First record of the wild bee *Eucera* (*Tetralonia*) *alticincta* (Lepeletier, 1841) in Luxembourg

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Abstract. On the 19th of July 2019, a regionally rare wild bee species was discovered in the context of the ongoing «Atlas of the wild bee fauna of Luxembourg» project at a dry meadow in Canach (commune of Lenningen). Morphological and genetic characters place the specimen into the *Tetralonia ruficornis*-group (Apidae, Eucerini), corresponding to a member of the species *Eucera* (*Tetralonia*) *alticincta* (Lepeletier, 1841). This finding not only adds a new species and subgenus to the list of wild bees of Luxembourg, but it also represents one of the northernmost findings of the species in Europe. Furthermore, the first European DNA barcode of the species has been generated. If this specimen is representative of an established population in Luxembourg or rather the result of an isolated finding due to natural dispersal from (unknown) neighbouring populations must be clarified by upcoming studies.

1. Introduction

Wild bees of Luxembourg

Luxembourg is home of 340 registered wild bee species in 39 different genera (Rasmont et al. 2017, Vereecken 2018, Schneider 2018). Compared to neighbouring countries, this amount is similar to the number of species registered for Belgium and the Netherlands (n=398 and 366, respectively), about one third of the species described in France (n=949) and more than half of the wild bees known from Germany (566 to 582 depending on the source) (Scheuchl & Schwenninger 2015, Rasmont et al. 2017, Vereecken 2018, Westrich 2018). In the context of the ongoing "Atlas of the wild bee fauna of Luxembourg" project, coordinated by the Musée national d'histoire naturelle de Luxembourg (MNHNL), it can be expected that further species will be added to the list. In particular, because the genetic identification performed via DNA (meta)barcoding will allow to address cryptic species, i.e. species which can not be separated using traditional morphological characters, which are known from some bee species in Central Europe (see e.g. Schmidt et al. 2015).

The long-horned bees and their taxonomic history

The commonly referred classifications of Michener (2000) split the Eucerini tribe in several genera such as *Eucera*, *Tetralonia* and *Tetraloniella*. Some of the authors here



Fig. 1. Illustration of a female *Eucera* (*Tetralonia*) *alticincta* from the *Tetralonia ruficornis*-group on Asteraceae. Drawn by F. Herrera-Mesías.

cited prefer this nomenclature to refer in their descriptions. *Tetralonia* differs from *Eucera* only in the number of submarginal cells with *Tetralonia* having three and *Eucera* two cells (Amiet et al. 2007). As a result, there was already a longer tendency to place *Tetralonia* within *Eucera* (Westrich & Dathe 1997). In the most current taxonomy revision of the *Eucera*-complex by Dorchin et al. (2018), *Tetralonia* is regarded as a subgenus of *Eucera* and *Tetraloniella* as a synonym of *Tetralonia* based on genetic and morphological data. For the species described in this article, *Tetralonia* and *Tetraloniella* are likewise considered synonyms and *Tetralonia* is regarded as a subgenus of *Eucera*, following the classification of Dorchin et al. (2018).

Bees of the subgenus *Tetralonia* are medium to large sized, with a wide abdomen (Fig. 1). As their common name 'long-horned bees' indicates, males have strikingly long antennae. The wings have three submarginal cells, from which at least the first and third are about the same size (Amiet et al. 2007). The abdomen is covered with velvety bandages of light hair and the clypeus is often brightly colored (Amiet et al. 2007). Very little is known about their biology. Species in this taxonomic group are ground nesting bees with one generation per year (univoltine), flying between early summer to autumn and some of them are hosts to the parasitic cuckoo bee species *Triepeolus tristis* Smith, 1854 (Amiet et al. 2007).

Most *Tetralonia* species seem to specialize on certain plant hosts for pollen collecting (Amiet et al. 2007). For example, the *Tetralonia ruficornis*-group (Müller 2008) consists of six closely related species in Europe (following Dorchin et al. 2018 now: *Eucera* (*Tetralonia) ruficornis* (Fabricius, 1804); *Eucera* (*T.*) *alticincta* (Lepeletier, 1841); *Eucera* (*T.*) *fulvescens* (Giraud, 1863); *Eucera* (*T.*) *inulae* (Tkalců, 1979); *Eucera* (*T.*) *iberica* (Dusmet, 1926); *Eucera* (*T.*) *julliani* (Pérez, 1879)). All species of this subgenus show a preference for the flowers of *Inula* and *Pulicaria* as pollen sources (Müller 2008, Tkalců 1979). They also share several morphological features, including specialized sternal pilosities in the form of robust hair-bands at the base of the sternites three to five,considered potential morphological adaptations for pollen harvesting (Müller 2008).

2. Material and methods

Sampling site

The single female specimen (MNHNL67132) was collected on 19th July 2019 in Canach (commune of Lenningen; WGS84 coordinates 49.6148N, 6.3245E). It was found on a south-exposed dry meadow classified as habitat type 6210 (Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia)) under the Fauna-Flora Habitats Directive (92/43/EEC). The meadow is a nature reserve of the Stiftung Hëllef fir d'Natur and frequently used as a pasture. The sampling site was visited during the pilot phase of the "Atlas of the wild bee fauna of Luxembourg" project. Specimens were actively collected and UV-yellow, UV-blue and white pan traps filled with 80% propylene glycol and soapy water deployed for three days (16.-19.07.2019).

Specimen identification

The specimen was morphologically identified using the keys of Amiet et al. (2007), Scheuchl (2000) and Tkalců (1979). Visualization of key characters was achieved using a Keyence VHX-S660E digital microscope. Additionally, the Cytochrome C Oxidase subunit 1 sequence (COI barcode) was sequenced using the Folmer primer pair (Folmer et al. 1994). DNA of the specimen was isolated from a single mid-leg, which was transferred to an individual 2 ml sterile Eppendorf tube containing a plastic bead (diameter: 3 mm) and 20 µl of ddH_2O. The tissue was homogenised by grinding at a frequency of 30 Hz for 6 minutes in a Retsch TissueLyser Mixer Mill model MM200. Overall DNA concentration was measured using a Microvolume Spectrophotometer Trinean Xpose®. DNA was isolated from the homogenised tissue using a modified salting out method for digestion (Miller et al. 1998). The PCR reaction consisted of 1x Master Mix (GoTaq® G2 Hot Start Colorless), 0.5 µM of each primer, 1 µl DNA (32.2 ng/µl) and nuclease-free H_2O to a final volume of 25 µl. The PCR started with an initial denaturation step at 95°C for 2 minutes, followed by 35 cycles of denaturation for 40 seconds at 95°C with annealing for 1 minute at 41°C and a 1 minute extension at 72°C;

finishing with an extension for 1.5 minutes at 72°C (Kharrat et al. 2015). A volume of 10 µl of PCR product was purified using the magnetic bead-based Agencourt® AMPure® XP purification system (Protocol 000601v024). Each sequencing reaction consisted of 0.5 µl BigDye®, 2 µl 5x reaction buffer, 3 µl ddH_2O, 0.5 µl of primer (5 µM; forward or reverse) and 4 µl of the purified PCR product. Traditional Sanger-Sequencing was performed in-house on an Applied Biosystems 3730xl DNA Analyzer. The resulting chromatograms were visually inspected using Geneious Prime 2019.1.1 (Kearse et al. 2012).

3. Results

Eucera (*Tetralonia*) *alticincta* (Lepeletier, 1841) from Luxembourg

Previous combinations: *Macrocera alticincta* Lepeletier, 1841; *Tetralonia alticincta* (Lepeletier, 1841); *Tetraloniella alticincta* (Lepeletier, 1841); *Eucera* (*Tetraloniella*) *alticincta* (Lepeletier, 1841).

Common names: Eucère des Pulicaires (French), Flohkraut-Langhornbiene (German).

Diagnosis (females): Medium-sized specimen (body length: 8.7 mm) presenting velvety patches of light hair in the basis of the tergites (Fig. 2c), a shiny first half of the tergite 1 (Fig. 2d), yellowish scopa at the outer side of the metatibia (Fig. 2e), antennae with a lighter coloration below (Fig. 2f) and a very distinctive yellow band in the lower half ot the clypeus (Fig. 2f & g, but see also Tkalců 1979 for images). Females can resemble *Eucera* (*T.*) *fulvescens, Eucera* (*T.*) *inulae* or *Eucera* (*T.*) *iberica,* but can be distinguished by the overall shape of the yellow band, the length of the antennal segments, the shape of the intraocular edges (convergent and not straight), the color of the bristles at the end of the tergites and the scopa (yellowish) and by the extensions of the pilosities on tergites 3 and 4 (Amiet et al. 2007, Tkalců 1979). In wet condition, the whitish velvety pilosities in our specimen were larger than the black surface area, matching the description of *Eucera* (*T.*) *alticincta,* but after drying out they have been partly contracted under the previous tergite (Fig. 2c).

DNA barcoding: The COI barcode (658 bp, BOLD-identifier MNHNL005-20) was compared to sequences stored in the Barcode of Life Data system (Ratnasingham & Hebert 2007). The molecular result supports the morphological identification with the specimen belonging to the *Tetralonia ruficornis*-group. The best percentages of sequence identity were achieved with *Eucera* (*Tetralonia*) *alticincta* (97.19%; GenBank-ID MG251111; BOLD-ID GBMIN142813-18) from Erzurum (Turkey) and with two specimens of *Eucera* (*Tetralonia*) *fulvescens* (96.60%, respectively, 96.54% with BOLD-IDs ABEE207-17 and ABEE208-17) from Sievering, Vienna (Austria) – the only two species from the group having reference sequences in the database. An intraspecific genetic distance of 2.81% (comparison of the specimen from Luxembourg with the specimen from Turkey) is within the range of genetic divergences observed for Central European wild bees (e.g. see Appendix S5 in Schmidt et al. 2015) and highlights the need for generating DNA barcodes throughout the distribution range of a species, thus to cover genetically diverse barcode variants (Bergsten et al. 2012).

Flight season: Starting from mid-July to early September (Scheuchl 2000, Amiet et al. 2007, Standfuss 2009). This agrees with the date of discovery of the Luxembourgish specimen.

Flowers visited: *Eucera* (*T.*) *alticincta* is an Asteraceae specialist. It has been observed on different species of the genus *Pulicaria* such as *Pulicaria dysenterica*, and is confirmed by pollen analysis (Westrich & Schmidt 1987, Westrich 1989) as well as visiting *Inula germanica* and *I. ensifolia* (Tkalců 1979, Amiet et al. 2007). At the sampling site and in the wider area, *Inula conyzae* and *Tanacetum vulgare* are present but no *Pulicaria* spp. is available (Helminger et al. 2019, pers. comm. Claudine Felten, natur&ëmwelt asbl.).

Conservation status: According to the European Red List of Bees, *Eucera* (*T.*) *alticincta* is placed in the IUCN category "least concern" (Nieto et al. 2014). The species is critically endangered in the Czech Republic (Farkač et al. 2005), extinct in Germany (Westrich et al. 2011), rare in Slovenia (Anonymous 2002) and possibly extinct in Switzerland (Amiet et al. 2007). The IUCN (2020) highlights that

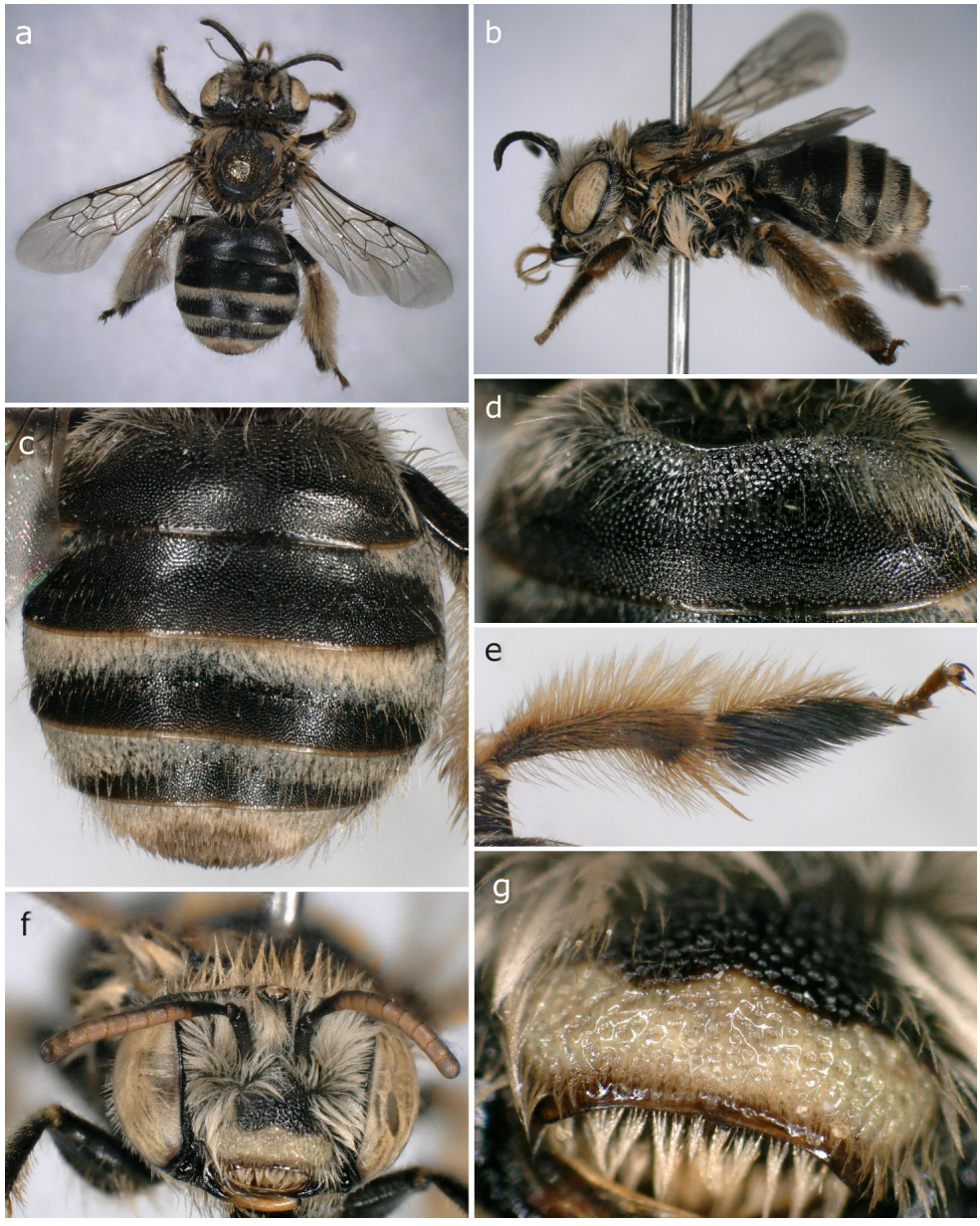


Fig. 2. *Eucera* (*Tetralonia*) *alticincta* (Lepeletier, 1841) (Canach, specimen MNHNL67132). a) Dorsal view with wings showing three submarginal cells (overall length = 8.7 mm, middle legs were used for DNA extraction); b) Lateral view; c) Abdomen, the dimensions of the pilosity patches in tergites 3 and 4 compared to the hairless black area can be observed. Due to drying conditions, the upper part of the tergites is somewhat contracted under the previous tergite; d) Detailed view of punctures and shiny interspaces in tergite 1; e) pollen-collecting hairs on the hind leg (notice coloration change in basitarsus); f) head (intraocular edges converging towards the centre, color of the antennae is a bit faded); g) clypeus with details on the form of the yellow band (slightly zigzagging, almost in-line with the rounded borders). Photos: MNHNL.

research should be performed regarding its population size and trends, habitat, distribution and ecology as well as threats.

Distribution: The species was originally described from France (type locality near Paris), but records commonly come from the Mediterranean area and Black Sea (Albania, Bulgaria, S-France, Greece, Italy, Romania, Spain, Ukraine), and only very sporadically from more Central and Eastern Europe (Austria, Czech Republic, Central France, S-Germany, Hungary, Slovakia, Switzerland) (Tkalců 1979, Westrich & Schmidt 1987, Amiet et al. 2007, Rasmont et al. 2017, IUCN 2020, Kuhlmann et al. 2020). According to the red list of Germany, it is treated as 'extinct' and has been last observed in 1926 near the southern Kaiserstuhl in Baden-Württemberg (Strohm 1925, Westrich et al. 2011, Scheuchl & Schwenninger 2015). Additional geographical records are very likely to be obtained when re-investigating older European specimens housed in museum collections referring to *Eucera* (*T.*) *ruficornis* and previous combinations thereof. A thorough revision of the actual distribution area covered by *Eucera* (*T.*) *alticincta* in and outside of Europe is more than needed, as Kuhlmann et al. (2020) refer to additional findings in Morocco, Tunisia, Russia and Turkey (the latter can be confirmed here by the specimen deposited in BOLD), Pagliano (1994) to findings in Algeria and the IUCN (2020) to the species absence in Spain and on the Balkan Peninsula.

4. Conclusions

The regionally rare wild bee species *Eucera* (*Tetralonia*) *alticincta* (Lepeletier, 1841) has been discovered in Luxembourg, representing a subgenus with no previous records in the area. At the same time, this is one of the northernmost findings for this species to date. The identification is supported by both morphological and molecular evidence and has relevant implications for understanding local wild bee biodiversity. Further sampling efforts must be conducted in order to evaluate the ecological importance of this evidence and to draw conclusions regarding the presence of this and other species of the *Tetralonia ruficornis*-group in the area. Since the specimen was still carrying pollen, a future pollen analysis might reveal whether *Pulicaria* spp. is present near the sampling site or if the female specimen collected other Asteraceae pollen (e.g. *Inula* spp.).

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References

Amiet, F., M. Herrmann, A. Müller & R. Neumeyer, 2007. Apidae 5: *Ammobates*, *Ammobatoides*, *Anthophora*, *Biastes*, *Ceratina*, *Dasypoda*, *Epeoloides*, *Epeolus*, *Eucera*, *Macropis*, *Melecta*, *Melitta*, *Nomada*, *Pasites*, *Tetralonia*, *Thyreus*, *Xylocopa* (Vol. 5). Centre suisse de cartographie de la faune. *Fauna Helvetica* 20, 356 pp.

Anonymous, 2002. Uradni list from Republic of Slovenia. Official Gazette, Minister for the Environment and Spatial Planning 56/99 and 31/00: Annex 14.

Bergsten, J., D. T. Bilton, T. Fujisawa, M. Elliott, M.T. Monaghan, M. Balke & I. Ribera, 2012. The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology* 61 (5): 851-869.

Dorchin, A., M. M. López-Uribe, C. J. Praz, T. Griswold & B. N. Danforth, 2018. Phylogeny, new generic-level classification, and historical biogeography of the *Eucera* complex (Hymenoptera: Apidae). *Molecular Phylogenetics and Evolution* 119: 81-92.

Farkač, J., D. Král & M. Škorpík, 2005. List of threatened species in Czech Republic. Invertebrates. Available at (Accessed: 30th January 2020): http://www.nationalredlist.org/site.aspx?&species=18217&pageid=116.

Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Helminger, T., G. Moes, E. Granda Alonso, F. Hans & O. Weber, 2019. Semi-dry grasslands and forests of slopes, screes and ravines in eastern Luxembourg. *Tuexenia* 12: 85-135.

IUCN, 2020. The IUCN Red List of Threatened Species. Version 2019-3. http://www.iucnredlist.org. (Accessed: 30th January 2020).

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S., ... & T. Thierer, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647-1649.

Kharrat, I. Mezghani, M. Casse, N. Denis, F. Caruso, A. Makni, H. ... & M. Makni, 2015. Characterization of mariner-like transposons of the mauritiana subfamily in seven tree aphid species. *Genetica*, 143 (1) 63-72.

Kuhlmann, M., J. S. Ascher, H. H. Dathe, A. W. Ebmer, P., Hartmann, D., Michez, A. Müller, S. Patiny, A. Pauly, C. Praz, P. Rasmont, S. Risch, E. Scheuchl, M. Schwarz, M. Terzo, P. H. Williams et al., 2020. Checklist of the Western Palaearctic bees: http://westpalbees.myspecies.info (accessed: 30th January 2020).

Michener, C. D., 2000. The bees of the world (Vol. 1). JHU press, 913 pp.

Miller, S. A., D. D. Dykes & H. F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 16 (3): 1215.

Müller, A., 2008. A specialized pollen-harvesting device in European bees of the genus *Tetraloniella* (Hymenoptera, Apidae, Eucerini). *Linzer biologische Beiträge* 40: 881-884.

Nieto, A., S. P. M. Roberts, J. Kemp, P. Rasmont, M. Kuhlmann, M. García Criado, J. C. Biesmeijer, P. Bogusch, H. H. Dathe, P. De la Rúa, T. De Meulemeester, M. Dehon, A. Dewulf, F. J. Ortiz-Sánchez, P. Lhomme, A. Pauly, S. G. Potts, C. Praz, M. Quaranta, V. G. Radchenko, E. Scheuchl, J. Smit, J. Straka, M. Terzo, B. Tomozii, J. Window & D. Michez, 2014. European Red List of bees. Luxembourg: Publication Office of the European Union.

Pagliano, G., 1994. Catalogo degli Imenotteri italiani. IV. (Apoidea: Colletidae, Andrenidae, Megachilidae, Anthophoridae, Apidae). *Memorie della Societá Entomologica Italiana* 72: 331-467.

Rasmont, P., D. Genoud, S., Gadoum, M., Aubert, E. Dufrene, G. Le Goff, G. Gilles Mahe, D. Michez & A. Pauly, 2017. Hymenoptera Apoidea Gallica: liste des abeilles sauvages de Belgique, France, Luxembourg et Suisse. Edition Atlas Hymenoptera, Université de Mons, Mons, Belgium.

Ratnasingham, S. & P. Hebert, 2007. BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes* 7: 355-364.

Scheuchl, E., 2000. Illustrierte Bestimmungstabellen der Wildbienen Deutschlands und Österreichs. Band I: Anthophoridae. 2. erweiterte Auflage. Eigenverlag, Velden, 158 pp.

Scheuchl, E., & H. R. Schwenninger, 2015. Kritisches Verzeichnis und aktuelle Checkliste der Wildbienen Deutschlands (Hymenoptera, Anthophila) sowie Anmerkungen zur Gefährdung. *Mitteilungen des Entomologischen Vereins Stuttgart* 50: 3-225.

Schmidt, S., C. Schmid-Egger, J. Morinière, G. Haszprunar & P. D. Hebert, 2015. DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea partim). *Molecular Ecology Resources* 15(4): 985-1000.

Schneider, N., 2018. Recension d'ouvrage: Découvrir & protéger nos abeilles sauvages. *Bulletin de la Société des naturalistes luxembourgeois* 120: 163-164.

Standfuss, K., 2009. Zur aktuellen Bienenfauna der Ölbaumzone in Südost-Thessalien/ Griechenland (Hymenoptera: Apoidea: Apiformes). 3. Colletidae, Melittidae, Apidae pro parte. *Entomofauna – Zeitschrift für Entomologie* 30 (12): 197-208.

Strohm, K., 1925. Insekten der badischen Fauna. I. Beitrag. *Mitteilungen der Badischen Entomologischen Vereinigung* 1 (7): 204-220.

Tkalců, B., 1979. Revision der europäischen Vertreter der Artengruppe von *Tetralonia ruficornis* (FABRICIUS) (Hymenoptera, Apoidea). *Acta Musei Moraviae* 64: 127-152.

Vereecken, N., 2018. Découvrir & protéger nos abeilles sauvages. Editions Glénat, Grenoble, 192 pp.

Westrich, P., & K. Schmidt, 1987. Pollenanalyse, ein Hilfsmittel beim Studium des Sammelverhaltens von Wildbienen (Hymenoptera, Apoidea). *Apidologie* 18 (2): 199-214.

Westrich, P., 1989. Die Wildbienen Baden-Württembergs. Volumes 1-2. Ulmer Verlag, Stuttgart, 972 pp.

Westrich, P., & H. H. Dathe, 1997. Die Bienenarten Deutschlands (Hymenoptera, Apidae). Ein aktualisiertes Verzeichnis mit kritischen Anmerkungen. *Mitteilungen des Entomologischen Vereins Stuttgart* 32: 3-34.

Westrich, P., U. Frommer, K. Mandery, H. Riemann, H. Ruhnke, C. Saure & J. Voith, 2011. Rote Liste und Gesamtartenliste der Bienen (Hymenoptera, Apidae) Deutschlands - (5. Fassung, Dezember 2011) [Red List and complete species list of bees in Germany]. Bundesamt für Naturschutz (ed.), Rote Liste der gefährdeten Tiere, Pflanzen und Pilze Deutschlands. Band 3: Wirbellose Tiere (Teil 1) [Red List of threatened animals, plants and fungi of Germany]. *Naturschutz und Biologische Vielfalt* 70 (3): 373–416.

Westrich, P., 2018. Die Wildbienen Deutschlands. Eugen Ulmer, 824 pp.