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THE STATUS OF THE JAPANESE *CROCOTHEMIS SERVILIA* (DRURY) AS REVEALED BY KARYOTYPIC MORPHOLOGY (ANISOPTERA: LIBELLULIDAE)*

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Close to 30 populations pertaining to *C. servilia* s.l. from India, Nepal, Thailand, Singapore, continental China, the Philippines, Taiwan and Japan were examined cytologically. The type locality of the sp. is "China", and its karyotypic morphology is uniform throughout continental Asia, the Philippines and Taiwan (2n ♂ = 25, n ♂ = 13, *m*, XO). The Japanese material, which has been for a long time noticed for the "usually distinctly large size" of the individuals, is essentially distinct from all other populations, deviating from these in chromosome number (2n ♂ = 24, n ♂ = 12) and in mode of sex determination (neo-XY). Since these features are geographically limited to Japan, consistently expressed in all individuals examined, not subject to variation among individuals, do not show intrinsic genetic variability, and are not conditioned by the environment, the Japanese population is separated under the name *C. servilia mariannae* ssp. n. The type series originates from Osaka, and all material examined is of Honshu provenience. This is the first case of erection of a new taxon in the Odonata on the basis of karyomorphological diagnostic characters.

INTRODUCTION

There are but few libellulids possessing a geographic range of a similar extension as *C. servilia*. It occurs from the Middle East, throughout southern Asia, into China, Australia, the Sundaic Archipelago, and Japan. Within this territory, it shows a considerable amount of geographic variation, though any clear infraspecific forms are not easy to define, and the problem of its infra-

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* Though a bit early, this paper is dedicated to the 80th birthday of my teacher and friend, Dr MAURITS ANNE LIEFTINCK, to be celebrated on February 18, 1984, as a small token of thanks for his guiding me along the fascinating paths of Odonatology for more than two decades.

speciation has received but little attention.

KRÜGER (1902) was probably the first to have noticed the pronounced morphological variation between different geographic populations. Mainly on the criterium of the general body size, he suggested the existence of at least two "races", a northern "Sino-Japanese", and a southern "Indo-Malayan" one. All subsequent workers, from e.g. RIS (1911) to ASAHINA (1961), have emphasized the usually distinctly larger size of the Japanese specimens, which differ from the continental and southern populations also by some other, not easily definable features. Althoug the peculiar general appearance of the Japanese material is obvious, it is very difficult to define it in terms of adequate diagnostic morphological characters.

During the past five decades the karyotypic morphology has been examined of almost 30 *servilia* populations, covering a substantial portion of its range, viz. India, Nepal, Thailand, Singapore, the Philippines, Taiwan, and Japan. It became apparent that within this area there are two karyomorphologically and geographically clearly distinct and sharply defined forms. However, due to the circumstance that *Crocothemis servilia* has been originally described from "China" (DRURY, 1770), the karyomorphological features could not be used in taxonomic considerations, without evidence on the cytological features of the "topotypic", i.e. continental Chinese population (cf. LOHMANN, 1981). Only recently did Dr Hui-qian Zhu (Taiyuan, Shanxi, China) succeed in examining the continental Chinese material (pers. comm., dated Sept. 10, 1983), thus enabling me to venture an attempt to separate the two forms taxonomically and nomenclaturally.

The information provided by Dr Zhu makes it clear that, as far as the area studied is concerned, the overall chromosome morphology of *C. servilia* is practically identic throughout continental Asia, and on the Philippines and Taiwan, and that only the Japanese *servilia* is significantly distinct from all other populations. It deviates from these in chromosome number and in the mode of sex determination. These characters are peculiar to the Japanese form, are consistently expressed in all individuals examined, they are neither subject to variation among individuals, nor do they appear to show any intrinsic genetic variability, and, it goes without saying, they are not-conditioned by the environment.

All cytologically examined Japanese material originates from various localities in Honshu, the largest island of the Japanese "mainland". For the sake of convenience (but not entirely correctly), in the below text the terms, "Japan" and "Japanese", actually stand for "Honshu" only, while nothing is known on the cytology of other Japanese populations.

Although, for obvious reasons, the application of cytotaxonomic diagnostic characters is but seldom practised in taxonomy and has so far never been used in the Odonata, I am of the opinion that the pronounced and biologically essential distinctions, coupled with the sharply defined geographic distribution, in this case do justify the taxonomic and nomenclatural separation of the Japanese form. It certainly seems to be too rash to claim a separate specific status for the Japanese *servilia* at this time; a separate infraspecific status, however, does appear justified. Since it clearly and consistently deviates from all other geographic populations, including the "topotypic" one from "China", I am proposing here to separate the Japanese form under the name *Crocothemis servilia mariannae* ssp. n.

The taxon is named after Marianne Kiauta, who participated in the cytotaxonomic study of most of the populations examined, both in the field and in laboratory, recognized that the *servilia* case was unique in the Order, and prepared hundreds of micrographs, including those reproduced in this paper.

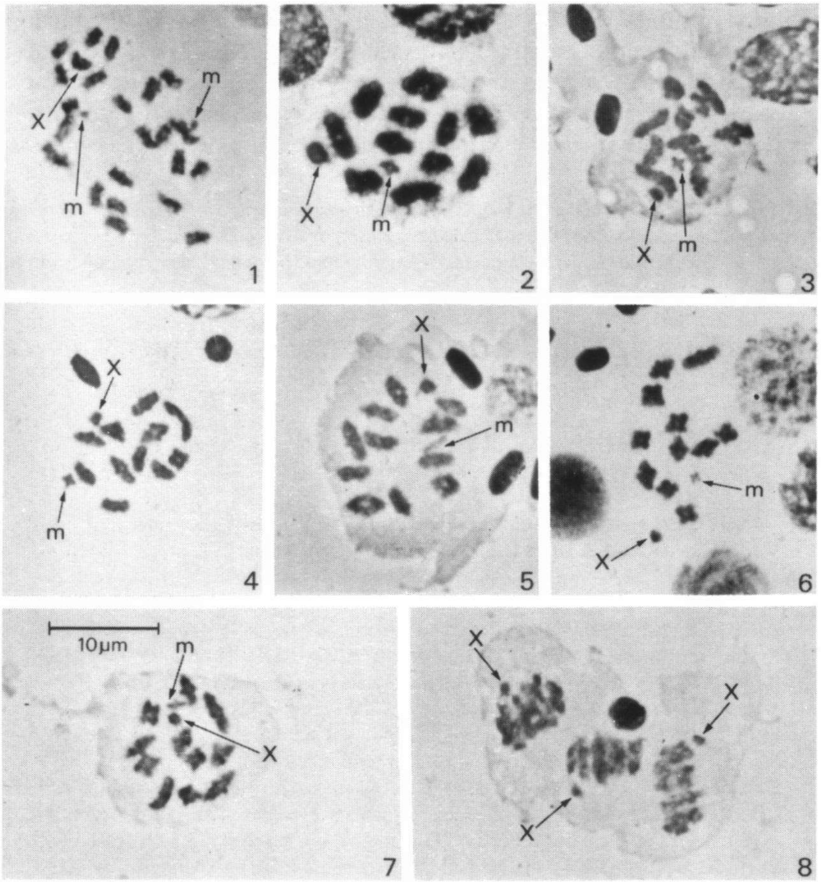
KARYOTYPIC MORPHOLOGY OF THE *CROCOTHEMIS SERVILIA* COMPLEX

CROCOTHEMIS SERVILIA (DRURY) SENSU LATO (non-Japanese populations)

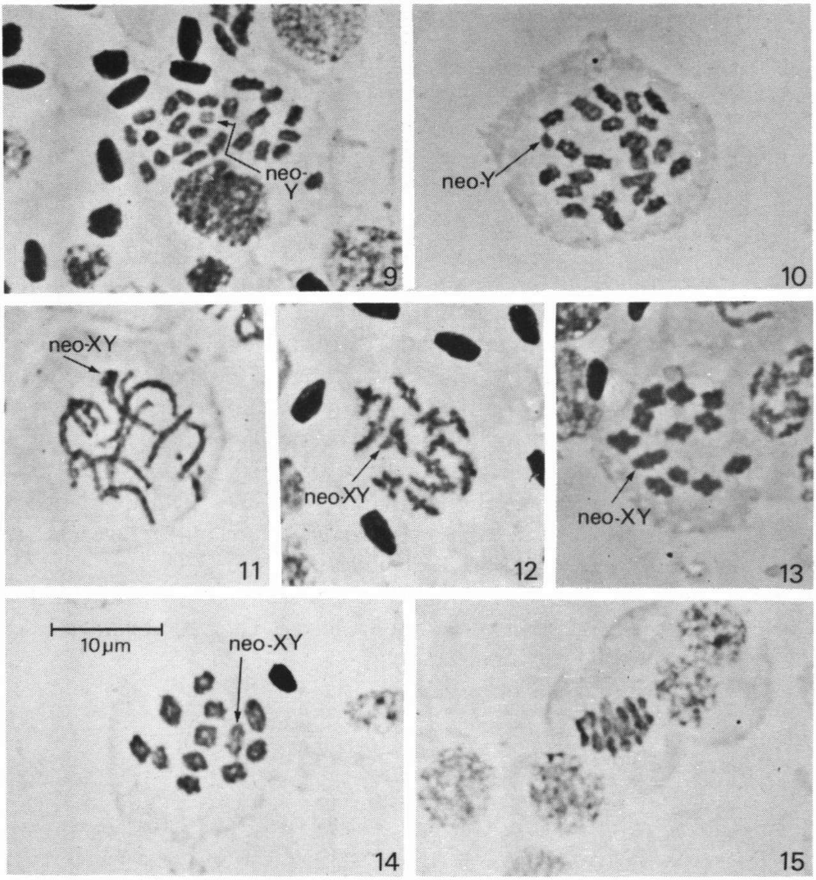
Figures 1-8

[Annotated references; all under *C. servilia* (Drury), unless stated otherwise].

India — ASANA & MAKINO, 1935: 69, 76-77, 84, 86, fig. 6 (♂ M m, I m, II m, m-a; drawings, 4200 X): Jogeshwari, Bombay (description of spermatogenetic stages); — MAKINO, 1935: 234 (abstract from Asana & Makino, 1935); — KICHIJO, 1942: 1090, 1091, fig. 23 (note on spermatocyte chromosomes, with fig. of ♂ I m from Asana & Makino, 1935); — RAY CHAUDHURI & DAS GUPTA, 1949: 82, 86-87, 92, fig. 3 (♂ I M m, I d, m, II m-a; drawings, 3000 X): Calcutta (full description of spermatogenetic stages); — BAGGA, 1961: 9, 22-23, 32, pl. 3: fig. 1 (♂ M m, I d, m, a. II m, a; drawings, magnification not stated), pl. 6 (♂ I m idiogram), tab. 3 (chiasma frequency): Delhi (description of spermatogenetic stages); — GANDHI, 1973: 46-47, 51-52, 60, 53-55): Chandigarh; Punjab (description of spermatogenetic stages, chiasma frequency, and karyotype morphology); — TYAGI, 1978:154-158, textfigs 21 (idiogram), 35 (ratio longest-bivalent : X), 36 (absolute length X), pl. 37: figs 273-279 (♂ M m, I p, d, m, m-a, II m, t; micrographs, 4000 X), tabs 13 (TCL), 15 (length X): Dehradun, Sulphur Springs, Goolar Ghati, Raiwala; all Uttar Pradesh (karyotypic morphology and chromosome behaviour; sub "C. *servilia* ssp."); — YADAV, 1979: 77-79, fig. 2 (♂ M m, I m; micrographs, 1500 X), Kurukshetra (notes on the karyotype, discussion on karyotypic variation in various geographic populations); — MITTAL & GANDHI, 1982: 16, figs 1, 3-4 (♂ M m, I m; micrographs and drawing; magnification not stated), Chandigarh; Punjab (notes on spermatogonial and spermatocyte I complements). — Nepal — KIAUTA, 1975: 45, 74, fig. 27 (♂ I m, micrograph, 1500 X): Bodnath, Chauni, both Kathmandu Valley (chromosome number and note on the karyotype); — KIAUTA & KIAUTA, 1982a: 145, Hile-Tumlingtar, in eastern Nepal (chromosome number and note on the karyotype). — Thailand — KIAUTA & KIAUTA, 1983b: 28, Huey-Kaew (Chiang Mai) Phai-Hin (Chiang Mai), Bangkok (2 populations) (chromosome number and note on the karyotype). — Singapore — KIAUTA & KIAUTA 1982b: 164 (chromosome number and notes on the karyotype). — China (Peoples' Republic; continental) — Personal communication from Dr Hui-qian Zhu, dated September 10, 1983: Hangchow. [Verbatim text, grammatically slightly corrected]:



Figs 1-8. *Crocothemis servilia* (Dru.) s.l., spermatogenetic stages in various non-Japanese populations, all characterized by 2n=25, n=13 chromosome numbers, and by the presence of a small *m*-pair bivalent: (1) Spermatogonial metaphase (Manila, the Philippines); – (2) Metaphase I (Kathmandu Valley, Nepal); — (3) Early metaphase I (Arun Valley, eastern Nepal); — (4) Early metaphase I (Bangkok, Thailand); — (5) Early metaphase I (Singapore); — (6) Late diakinesis / early metaphase (Manila, the Philippines); — (7) Early metaphase I (Taipei, Taiwan); — (8) Early anaphase II, showing the formation of an "accessory plate", by the X (Taipei, Taiwan). — [1500 X; – Fig. 2 is a lacto-acetic-orcein squash, photographed several weeks after fixation, hence the unusually large size of all elements; all other figs are Feulgen squashes].



Figs 9-15. *Crocothemis servilia mariannae* ssp. n., various spermatogenetic stages, characterized by 2n=24, n=12 chromosome numbers, lack of *m*-chromosomes, and by the presence of neo-X and neo-Y elements (Osaka; Feulgen squash, 1500 X): (9-10) Spermatogonial metaphase; — (11) Pachytene (note the heteropycnotic section on the neo-sex trivalent, representing the original X); — (12) Late diakinesis; (13-14) Metaphase I; (15) Anaphase II, during which the neo-sex element does not form an "accessory plate". [All micrographs from the holotype].

"We were going to Hangchow [...] in July and August, [and have] collected [...] there a specimen of *Crocothemis servilia* (Drury). The preparations and micrographs were made by me. The spermatogonial elements, save for the *m*'s, are gradually decreasing in magnitude, the *m* and X are similar in size. [The karyotype] is similar to fig. 2 of Yadav, 1979". The Philippines KIAUTA & KIAUTA, 1980: 242-243, figs 13-15 (♂ M m, I m; micrographs, 1500 X): Manila (karyotypic morphology and comparison with the continental populations); — KIAUTA & KIAUTA, 1983a: 14: La Trinidad nr Baguio, Luzon (notes on the karyotype).

Additional material — Taiwan (insular province of China): 1 ♂, Waisuangshi (Taipei), 12-VIII-1980; 1 ♂, Shihting (Hsien Prov.), 13-VIII-1980. — [4 slides, 43 micrographs].

Within this range, the species possesses a typical libellulide karyotype, 2n ♂ = 25, n ♂ = 13, *m*, XO. The autosomes and primary spermatocyte metaphase bivalents show little size gradation. The X is small, and the *m*'s are minute. At I m, the *m*-bivalent is similar in size to X, but more often its length and volume are slightly or considerably inferior to those of the sex element at this stage. The TCL values, the absolute lengths of various marker elements (longest bivalent, *m*-bivalent, X), and various ratios between these were stated for a number of Indian populations. It is believed that the considerable variation of these figures is due to different fixation techniques and to the lack of uniformity of the criteria of measuring rather than to an actual variation between geographic populations. It is likely, however, that the absolute and relative sizes of X and *m* are subject to geographic variation. In view of their minute size, an adequate statistical treatment would present considerable technical difficulties, and a huge material would be needed to obtain statistically significant values.

Including the material on which our earlier publications were based, almost 600 diakinetic and metaphase-I figures were examined. The recombination index in the male is 24, with few exceptions, if any.

The anaphase-II behaviour of X is normal (cf. Fig. 8).

CROCOTHEMIS SERVILIA MARIANNAE SSP. NOV.

Figures 9-15

Crocothemis servilia (Drury): OMURA, 1955: 96, 97, 112-113, 129, 135 fig. 7 (♂ M, I, II; drawings, 1790 X): Okayama, Japan (full description of spermatogenetic cycle).

Material — Japan: 3 ♂, Nagai Park, Osaka, 1-VIII-1980 (microscopic slides and corresponding specimen Nos OB 1a-c, OB 2a-b, OB 3a-b resp.); — 1 ♂, Hachigamine Hills, Sakay City, Osaka Prefecture, 2-VIII-1980 (No. HV 1a-c); — 1 ♂, lake behind the Kyoto International Conference Hall, 9-VIII-1980 (No. KL 1a-b). — [24 slides, 154 micrographs].

The specimen No. OB 3a-b is the holotype, the other are paratypes. All matarial is deposited in the author's collection.

The new taxon is distinct from all other known populations of the *Crocothemis servilia* group in the following karyomorphological features: (1) the chromosome number is 2n ♂ = 24, n ♂ = 12, without any distinctly small elements in the set at any stage; — (2) sex determination is of the neo-XY mode; and — (3) the sex element does not form an accessory plate at metaphase II. These features invariably occur in all specimens examined, are not subject to variation, and are expressed in, and restricted to the Japanese material only.

It goes without saying that the *C. s. mariannae* ssp. n. complement is of a secondary origin, the result of a fusion of the X of the primary complement with one of the autosomes. The fusion is permanent and irreversible in all material examined.

The *mariannae* complement is characterized by small gradation of elements. This applies to mitotic metaphase (Figs 9-10), as well as to primary spermatocyte metaphase bivalents (Figs 12-15). As apparent from the magnitude of the heteropycnotic section appearing in one of the elements at pachytene (Fig. 11) and representing the original X, the latter was small, not unlike that known in the non-Japanese forms. On the other hand, the karyotype of the direct ancestor of *mariannae* must have been already clearly distinct from all other known forms in having no *m*-chromosomes. This is demonstrated by the relatively large size of the neo-Y, which is but slightly inferior to the other mitotic metaphase elements (autosomes).

Since the original sex element is involved in fusion, causing trivalent formation at diakinesis, the recombination potential is slightly increased, thus promoting the flexibility of the Japanese form, i.e. the ability of its genotype to vary and adapt to changing conditions. This would be in agreement with a tentative assumption that *mariannae* represents a phylogenetically young offshoot of the continental *servilia* stock.

It is interesting that, cytogenetically, the Philippine and Taiwanese populations are identic to the continental populations, and show no affinities with the Japanese form. The contact zone between the continental and Japanese subspecies must be somewhere in the area of the southern Japanese island chain, between Taiwan and the Japanese "mainland". We had no opportunity to examine these populations.

If one could assume the centre of origin of *servilia* to lie somewhere in southeastern Asia, it would be interesting to know whether or not karyomorphologically different forms have evolved elsewhere in the temperate conditions of the marginal areas of the *servilia* range.

ACKNOWLEDGEMENTS

I am thankful to Dr M.A. LIEFTINCK (Rhenen) for numerous discussions on the problem of infraspeciation in *C. servilia*, and for his encouragement during the preparation of this paper. He also brought me in touch with Professor Dr L.B. HOLTHUIS of the International Commission on Zoological Nomenclature, whose positive opinion was considered decisive for the publication of this note. In selection of the new name I did not follow Dr Lieftinck's suggestion to introduce a term indicating the peculiar karyomorphological features of the new taxon, but have decided to rather name it after my wife, MARIANNE, without whose help I would not have been able to sort out the cytology of this interesting case. It goes without saying that warm thanks are also due to my friends, Dr SYOZIRO ASAHINA (Tokyo) and Mr KIYOSHI INOUE (Osaka) and to the colleagues of the

Kansai Research Group of Odonatology, whose hospitality and assistance during our two visits to Japan enabled us to examine the cytology of the Japanese populations. Last but not least, it should be strongly emphasized that without the help of Dr ZHU HIU-QIAN (Taiyuan, Shanxi), the erection of a new taxon for the Japanese population would be entirely impossible.

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