

Two new distribution records of *Aedes (Rusticoides) refiki* Medschid, 1928 (Diptera: Culicidae) from Germany

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Abstract: Although relatively rare, the culicid species *Aedes refiki* is considered widely distributed throughout Europe. It has also been described to occur over large parts of Germany, but reports are scarce and date back several decades. The last time *Ac. refiki* was documented for Germany was in 1980 when the species was found in the central northern part of the country. During larval sampling activities, *Ac. refiki* was rediscovered at two locations in the German federal state of Thuringia in spring 2016. The collection sites, method of species identification and species characteristics are described and discussed. *Journal of the European Mosquito Control Association* 35: 18-24, 2017

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Introduction

The culicid species *Aedes refiki* was described by Medschid (1928) in Anatolia. According to Reinert (1999) and Wilkerson & Linton (2015), *Ae. refiki* Medschid, 1928 is included in the aedine subgenus *Rusticoides* Shevchenko & Prudkina, 1973. Because no primary type had been designated, Reinert (2000) selected a lectotype from the collection of the Natural History Museum, London.

Aedes refiki is considered widely distributed throughout Europe, its distribution area extending from the Iberian Peninsula to Asia Minor, and from Sweden to Italy (Mohrig, 1969; Gilot *et al.*, 1971; Becker *et al.*, 2010). According to Mohrig (1969), *Ac. refiki* is also widespread in Germany and, although generally rare, can be annoying locally. It was reported from Germany for the first time by Vogel (1931), who found specimens in the field in Baden-Württemberg in 1929 but recognised larvae among museum material which had already been collected in 1897 and 1910 in the same federal state¹. Later documents refer to collections carried out in Hesse and Lower Saxony as early as 1904, 1928 and 1929 (Peus, 1951; Scherpner, 1960). Until 1980, the species was recorded from additional localities in northeast and central Germany (Table 1, Fig. 1).

The larval biology of *Ac. refiki* seems to be similar to that of *Ac. rusticus* (Rossi, 1790). *Aedes refiki* is a monocyclic snow-melt mosquito, which can overwinter in the larval or egg stage. Larvae may be found in winter when rainfall was sufficient in autumn and the water of the breeding site is not entirely frozen (Mohrig, 1969). In the case of overwintering eggs, the larvae hatch during the snow-melt early in the year. Breeding sites can be found both in open meadows and swampy woodlands and among temporary, semi-permanent or permanent water bodies with a neutral to alkaline pH (Vogel, 1931, 1940; Mohrig, 1965, 1969; Gilot *et al.*, 1971; Schuster & Mohrig, 1971; Dix, 1972; Franke, 1981). In central Europe, adults occur at the end of April and are active until mid-July. Females

feed on mammals including humans (Becker *et al.*, 2010). *Aedes refiki* is not known to be a vector of pathogens.

Here we describe and discuss new collection sites of *Ae. refiki* in Germany from 2016, i.e. after decades without documentation, and relate them to historic collection sites. We also present morphological and genetic characteristics of specimens collected at the two new collection sites.

Materials and Methods

During monitoring activities, mosquito larvae were collected in the German federal state of Thuringia on 14th April 2016 in the city forest of Mühlhausen (N51.206944, E10.387500, altitude 330m) and on 20th April 2016 in the Pennicken Valley close to Jena-Wöllnitz (N50.906389, E11.617778, altitude 241m) (Fig. 1, Table 1).

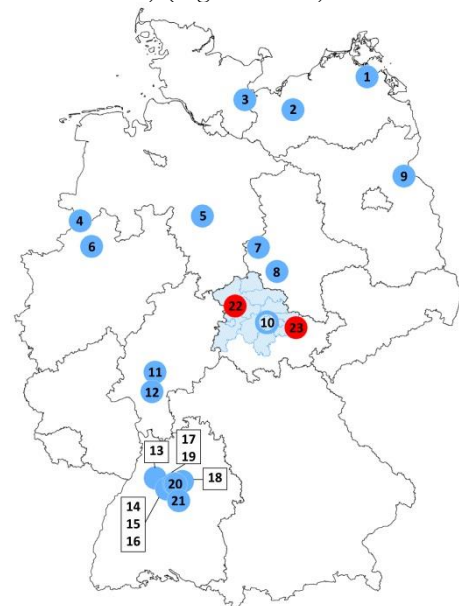


Figure 1: Geographical distribution of historic (blue dots and hatched area) and new findings (red dots) of *Ae. refiki* in Germany.

¹ The findings described are allocated to the present-day federal states of the Federal Republic of Germany.

Table 1: *Aedes refiki* documentations from Germany based on published literature and museum collections. ^a Geographic locations of collection sites as shown in Fig. 1 based on literature records and specimens in the Peus collection in the Senckenberg Museum in Frankfurt, Germany. When the specific collection site could not be geo-referenced with some precision, the centre of the town was used for geo-referencing; ^b as no specific sites were identifiable, the whole district is hatched in Figure 1; ^c specific town not identifiable due to existence of several settlements with the same name.

Federal state	Location	Position in Fig. 1 ^a	Collection time	Developmental stage found	Reference
Mecklenburg-Western Pomerania	Greifswald	1	June 1961	adults (♀, ♂)	Mohrig, 1965
	Brüel	2	June 1980	adult (♀)	Sommer, 1983
Schleswig-Holstein	Lübeck	3	June 1929	not provided	Peus, 1951
Lower Saxony	Bentheim	4	June 1929	adults (♀)	Peus, 1951
	Hannover	5	April, May 1935	adults (♀, ♂)	Martini (Diptera collection of the Natural History Museum, Berlin)
North Rhine-Westphalia	Havixbeck	6	April 1940	not provided	Peus, 1951
Saxony-Anhalt	Badersleben	7	April 1968	larva	Schuster & Mohrig, 1971
	Pansfelde	8	June 1970	adult (♀)	Dix, 1972
		8	May 1971	larvae/pupae	Dix, 1972
Brandenburg	Bad Freienwalde	9	mid-June, year not identifiable	adult (♀)	Harksen, 1976
Thuringia	District of Erfurt	10 ^b	March-June 1970-1976	larvae/adults	Franke, 1981
Hesse	Bad Nauheim	11	May 1928	adults (♀)	Peus, 1951
	Frankfurt	12	April 1904	adult (♂)	Scherpner, 1960
Baden-Württemberg	Maulbronn	13	April 1910	larvae	Vogel, 1929
	Heimerdingen	14	March 1897	larvae	Vogel, 1929
		15	April 1929	larvae	Vogel, 1929
		16	March 1930	larvae	Vogel, 1931
		17	April 1930	larvae	Vogel, 1931
	Marbach	18	March 1936	larvae	Vogel, 1940
	Tamm	19	April 1930	larvae	Vogel, 1933
	Asperg	20	May 1930	larvae	Vogel, 1933
	Hohenheim	21	not identifiable	larvae	Vogel, 1933
	Unterhaslach	- ^c	not identifiable	larvae	Vogel, 1933
Thuringia	Mühlhausen	23	April 2016	larvae	this study
	Jena-Wöllnitz	24	April 2016	larvae	this study

In Mühlhausen, the larvae occurred in a shaded, eutrophic pool in a *Pinus sylvestris* forest with some *Fagus sylvatica* undergrowth. The water body near Jena-Wöllnitz was a moderately shaded, semi-permanent small pool with abundant submerged foliage and branches. It was located near a stream in a stand of trees primarily consisting of *Acer pseudoplatanus*, *Fraxinus excelsior* and *Corylus avellana*.

The larvae were collected by repeated dipping with a standard dipper (Bioquip, CA, USA) at various sites of the pools, not following a specific sampling regime. They were brought into the laboratory in jars containing water from their breeding site, which were placed in insect rearing cages kept outdoors under natural climatic conditions, except that they were sheltered from rain. Until adult emergence, the larvae were fed with fish food flakes (Guppy, Tetra, Germany). Adults were killed by overnight freezing (-20°C) and were dry-

pinned on minute pins using the double-mounted method (Becker *et al.*, 2010). Morphological identification was performed using the keys of Mohrig (1969) and Becker *et al.* (2010). For long-term conservation, the specimens are stored in the mosquito reference collection of the Leibniz-Center for Agricultural Landscape Research in Müncheberg, Germany.

Single legs of 17 specimens (10 males and 6 females from Jena-Wöllnitz, 1 female from Mühlhausen) were processed to obtain COI mtDNA barcodes as described by Ibáñez-Justicia *et al.* (2014).

Results

Of 120 larvae collected in Mühlhausen and reared to adults, a single female mosquito was morphologically identified as *Ae. refiki*.

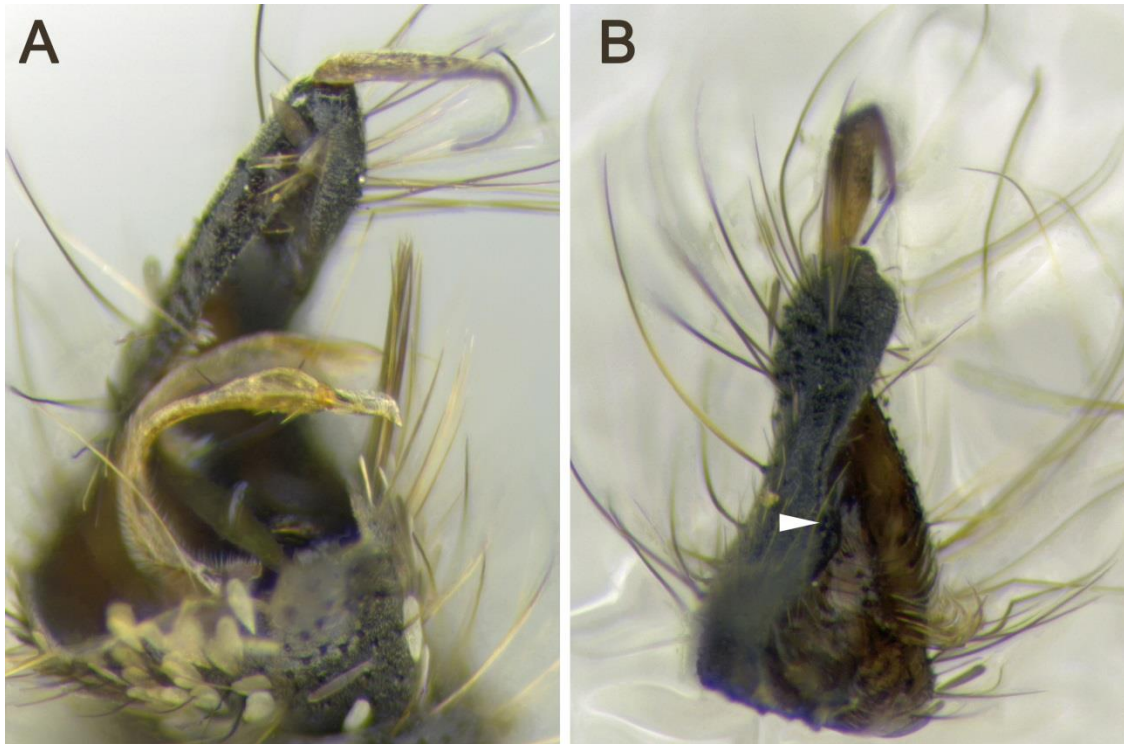


Figure 2: Hypopygium of *Ae. refiki* male. A: Lateral view of claspette (right gonocoxite removed), B: gonocoxite showing the basal dorsomesal lobe, white arrow: alveoli of the two missing strong setae of the dorsal part of basal dorsomesal lobe.

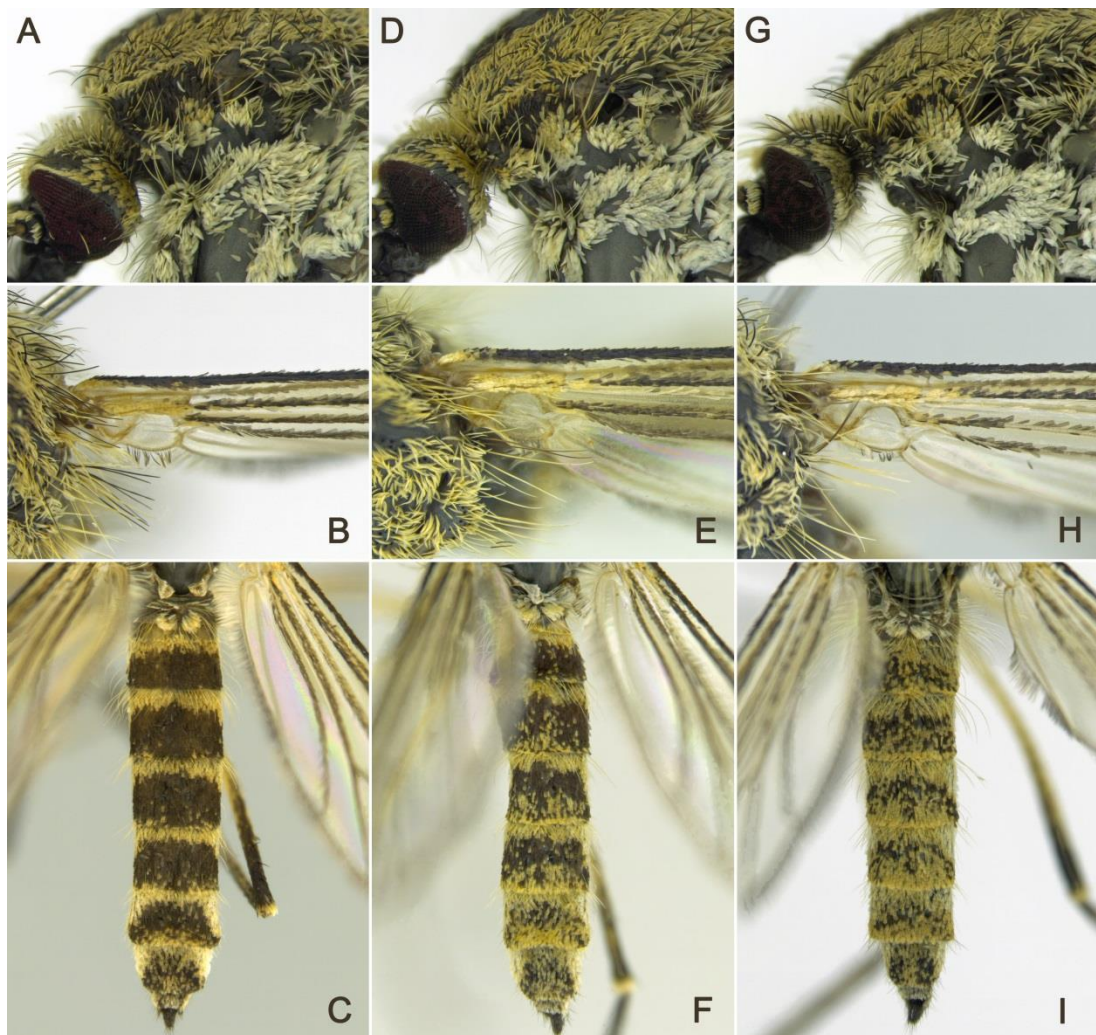


Figure 3: Dark (A-C), intermediate (D-F) and light (G-I) variant of *Ae. refiki* females showing lateral views of the thorax (A, D, G), the basal part of the wing (B, E, H) and the abdomen (C, F, I).

The adults developing from the other larvae were identified as species *Ae. cantans* (Meigen, 1818) (*n*=79), *Ae. cataphylla* Dyar, 1916 (*n*=7), *Ae. communis* (de Geer, 1776) (*n*=8), *Ae. leucomelas* (Meigen, 1804) (*n*=1), *Ae. riparius* Dyar & Knab, 1907 (*n*=20) and *Ae. rusticus* (*n*=4). Forty-six adult mosquitoes reared from the larvae collected in Jena-Wöllnitz included 27 males and 14 females of *Ae. refiki*. Other species from the same pool were *Ae. cantans* (*n*=4) and *Ae. cinereus* group (*n*=1).

Aedes refiki males were identified by the divided basal dorsomesal lobe of the gonocoxite, which is characterised by several slightly curved, lanceolate, flattened setae on the ventral part of the lobe and two long, strong and apically directed setae on the dorsal part of the lobe, a claspette with an apically broadened stem, a distinct transversely striated filament and the straight apical spine of the gonostylus (Fig. 2). The females were identified by the black, broad upper postpronotal scales, the pale-scaled remigium and the pale basal bands of the abdominal terga, which lack a median longitudinal stripe (Fig. 3).

The specimens exhibited a variable dark to lighter scale pattern, with the latter being less frequent. This variability was primarily due to the scaling of the posterior area of the terga which had some scattered pale scales (Fig. 3C), narrow apical bands (Fig. 3F) or were predominantly pale-scaled (Fig. 3I). In the lighter specimens, the dorsal stripes and the pale scaling on the scutum were also lighter, and the dark scales on

the postpronotum were less numerous than in the dark variant (Fig. 3A, 3G). The COI mtDNA sequences generated (709 bp) were identical in 14 specimens, both light and dark forms, from Jena-Wöllnitz and the one specimen from Mühlhausen. Two specimens from Jena-Wöllnitz showed one nucleotide difference each (positions 56 and 653, respectively, in Table 2). Alignment of the three sequence haplotypes with GenBank (<https://www.ncbi.nlm.nih.gov/genbank>) and BOLD (<http://www.boldsystems.org/>) database entries produced a maximum identity of about 97% with *Ae. provocans* (Walker, 1848), another species of the subgenus *Rusticoides* which occurs in North America. After cross-checking, it turned out that sequence data of *Ae. refiki* were not present in the BOLD database, and only one COI sequence was included in GenBank. The latter one belonged to a mosquito collected in Sweden, was significantly shorter (467 bp) than those generated in the present study and was shifted, due to using different PCR primers (Lilja *et al.*, 2017). Within the corresponding DNA region of 449 bp, the Swedish specimen differed by 5 and 6 nucleotides (1.1 and 1.3%; positions 268, 279, 341, 653, 695 and 701 in Table 2), respectively, from the German specimens (Table 2).

The three *Ae. refiki* COI haplotypes from the two German collection sites from 2016 are deposited in GenBank (accession nos.: KY646204-KY646206).

Table 2: COI sequence alignment of *Ae. refiki* from Germany (KY646204-6) and from Sweden (KP942731) (highlighted green: non-matching nucleotide sites).

	12345678911111111111111112222222222233333333333444444444455555555556 0123456789012345678901234567890123456789012345678901234567890
KY646204	GGTCAACAAATCATAAAGATATTGGAACATTATATTTTATTTTCGGGGTATGATCGGGAA
KY646205	GGTCAACAAATCATAAAGATATTGGAACATTATATTTTATTTTCGGAGTATGATCGGGAA
KY646206	GGTCAACAAATCATAAAGATATTGGAACATTATATTTTATTTTCGGAGTATGATCGGGAA
KP942731	-----
	6666666667777777777888888888899999999911111111111111111111111111111111 123456789012345678901234567890123456789000000000011111111111111111112 012345678901234567890
KY646204	TAGTGGTACTTCATTAAGTATATTAATTCGTGCTGAATTAAGTCAACCAGGAATATTTA
KY646205	TAGTGGTACTTCATTAAGTATATTAATTCGTGCTGAATTAAGTCAACCAGGAATATTTA
KY646206	TAGTGGTACTTCATTAAGTATATTAATTCGTGCTGAATTAAGTCAACCAGGAATATTTA
KP942731	-----
	11 222222222333333333344444444445555555556666666666777777777778 1234567890123456789012345678901234567890123456789012345678901234567890
KY646204	TTGGAAATGACCAAATTTATAACGTAATTGTTACAGCTCATGCATTTTATAATTTTTT
KY646205	TTGGAAATGACCAAATTTATAACGTAATTGTTACAGCTCATGCATTTTATAATTTTTT
KY646206	TTGGAAATGACCAAATTTATAACGTAATTGTTACAGCTCATGCATTTTATAATTTTTT
KP942731	-----
	1111111111111111111111111222 888888888999999999900000000001111111111222222222333333333334 1234567890123456789012345678901234567890123456789012345678901234567890
KY646204	TCATAGTAATACCTATTATAATTGGAGGATTTGGAAATGATTAGTTCCCTTAAATATTAG
KY646205	TCATAGTAATACCTATTATAATTGGAGGATTTGGAAATGATTAGTTCCCTTAAATATTAG
KY646206	TCATAGTAATACCTATTATAATTGGAGGATTTGGAAATGATTAGTTCCCTTAAATATTAG
KP942731	-----
	2223 4444444445555555555666666666677777777788888888899999999990 1234567890123456789012345678901234567890123456789012345678901234567890
KY646204	GAGTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTCCAT
KY646205	GAGTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTCCAT
KY646206	GAGTCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTTTGAATACTACCTCCAT
KP942731	-----CCTCGAA ^{AAATAATATA} GTTTTTGAATACTACCTCCAT
	33 000000000111111111122222222233333333334444444445555555556 1234567890123456789012345678901234567890123456789012345678901234567890
KY646204	CATTAACACTTCTGCTTCAAGTAGTATAGTAGAAAATGGATCTGGGACAGGATGAACAG
KY646205	CATTAACACTTCTGCTTCAAGTAGTATAGTAGAAAATGGATCTGGGACAGGATGAACAG

case, the ecological requirements of this species seem to be very complex (Mohrig, 1965; Gilot et al., 1971).

Although adults and immature stages of mosquitoes have been extensively collected within the frameworks of the German mosquito monitoring programme and the citizen science project 'Mueckenatlas' (Kampen et al., 2015) since 2011, *Ae. refiki* was only found in 2016. Elsewhere in Europe and prior to 2000, *Ae. refiki* was reported from Bosnia-Herzegovina, Czech Republic, France, Hungary, Italy, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and Ukraine (Trpiš & Tovornik, 1958; Parrish, 1959; Snow & Ramsdale, 1999). More recent reports exist from the Czech Republic (Rettich et al., 2007), Hungary (Kemenesi et al., 2015), Slovakia (Bocková & Kočišová, 2016) and Sweden (Lundström et al., 2013), all recording very few specimens.

The limited number of specimens encountered can be explained by the fact that the species usually occurs very locally in low densities and does not disperse far from larval habitats (Becker et al., 2010). Thus, *Ae. refiki* must continue to be ranked as a very rare species in Europe.

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References

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M., Dahl, C. & Kaiser, A. (2010) Mosquitoes and their control, 2nd edn. Springer, Berlin, Heidelberg.

Bocková, E. & Kočišová, A. (2016) Species composition of mosquitoes (Diptera: Culicidae) in relation to climate conditions in South-Eastern Slovakia. *Biologia*, **71**, 204-211.

Dix, V. (1972) Beiträge zur Stechmücken-Fauna (Dipt., Culicidae) der Landschaften zwischen Unterharzhochfläche, Unstrutniederung und mittlerer Elbe. - 5. Jahreszeitliche Abundanz, Biotopbindung, biogeographische Verteilung und Tagesaktivität der Aedesarten. *Hercynia N.F.*, **9**, 423-436.

Franke, I. (1981) Faunistisch-ökologische Untersuchungen an Stechmücken (Diptera, Culicidae) des Bezirkes Erfurt unter besonderer Berücksichtigung der Gattung *Aedes*. *Hercynia N.F.*, **18**, 65-86.

Gilot, B., Ain, G., Pautou, G. & Vigny, F. (1971) Répartition d'*Aedes refiki* Medschid 1928 (Dipt. Culicidae). *Ecologie de cette espèce dans la région Rhône-Alpes*. *Cahiers ORSTOM, Entomologie Médicale et Parasitologie*, **9**, 183-196.

Harksen, E., Mönke, R. & Schumann, H. (1976) Faunistisch-ökologische Untersuchungen zur Stechmückenfauna Berlins. *Deutsche Entomologische Zeitschrift*, **23**, 367-406.

Ibáñez-Justicia, A., Kampen, H., Braks, M., Schaffner, F., Steeghs, M., Werner, D., Zielke, D., den Hartog, W., Brooks, M., Dik, M., van de Vossen, B. & Scholte, E.-J. (2014) First report of established population of *Aedes japonicus japonicus* (Theobald, 1901) (Diptera, Culicidae) in the Netherlands. *Journal of the European Mosquito Control Association*, **32**, 9-13.

Kampen, H., Medlock, J.M., Vaux, A.G., Koenraadt, C.J., van Vliet, A.J., Bartumeus, F., Oltra, A., Sousa, C., Chouin, S. & Werner, W. (2015) Approaches to passive mosquito surveillance in the EU. *Parasites & Vectors*, **8**, 9.

Kemenesi, G., Kurucz, K., Kepner, A., Dallos, B., Oldal, M., Herczeg, R., Vajdovics, P., Bányai, K. & Jakab, F. (2015) Circulation of *Dirofilaria repens*, *Setaria tundra*, and Onchocercidae species in Hungary during the period 2011-2013. *Veterinary Parasitology*, **214**, 108-113.

Lilja, T., Nylander, J.A.A., Troell, K. & Lindström, A. (2017) Species identification of Swedish mosquitoes through DNA metabarcoding. *Journal of the European Mosquito Control Association*, **35**, 1-9.

Lundström, J.O., Schäfer, M.L., Hesson, J.C., Blomgren, E., Lindström, A., Wahlqvist, P., Halling, A., Hagelin, A., Ahlm, C., Evander, M., Broman, T., Forsman, M. & Persson Vinnersten, T.Z. (2013) The geographic distribution of mosquito species in Sweden. *Journal of the European Mosquito Control Association*, **31**, 21-35.

Medschid, E. (1928) Über *Aedes lepidonotus* Edw. und *Aedes refiki* n.sp. *Archiv für Schiffs- und Tropenhygiene*, **32**, 306-315.

Mohrig, W. (1965) Ergänzungen zur Culiciden-Fauna der Umgebung von Greifswald. *Deutsche Entomologische Zeitschrift*, **12**, 325-328.

Mohrig, W. (1969) Die Culiciden Deutschlands. Untersuchungen zur Taxonomie, Biologie und Ökologie der einheimischen Stechmücken. *Parasitologische Schriftenreihe*, **18**, 1-260.

Parrish, D.W. (1959) The mosquitoes of Turkey. *Mosquito News*, **19**, 264-266.

Peus, F. (1951) Stechmücken. Die neue Brehm-Bücherei, **22**, 1-80.

Reinert, J.F. (1999) The subgenus *Rusticoides* of genus *Aedes* (Diptera: Culicidae) in Europe and Asia. *European Mosquito Bulletin*, **4**, 1-7.

Reinert, J.F. (2000) Selection of a lectotype for *Aedes refiki* Medschid (Diptera: Culicidae) and redescription of the male, females and fourth-instar larvae of the type series. *European Mosquito Bulletin*, **8**, 1-6.

Rettich, F., Imrichova, K. & Šebesta, O. (2007) Seasonal comparisons of the mosquito fauna in the flood plains of Bohemia and Moravia, Czech Republic. *European Mosquito Bulletin*, **23**, 10-16.

Scherpner, C. (1960) Zur Ökologie und Biologie der Stechmücken des Gebietes von Frankfurt am Main (Diptera, Culicidae). *Mitteilungen des Zoologischen Museums Berlin*, **36**, 49-99.

Schuster, W. & Mohrig, W. (1971) Stechmücken und ihre Bekämpfung im DDR-Bezirk Magdeburg. *Angewandte Parasitologie*, **12**, 11-19.

Snow, K., Ramsdale C. (1999) Distribution chart of European mosquitoes. *European Mosquito Bulletin*, **3**, 14-31.

Sommer, S.H. (1983) Die Stechmückenfauna (Diptera, Culicidae) des DDR-Bezirk Schwerin und ihre Bedeutung als Plageerreger. *Angewandte Parasitologie*, **24**, 11-19.

Trpiš, M. (1958) Poznámky k ekológii a zoogeografii druhu *Aedes (O.) refiki* (Diptera, Culicidae). *Biologia (Bratislava)*, **8**, 305-310.

Trpiš, M. & Tovornik, D. (1958) Faunistische, ökologische und zoogeographische Bemerkungen zu den Stechmücken Sloweniens (Jugoslawien). *Biologia (Bratislava)*, **13**, 721-739.

Vogel, R. (1929) Zur Kenntnis der Stechmücken Württembergs. I. Teil. *Jahreshefte des Vereins für vaterländische Naturkunde in Württemberg*, **85**, 258-277.

Vogel, R. (1931) Eine für Deutschland neue Stechmücke, *Aedes refiki* Medschid. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, **25**, 257-268.

Vogel, R. (1933) Zur Kenntnis der Stechmücken Württembergs. II. Teil. Jahreshefte des Vereins fürvaterländische Naturkunde in Württemberg, **89**, 175-186.

Vogel, R. (1940) Zur Kenntnis der Stechmücken Württembergs. III. Teil. Jahreshefte des Vereins fürvaterländische Naturkunde in Württemberg, **96**, 97-116.

Wilkerson, R.C. & Linton, Y.-M. (2015) Elevation of *Pseudokusea*, *Rusticoidus* and *Protomacleaya* to valid subgenera in the mosquito genus *Aedes* based on taxon naming criteria recently applied to other members of the tribe Aedini (Diptera: Culicidae). *Parasites & Vectors*, **8**, 668.