

A new European mosquito species: *Culex (Neoculex) europaeus* (Diptera: Culicidae)

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Abstract

The male, female, larva and pupa of *Culex (Neoculex) europaeus*, sp. nov. are described on the basis of the examination of material from Portugal and comparison with reared specimens of *Culex (Neoculex) territans* from North Carolina, USA. The recognition of the new taxon is discussed and diagnostic identification keys to the male and female genitalia and pupa are provided.

Introduction

Culex territans Walker was described from the “United States” (Walker, 1856), but the type locality was restricted later to Charleston (Charleston County), South Carolina, by Belkin *et al.* (1966). Furthermore, Belkin (1968) was unable to locate the type specimen of *Cx. territans* and considered it “presumably lost” and non-existent.

Sirivanakarn (1971) considered *Cx. territans* the most widely spread member of the subgenus *Neoculex* Dyar, since it occurs in both the Eastern and Western Hemispheres. In North America, *Cx. territans* has a very wide distribution to include parts of Alaska and Canada, and most of the contiguous United States except for several southwestern states (Darsie & Ward, 1981). Ross (1964) proposed that all of the North American *Neoculex* species originated in the New World, except for *Cx. territans*, which originated in the Eastern Hemisphere (Eurasia) and came to North America via the Bering land bridge. We consider it likely that specimens of the European and Mediterranean *territans*-like species were transported to North America in water barrels along with *Culex pipiens* L., during the last five hundred years. If this occurred, new DNA techniques should be able to detect the introgression of European genes among the American representatives of *Neoculex*.

In Europe, *Cx. territans* was initially misidentified as *Culex apicalis* Adams, 1903 (Martini, 1931; Braga, 1931; Marshall, 1938), a species now known to be endemic to the Nearctic Region (Mattingly, 1953; Callot, 1954; Carpenter & LaCasse 1955; Gutsevich *et al.*, 1974; Knight & Stone, 1977). In the Eastern Hemisphere, *Culex territans* is recorded as widely distributed in the European and Mediterranean Subregions of the Palaearctic, from the Iberian Peninsula across the Palaearctic to Siberia. However, these records need comprehensive review as some (e.g. the *Cx. territans* of Nilsson, 1983) may possibly represent additional undescribed species, and in other areas specimens of *Cx. territans* are easily confused with *Cx. impudicus* Ficalbi, *Culex judaicus* Edwards, *Culex martinii* Medschid, and *Culex rubensis* Sasa & Takahasi.

In Portugal, *Cx. territans* is fairly widespread, but more common in the northern half of the country (Ribeiro *et al.*, 1988). A close examination of Portuguese specimens, however, revealed differences in characters from those described for *Cx. territans* in North America, even when the excessive variation known for *Cx. territans* (Michener, 1945 [as *Cx. apicalis*]; Bohart, 1948; Darsie, 1951; Bickley & Harrison, 1989) is considered. After a comparison of associated larvae, pupae and adults from individual rearings from Portugal with North American (North Carolina) individually reared associated specimens, we concluded that the Portugal specimens belong to a new species, *Culex (Neoculex) europaeus*, sp. nov., which is described below. The nomenclature adopted for this description is that of Harbach & Knight (1980, 1982).

Culex (Neoculex) europaeus, sp. nov.

(Figs. 1-5)

Male. Wing length about 3 mm. **Head.** Vertex clothed with narrow whitish decumbent scales; usually with a few forked erect pale scales on the occiput. Proboscis long, dark. Maxillary palpus dark brown. Distal palpomere with numerous long, dark setae. **Antenna.** Pedicel dark brown; flagellar whorls with about 30 setae. Clypeus dark. **Thorax.** Integument mainly dark brown. Scutum mainly with narrow golden-brown scales; prescutellar, supralar and antealar areas, lateral fossal border, and anterior promontory with narrow whitish scales; prescutellar patch of whitish scales either extending to midline of prescutellar area or more reduced, with only a pair of small isolated patches on posterior dorsocentral areas of scutum. Integument of scutellum entirely light brown; all lobes with narrow white scales. Antepronotum dark brown, with narrow pale scales on upper half. Postpronotum dark brown, also with numerous narrow pale scales on upper 0.33-0.5. Postspiracular scales absent. Integument of mesokatepisternum paler on lower half; with conspicuous prealar and lower mesokatepisternal patches of dense broad white scales. Mesepimeron with a dense patch of broadish white scales on upper half, without lower mesepimeral seta. **Legs.** Mainly dark. Femora paler below, more extensively so on hindfemur, with an almost complete pale stripe. Tibiae with a variable pale streak behind, usually more distinct on hindtibia, which also bears moderately developed spinulate setae on outer and inner borders. Hind tarsomere I with a somewhat indistinct posterior pale streak. Foreungues unequal, one stronger and toothed; mid- and hindungues smaller, subequal, simple. **Wing.** All veins dark-scaled, with costa, subcosta and radius obviously darker; cell R_2 longer than R_{2+3} and M_1 . **Halter.** Capitellum brown, with pale scales; pedicel and scabellum yellowish, bare. **Abdomen.** Tergum I dark-scaled. Terga II-VII with narrow apical bands of white scales, not widened at sides. Sterna II-VIII with well developed triangular patches of pale scales which may reach anterior border of segment. **Genitalia** (Figs. 1-3) Gonocoxite length about 0.33 mm; gonostylus length 0.16 mm; gonostylar claw length about 0.017 (0.015-0.018) mm. Tergum IX with distal margin slightly concave, interlobal space wide, lobes rounded, strongly sclerotized, with 7-13 (usually about 10) thin, relatively long setae. Aedeagal lateral plates minutely tuberculate at rounded apex, joined at base and by a distinct dorsal bridge at about 0.60-0.70. Paraproct without basal arm, with apical blunt spines and 4-6 minute cercal setae. Gonocoxite about twice as long as broad, with long setae on outer surface. Subapical lobe clearly divided, bearing 9, at times 10, more or less modified setae: 2 rods, *a* and *b*, the most proximal ones, 2 or 3 small straight setae (*c*, and *c'*, rarely also *c''*), 3 longer, somewhat flattened setae with membranous curved tips (*d*, *e*, *f*), the most proximal of which (*d*, *e*) are slightly serrated, seta *g*, straight, slightly longer than *f* and somewhat flattened, and seta *h*, longer than *g*, strong but delicate and pointed distally. Inner aspect of gonostylus with usually 2(1-3) small setae at about 0.33-0.67, without microtrichia or almost so; a few small membranous lobes sometimes discernible along dorsal border. Gonostylar claw blunt-tipped and relatively short.

Female. Wing length about 4 mm. Very similar to male except as follows. **Head.** Maxillary palpus very short, only about 2x length of clypeus. **Legs.** All unguis small, equal, simple. **Genitalia** (Figs. 4, 5). Cerci relatively short, ratio length to width 1.6-1.8. Postgenital lobe relatively wide, with margin almost evenly rounded. Ninth tergal lobes well sclerotized, each with 6-9 (mean 8) setae. Insula with 10-13 setae.

Pupa. Integument moderately dark. **Cephalothorax.** Seta 8-CT with 2-5 branches, usually 3 or 4; 9-CT always double. Respiratory trumpet very dark, somewhat narrowed at middle, tracheoid area extending to about 0.5 its length; pinna short, with index about 0.15. Trumpet index 8-10, usually about 9. **Metathorax.** Seta 10-MT with 5-8 branches; 11-MT double; 12-MT with 2-4 branches. **Abdomen.** Integument tomentous. Seta I-III with 8-13 branches; 1-IV with 8-10 branches; 1-V with 5-8 branches; 1-VI with 4-5 branches; 1-VIII 2-4 branched; 4-I with 4-6 branches; 5-II and 5-III with 4-7 branches and 5-7 branches, respectively; 5-IV with 3 or 4 long branches, clearly reaching beyond posterior border of segment V; 5-V with 2 long, strong branches reaching beyond middle of segment VII; 5-VI also with 2 strong and long branches reaching beyond middle of segment VIII; 6-IV with 3-5 branches; 6-VI 4- or 5-branched; 6-VII with 2-4 branches; 9-VII with 2 small branches, about half length of segment; 9-VIII with 3 branches, also about half length of segment. **Paddle.** Unpigmented; midrib not reaching apex; outer border minutely and sparsely spiculate on distal half, spicules not easily seen owing to lack of pigmentation. Paddle seta (1-Pa) always present, very small; accessory seta (2-Pa) minute, always present. Paddle index 1.59-1.74 (mean 1.68).

Fourth instar larva. **Head.** Antenna obviously spiculate, almost as long as head, infusate at tuft insertion and beyond, as well as at base. Seta 1-C thin, long, pointed, heavily sclerotized. Seta 4-C single, delicate, long; 5-C with 1 or 2 branches about 0.5-0.7 of 6-C; 6-C single, very long; 7-C with 6-9 delicately feathered branches; 8-C with 3 or 4 relatively long branches; 9-C with 6 or 7 branches; seta 10-C 2-branched. **Thorax.** Integument obviously and densely spiculate. Submedian group of prothoracic setae: 1- and 2-P single, very long, strong, feathered; 3-P with 3 or 4 delicate and much shorter

branches. **Abdomen.** Integument spiculate. Abdominal seta 6-I, II with 3 or 4 branches; 6-III-VII with 2 branches; 1-VIII with 5-8 branches, usually 6 or 7; 2-VIII always single; 3-VIII with 8-10 branches, usually 9; 4-VIII always single; 5-VIII with 4 branches, occasionally 5. Comb of segment VIII with 50-65 scales, individual scales with rounded apical fringe. Siphon darkened, tomentous, index (length to width at base) 6.5-8.8. Siphon pecten with 12-18 (mean 14.5) spines, most distal clearly more widely spaced; spines usually with only one small denticle at base; with 10 unpaired, 2- or 3-branched subventral tufts (seta 1-S) inserted distal to pecten, about as long or slightly longer than width of siphon at point of attachment. Saddle well sclerotized, strongly spiculate toward distal upper angle; seta 1-X delicate, usually 2-branched but occasionally 3-branched, shorter than saddle; seta 2-X with 4 unequal branches; seta 3-X single. **Anal papillae.** Dorsal pair usually about as long as saddle, ventral pair slightly shorter.

Type data. **Holotype:** Male labelled "*Culex (Ncx) europaeus* sp. nov. **Holótipo.** Avô, Prov.Beira-Baixa.1977.09.02. No. E48137". **Paratypes:** 4 males and 7 females with genitalia mounted on slide, plus 10 pupal exuviae and pupae and 9 fourth-instar larvae, nos. E48138 to E48167, all with the same data and from the same breeding place as the holotype, a small vegetated niche in the shallow margins of the Alva River, at Avô. **Type locality,** Avô, in northern Portugal, at about 40° 17' 20"N and 7° 53' 40"W, and 300 m in altitude.

Depository for types. The type specimens are deposited at the Insect Collection of the Unidade de Entomologia Médica, Instituto de Higiene e Medicina Tropical, UNL, Lisboa, Portugal, except for one male and one female paratypes to be deposited in the Smithsonian Institution, Washington, DC, USA.

Etymology. The new species is named for Europe, its area of occurrence.

Discussion

The only representatives of subgenus *Neoculex* Dyar in continental Portugal are *Cx. impudicus* and what was identified previously as *Cx. territans* (Ribeiro *et al.*, 1988). The pupae of these two species cannot be separated (unpublished data) and the larvae are not always distinguishable (Ribeiro & Ramos, 1999). The only diagnostic characters for these two species are on the male genitalia. A very similar situation exists in North America, where *Cx. territans* often cannot be separated from other sympatric *Neoculex* species unless male genital characters are used (Bohart, 1948; Darsie, 1951; Carpenter & LaCasse, 1955; Bickley & Harrison, 1989). Certain characters of the adult habitus of *Cx. territans* are highly variable, e.g. integument colour (including pedicel and scutellum), location of scales on the postpronotum, extent of apical banding on the abdominal terga, and the size, shape, and colour of scutal scales. Likewise, the following larval characters have also been found highly variable: siphon index; the number and size of seta 1-S on the siphon; the number of branches on setae 5- and 6-C; the length of seta 5-C in relation to 6-C; the number of denticles on individual pecten spines and the presence or absence of pigmented banding patterns on the abdominal segments. Michener (1945) pointed out that these adult and larval variations are probably influenced by seasonal temperatures. Danilov (1978; 1980) documented that variation in the branching of head seta 5-C and the siphon index on *Cx. territans* in the Soviet Palaearctic were clinal, with seta 5-C branches decreasing from western Siberia to far-eastern Asia, and the siphon index declining from south to the north in the Palaearctic. In the Siberian region, the siphon index declined from west to east in correlation with the transition into the permafrost zone (Danilov, 1980). An example of clinal changes in adults of other species is recorded by Harrison *et al.* (1981) who found south to north clinal changes in the scutal scale colours on *Ochlerotatus (Finlaya) albotaeniatus* (Leicester) and *Oc. (Fin.) greenii* (Theobald) that resulted in the synonymy of a subspecies of each of these species.

Gutsevich *et al.* (1974) suggested that *Cx. rubensis*, from Japan and Korea, was probably a synonym or subspecies of *Cx. territans*. However, Tanaka *et al.* (1979) clearly showed that *Cx. rubensis* is a valid species. Furthermore, while addressing this problem, Tanaka *et al.* (1979) discussed finding certain characters in European *Cx. territans* that differed from North American *Cx. territans*, and suggested that "Further study will be necessary to evaluate the significance of these differences". We have addressed the characters mentioned in Tanaka *et al.* (1979), and for some characters, have confirmed their findings.

While trying to find diagnostic adult and larval characters to separate North American *Cx. territans* and the new species described here, the environmental influences of latitude and seasonality on adult and larval characters were of paramount importance. Although the Portuguese specimens consistently had more pale scales on the scutum and the postpronotum than North Carolina specimens, specimens described and illustrated by Wood *et al.* (1979) from Canada normally have more pale scales on those structures and appear similar to Portuguese specimens. Also, even in North Carolina infrequent

specimens of *Cx. territans* are found that have more pale scutal scales, even two white-scaled spots on the scutum much like those on *Culex restuans* Coquillett. Southern Canada and northern Portugal are located at approximately 42° north latitude, whereas the most northern part of North Carolina is located considerably further south at approximately 36.5° north latitude. Thus, specimens from North Carolina would be expected to possess characters more like the characters found by Michener (1945) in specimens from the southern United States.

Because of problems in dealing with overlapping variation, it was decided to try to define the new species on the basis of male and female genital characters. Male genital characters are currently considered the most reliable for separating the currently recognised species of *Neoculex* (Bohart, 1948; Carpenter & LaCasse, 1955; Sirivanakarn, 1971; Bickley & Harrison, 1989). Accordingly, the male genital characters noted here, the allopatric distributions of the two taxa (Mayr & Ashlock, 1991), differences in female genital characters, and promising characters on the pupae justify the recognition of *Cx. europaeus* as a separate species. Unfortunately, we were unable to find habitus characters that will separate the adult females and larvae of these two species with a high level of reliability. *Culex europaeus* can be separated from *Cx. territans* using the following keys.

KEY TO MALE GENITALIA

- Gonostylus (Fig. 7) minutely pilose on internal surface, particularly on distal half; gonostylar claw about 0.024 mm; tergum IX lobes (Fig. 8) weakly sclerotized, each with 2-7 setae (usually 3-5); subapical lobe of gonocoxite (Fig. 6) with 4 small flattened setae with curved apices (d, d', e and f)*territans*
- Gonostylus not obviously pilose on internal surface (Fig. 2); gonostylar claw about 0.017 mm; tergum IX lobes strongly sclerotized, with 7-13 setae (Fig. 3); subapical lobe of gonocoxite (Fig. 1) with 3 small flattened setae with curved apices (d, e and f).....*europaeus*

KEY TO FEMALE GENITALIA

- Tergum IX lobes with 4-7 setae (Fig. 9); postgenital lobe somewhat elongated (Fig. 10).....*territans*
- Tergum IX lobes with 6-9 setae (Fig. 4); postgenital lobe nearly rounded (Fig. 5).....*europaeus*

KEY TO PUPAE

- Seta 1-III with 6-9 branches; seta 1-IV with 5-7 branches; seta 1-V with 3-5 branches; paddle with outer border smooth*territans*
- Seta 1-III with 9-12 branches; seta 1-IV with 8-10 branches; seta 1-V with 5-8 branches; paddle with outer border minutely and sparsely spiculate on distal half.....*europaeus*

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References

- Belkin, J.N. (1968) Mosquito Studies (Diptera, Culicidae) IX. The type specimens of World mosquitos in European museums. *Contributions of the American Entomological Institute (Ann Arbor)* 3 (4), 1-69.
- Belkin, J.N., Schick, R.X. & Heinemann, S.J. (1966) Mosquito Studies (Diptera, Culicidae) VI. Mosquitoes originally described from North America. *Contributions of the American Entomological Institute (Ann Arbor)* 1 (2), 1-39.
- Bickley, W. E. & Harrison, B.A. (1989) Separation of variable *Culex territans* specimens from other *Culex* (*Neoculex*) in North America. *Mosquito Systematics* 21, 188-196.
- Bohart, R.M. (1948) The subgenus *Neoculex* in America North of Mexico (Diptera: Culicidae). *Annals of the American Entomological Society* 41, 330-345.
- Braga, J.M. (1931) *Culicídeos de Portugal*. Institute Zoologico da Universita de Porto. Porto, Portugal. 83pp.
- Callot, J. (1954) Notes et informations. *Annales de Parasitologie* 29, 25-326.
- Carpenter, S.J. & LaCasse, W.J. (1955) *Mosquitoes of North America (North of Mexico)*. University of California Press, Berkeley, USA. 360pp.
- Danilov, V.N. (1978) On branching of frontal hairs in the *Culex* (*Neoculex*) *territans* Walker larvae (Diptera, Culicidae). *Zoological Journal* 57, 1581-1583 (English translation).
- Danilov, V.N. (1980) Variability of siphon index in mosquito larvae of the subgenus *Neoculex* (Diptera, Culicidae) of the Palaearctic fauna. *Zoological Journal* 59, 234-238 (English translation).
- Darsie, R.F., Jr. (1951) Pupae of the culicine mosquitoes of the northeastern United States (Diptera, Culicidae, Culicini). *Memoirs of the Cornell Agricultural Experimental Station* 304, 1-67.
- Darsie, R.F., Jr. & Ward, R.A. (1981) Identification and geographical distribution of the mosquitoes of North America, north of Mexico. *Mosquito Systematics Supplement* 1, 1-313.
- Gutsevich, A.V., Monchadskii, A.S. & Shtakel'berg, A.A. (1974) *Fauna of the USSR. Vol.3. Part 4. Mosquitoes. Family Culicidae*. Academy of Sciences of the USSR, Leningrad (English translation).
- Harbach, R.E. & Knight, K.L. (1980) *Taxonomists' Glossary of Mosquito Anatomy*. Plexus Publishing, Inc., Marlton, New Jersey, USA. 415 pp.
- Harbach, R.E. & Knight, K.L. (1982) Corrections and additions to *Taxonomists' Glossary of Mosquito Anatomy*. *Mosquito Systematics* 13, 201-217.
- Harrison, B.A., Rattarithikul, R., Peyton, E.L. & Mongkolpanya, K. (1981) Taxonomic changes, revised occurrence records and notes on the Culicidae of Thailand and neighboring countries. *Mosquito Systematics* 22, 196-227.
- Knight, K.L. & Stone, A. (1977) A catalog of the mosquitoes of the world (Diptera: Culicidae). 2nd Edition. Thomas Say Foundation. *Entomological Society of America* 6, 1-611.
- Marshall, J.F. (1938) *The British Mosquitoes*. British Museum (Natural History). London. 341 pp.
- Martini, E. (1931) 11u.12. Culicidae. pp.321-398. In Lindner, E. *Die Fliegen der Palaearktischen Region*. Stuttgart, Germany.
- Mattingly, P.F. (1953) A change of name among the British mosquitoes (Diptera, Culicidae). *Proceedings of the Royal Entomological Society of London (B)* 22, 106-108.
- Mayr, E. & Ashlock, P.D. (1991) *Principles of Systematic Zoology*. 2nd Edition. McGraw-Hill Inc., N.Y. 475 pp.
- Michener, C.D. (1945) Seasonal variations in certain species of mosquitoes (Diptera, Culicidae). *Journal of the New York Entomological Society* 53, 293-300.
- Nilsson, C. (1983) Stickmyggan *Culex territans* - belagd fran Sverige. *Entomologisk Tidskrift* 104, 41-43.
- Ribeiro, H. & Ramos, H.C (1999) Identification keys of the mosquitoes (Diptera: Culicidae) of Continental Portugal, Açores and Madeira. *European Mosquito Bulletin* 3, 1-11.
- Ribeiro, H., Ramos, H.C., Pires, C.A. & Capela, R.A. (1988) An annotated check-list of the mosquitoes of Continental Portugal (Diptera: Culicidae). *Actas III Congresso Ibérico Entomologia*, 233-253. Granada, Spain.
- Ross, H.H. (1964) The colonization of temperate North America by mosquitoes and man. *Mosquito News* 24, 103-118.
- Sirivanakarn, S. (1971) Contributions to the mosquito fauna of Southeast Asia XI. A proposed reclassification of *Neoculex* Dyar based principally on the male genitalia. *Contributions of the American Entomological Institute (Ann Arbor)* 7 (3), 62-85.
- Tanaka, K., Mizusawa, K. & Saugstad, E.S. (1979) A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera, Culicidae). *Contributions of the American Entomological Institute (Ann Arbor)* 16, 1-987.
- Walker, F. (1856) *Insecta Saundersiana: or Characters of Undescribed Insects in the Collection of William Wilson Saunders. Vol. 1. Diptera*. London. 474 pp.
- Wood, D.M., P. T. Dang, & Ellis, R.A. (1979) *The Insects and Arachnids of Canada. Part 6. The Mosquitoes of Canada. Diptera: Culicidae*. Agriculture Canada publication 1686. 390 pp.

Culex (Neoculex) europaeus sp. nov.

Figures 1-3. Genitalia of male holotype: Fig. 1. Setae $\underline{c-h}$ of the subapical lobe of gonocoxite. Fig. 2. Gonostylus and gonostylar claw. Fig. 3. Lobes of tergum IX.

Figures 4-5. Genitalia of a female paratype. Fig. 4. Tergum IX lobes. Fig. 5. Postgenital lobe.

Culex (Neoculex) territans Walker (North Carolina, USA)

Figures 6-8. Male genitalia. Fig. 6. Setae $\underline{c-h}$ of the subapical lobe of gonocoxite. Fig. 7. Gonostylus and gonostylar claw. Fig. 8. Tergum IX lobes.

Figures 9-10. Female genitalia. Fig. 9. Tergum IX lobes. Fig. 10. Postgenital lobe.

Scales = 0.050 mm.

