus mountains (State of Hesse, Germany).

### ***Eumenes pedunculatus* (Panzer, 1799)**

*Vespa pedunculata* Panzer, 1799: 63, 8; ♀, type destroyed (van der Vecht 1968: 68); «Habitat et apud nos in hortis, minus frequens. Dn. [Dominus] Iurine.» [Lives also in our area in gardens, infrequent. Mr Jurine].

♀, neotype, designated in van der Vecht (1968: 72); Ferreyres, [canton of] Vaud, Switzerland; 22 Aug 1962, de Beaumont leg.; MZL coll.

No type locality is explicitly mentioned in Panzer (1799: 63, 8) for *Vespa pedunculata*, but «Dn. Iurine» probably refers to the Genevan naturalist Louis Jurine (1751–1819) and not to a Swiss locality as presumed by Gusenleitner (1972: 81). So, given that «apud nos» means «in our area» and therefore the Genevan basin, the locality of Ferreyres (60 km NE of Geneva) is certainly an appropriate locality for the neotype selected by van der Vecht (1968).

The neotype has an interantennal yellow spot reaching the clypeus, which has a large yellow spot at the base. Moreover, the points on the second tergite are distinctly smaller and more wide-spaced than in Swiss female specimens of *E. coarctatus*, but not more so than on the second tergite of the Linnean type of *E. coarctatus*. However, while the second tergite is very bright in the Linnean type of *coarctatus*, it has a milky shininess in the neotype of *pedunculatus*, a character common in *pedunculatus* but unusual in *coarctatus*. All in all, we can exclude any confusion with the otherwise very similar *E. coarctatus*.

### ***Eumenes pomiformis* (Fabricius, 1781)**

*Vespa pomiformis* Fabricius, 1781: 467–468; ♀, lectotype (designated in Blüthgen 1938); Italia [Italy]; ZMUC coll. (Sammlung Kiel).

Like the other mentioned types of Fabricius this lectotype is in bad condition, probably due to damage by *Anthrenus* beetles. However, the characteristic short hairs on the propleurum are still clearly visible.

### ***Eumenes sareptanus* André, 1884:**

♀, lectotype, designated in Gusenleitner (2001: 214); Sarepta [Volgograd Oblast, Russia]; MHNN coll.

*Eumenes pomiformis* forma *insolata* Müller, 1923; ♀, ♂, types missing, not in the MFNB; Mark Brandenburg [Mark of Brandenburg], Germany.

*Eumenes dubius sareptanus* var. *germanica* Blüthgen, 1938: 469; ♀, holotype; Wasenweiler (Kaiserstuhl) in Germany. MFNB coll.

The lectotype in the MHNN is to some extent covered by a silky webbing, possibly mycelium, but its surface is still visible. In any case, it matches the original description (André 1884: 638) fairly well. However, there are three distinct deviations. First, the hairs on head and mesosoma are white instead of bright orange [roux]. Second, the flagella are not just «black» [noires], but partially black to

orange ventrally, especially on the first pairs of flagellomeres. Third, the punctuation of the second tergite is not «very fine» [très finement], but rather strong, as described for *Eumenes pomiformis* forma *insolata* by Müller (1923: 624). This morph [forma], considered as a subspecies (*E. sareptanus insolatus* Müller, 1923) since Gusenleitner (1972), differs from the nominate form (*sareptanus* André, 1884) by the golden yellow instead of sulfur-yellow, almost whitish integumental maculations (Gusenleitner 1972: 109) and by the female legs, which are not only red, but also partially yellow (Gusenleitner 1972: 109) and even black (Müller 1923: 627).

### *Eumenes subpomiformis* Blüthgen, 1938

♀, holotype; Austria, «O. Tirol» [East Tyrol], Huben, 850–1000 m; 27. Jul 1930, S.G. Bischoff leg; MFNB coll.

The holotype is in good condition and is identical to typical females of this species from Switzerland. However, the type locality, interpreted as «Huben» (Blüthgen 1938), «Huben (Tirol)» (Gusenleitner 1972: 101) or «Huben in Tirol» (Gusenleitner 1999: 574), remains ambiguous as several localities with this name are found in Tyrol. The indications on the holotype's label «O. Tirol: Huben 850–1000 m» (Fig. 6) allow this question to be answered. The well known Tyrolian resort Huben in the Tyrolian valley of Ötztal is apparently too high ( $\geq 1200$  m) to be the type locality. Moreover, this locality is not in East Tyrol (Osttirol), but in Western Tyrol. However, in East Tyrol where the river Schwarzach enters the river Isel there is another locality called Huben situated at 819 m, which corresponds fairly well to the altitude (850–1000 m) indicated on the label (Fig. 6). This should be considered the type locality. «O. Tirol» is more likely to be an abbreviation of Osttirol than of «Oesterreich [= Österreich] Tirol». Note that East Tyrol is a district of the Austrian federal state of Tyrol, but is geographically separated from the rest of the state.

### DISCUSSION

In accordance with Castro & Sanza (2009: 265) our data fail to show any genetic differences (Fig. 1) among the two morphs (Fig. 3, 5) of *E. coarctatus* in Switzer-



Fig. 6. Labels of the holotype of *Eumenes subpomiformis* Blüthgen, 1938 (MFNB coll.).

land. However, we can still question the morphological differences between the females from Switzerland and the Linnean holotype (LINN 2796, LINN 2797) from «Europa» (Linnaeus 1758: 573). Gusenleitner (1999: 569) regarded females of *coarctatus* with an entirely black clypeus and widely spaced points on the second tergite as typical but stressed that specimens of *coarctatus* become brighter with denser punctuation on the second tergite in populations found in more xerothermic habitats (Gusenleitner 1999: 570). Some individuals (*E. coarctatus*) from Scandinavia should also be sequenced to solve this problem. Lastly, sequencing of a nuclear marker may also be useful to know whether both clades in our COX1-tree represent two reproductively isolated populations or not.

Genetic distances correlating with geographic separation as appears to be the case within both *E. coarctatus* and *E. coronatus* (Fig. 1), are not really surprising (Podnar *et al.* 2014). Switzerland represents the meeting point of the several post-glacial recolonization routes identified in Europe (Hewitt 1999). The genetic differences seen in these two taxa may reflect distinct recolonisation patterns, e.g., from southwestern Europe through the Rhone Valley in the Valais populations and from Eastern Europe refugia in populations from Northern Switzerland. Yet, the Valais is connected to the rest of Switzerland at the shores of Lake Geneva in a region of low elevation (372 m a.s.l.). Thus, individuals from the region of Geneva and Lausanne would be interesting to sequence.

The Swiss specimens of *sareptanus* – considered to belong to the subspecies *E. sareptanus insolatus* Müller, 1923, like all European populations outside Russia (Gusenleitner 1999) – are not intermediate between *E. dubius* and *E. sareptanus sareptanus* from Southern Russia. Instead, Swiss specimens have particularly long pubescence (longer than typical *sareptanus* or *dubius*) and relatively dark flagella (darker than typical *dubius* or *sareptanus*). Furthermore, the femora of Swiss individuals are not only orange or yellow, but also partially black. Hence, there is no gradual cline of traits from Northern Africa (*dubius*) to Russia (*sareptanus*) here.

We thus reject the concept of one Afro-Russo-European species (*E. dubius* sensu Castro 1992) for now and regard *E. dubius* and *E. sareptanus* as different species, as already mentioned. We call the concept of two discrete subspecies (*sareptanus*, *insolatus*) of *E. sareptanus* into question, however, since Müller (1923: 626 ff.) also describes reared individuals from central Europe (thus belonging to the forma *insolata*) which showed exactly the same color traits as the nominate form (Gusenleitner 1972: 109).

#### ZUSAMMENFASSUNG

Von allen acht einheimischen Arten (*E. coarctatus*, (Linnaeus, 1758), *E. coronatus* (Panzer, 1799), *E. mediterraneus* Kriechbaumer, 1879, *E. papillarius* (Christ, 1791), *E. pedunculatus* (Panzer, 1799), *E. pomiformis* (Fabricius, 1781), *E. sareptanus* André, 1884 und *E. subpomiformis* Blüthgen, 1938) der Pillenwespen (*Eumenes* Latreille, 1802) sequenzierten wir den mitochondrialen Marker COX1. Insgesamt wurden 37 Ingroup-Individuen untersucht, 18 davon allein bei den einander besonders ähnlichen Arten *E. coarctatus* (11 Individuen) und *E. pedunculatus* (7). Bei *E. coarctatus* wurden dabei zwei Morphen unterschieden, die den früheren Unterarten *coarctatus* (7) und *lunulatus* (4) entsprechen. Die genetische Analyse erbrachte bei allen Arten wohldifferenzierte Kladen. Innerhalb von *E. coarctatus* gruppieren sich nicht die Individuen derselben Morphe, sondern geografische Nachbarn, unter denen sich zum Teil beide Morphen befanden. Ferner inspizierten wir von allen erwähnten Taxa die verfügbaren Typen und stiessen dabei auf Forschungsbedarf, was den Unterschied der Taxa *Eumenes sareptanus* und *E. dubius* de Saussure, 1852 betrifft.

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