THE OVARIAN POLYTENE CHROMOSOME OF THE TAXON *ANOPHELES* (CELLIA) *SUNDAICUS* (RODENWALD)

Supratman Sukowati *

ABSTRAK

Penelitian khromosom politen ovarium nyamuk Anopheles sundaicus *Rodenwald, 1925 telah dilakukan dari populasi alam di Pangha (Thailand Selatan), Trat (Thailand Timur) dan Pangandaran (Jawa Barat, Indonesia). Metode penelitian khromosom politen berdasarkan Green dan Hunt (1980). Pola penggelangan khromosom di dalam populasi dan antara populasi dibandingkan. Jumlah khromosom karyotipe metaphase spesies tersebut terbukti sama dengan jumlah khromosom nyamuk dari kelompok anopheline (2n = 6), yang terdiri dari satu pasangan khromosom-X dan dua pasang autosom. Khromosom politen* An. sundaicus *ditemukan dapat berkembang dengan baik, sehingga peta photo politen ovarium dapat disajikan sebagai peta baku khromosom politen, dan digunakan sebagai bahan acuan takson ini. Pola penggelangan yang jelas dan tanda-tanda yang konsisten, merupakan ciri-ciri dari setiap lengan khromosom. Perbandingan pola khromosom politen* An. sundaicus *di dalam populasi dan antara populasi Pangha, Trat dan Pangandaran dengan peta baku khromosom politen belum ditemukan adanya variasi atau polimorphisme khromosom.*

Introducton

The mosquito *Anopheles sundaicus* is the principal vector of human malaria in coastal areas of Indonesia [1,2,3,4,5]. It is a widely distributed species and occurs in India, Burma, Malaysia, Thailand, Indonesia, China and Indochina [6]. This species is primarily anthropophilic [3,7], even though in some places, e.g. Purworejo, Central Java and Kulon Progo, Yogyakarta they are known to be zoophilic [8,9]. It has been reported that *An. sundaicus* has two breeding types, i.e. brackish-water breeding [2] and fresh water breeding [10]. In almost all of the areas, this species has been found to be exophilic, except in Kampung Laut, Cilacap, where it was found to be endophilic and has become resistant to DDT [11]. Thus within brackish-water breeding *An. sundaicus*, there appears to be distinct population differing in feeding and resting behavior which in turn influence their vectorial capacity and response to control measure.

Therefore these observations seem to suggest that An. sundaicus is a cluster of some closely related species which play a major role in malaria transmission in their distributions.

Genetical aspect studies are of fundamental importance in understanding the biology of the species, and genetic studies on anopheline mosquitoes had been closely related

* Health Ecology Research Centre, National Institute of Health Research and Development. Jl. Percetakan Negara 29, Jakarta Indonesia.

to their medical importance. The practical value of mosquito cytogenetics has increased in recent years, particularly in connection with the problem of identification of members of the malaria vector. Pioneering work by Frizzi on Palearctic *An. maculipennis* complex and the studies of Kitzmiller and his collaborator on the Nearctic members of this group demonstrated the value of cytogenetic in identifying members of sibling species complexes and tracing their evolutionary relationships [12].

In 1968 Coluzzi found that good polytene chromosomes were present in ovarian nurse cells of adult female mosquitoes. Nurse cell polytene chromosomes have been shown to be useful for identifying the members of the An. gambiae complex[13,14,15,16]. *An. culicifacies*,[17,18] *An. maculatus*.[19]

Little is known about *An. sundaicus* and related species from the genetic point of view, in relation to their role as vector of malaria in Southeast Asia. The genetic studies including cytogenetic and isozymes of different populations of *An. sundaicus* were carried out.

We present a photomap of the ovarian polytene chromosomes of *An. sundaicus* which we have designated as "standard" for that taxon. We also report the results of a survey of the polytene chromosome arrangements relative to the "standard" arrangement in a natural population of An. sundaicus.

Materials and Methods

Wild cought females of *An sundaicus* were collected from the coastal areas of the following localities: (i) Pangha district, Southern part of Thailand on March 1992, (ii) Trat district, Eastern part of Thailand on April 1993 and (iii) Pangandaran district, West Java, Indonesia on August 1992. Collections were made at night time by using animal bait and landing collection on man.

The blood fed female mosquitoes were held at 20°C with relative humidity of 80%. About 24-36 hours later (half gravid), ovaries were dissected and put on labelled filter paper and placed in freshly prepared modified Carnoy's fluid and kept at low temperature (4°) for at least 24 hours.

The polytene chromosomes were spread and processed according to the methods of Green and Hunt (1980)[20]. The chromosome photographs were made by using a phase contrast microscope and made on Kodak Technical Pan Film 2415. A chromosome standard map was made according to methods of Hunt (1984) and Stalker (1964).[21,22]

Determination of banding sequence of unknown specimens was accomplished following the methods of Carson (1970)[23] by comparing the standard photomap and the chromosome specimen directly through the microscope with the aid of Camera Lucida attached to a Nicon microscope.

Readable polytene preparations were made from 57 females collected in Pangha, 6 females collected in Trat and 39 females collected in Pangandaran.

Results

The standard photomap of ovarian nurse cell polytene chromosome for the taxon An sundaicus is presented in figure 1. Polytene chromosome of *An. sundaicus*, present of the genus *Anopheles* revealed a karyotype of three pairs (2n = 6), comprises of three synapse elements: a short sex chromosome and two longer autosome (chromosome 2 and 3) labelling of 2R /arm 2, 2L /arm 3, 3R /arm 4 and 3L /arm 5. The right and left arm are almost equal in chromosome 2 and unequal in chromosome 3. The ovarian polytene chromosome complement of *An. sundaicus* has been divided into 50 zones arbitrarily, starting with free end of the x-chromosome. The x-

chromosome contains zone 1-6; 2R zone 7-19; 2L zones 20-30; 3R zones 31-39 and 3L zones 40-50. In the map, arrow indicates the positiion of centromere. A careful examination of chromosome in freshly stained slide has been done, and it is easier to recognize and characterize the band of each chromosome and is possible to describe any difference. There is no inversion nor polymorphism detected, all of the samples had the same chromosomal arrangement as the standard.

Description of chromosome (figure 1)

X-Chromosome

As usual the chromosome in the genus *Anopheles* the x-chromosome in *An. sundaicus* is identified by its shortest size. In the most preparation, this chromosome is very distinct with flare and large centromore.

Zone 1 marks the free end of x-chromosome, it is rounded in appearance and diffuse at its tip. The occurance of four faint dotted bands with the same curvature is constant feature on the tip. It shows a faint area at the terminal curvature, and followed by a short dark band, and makes a constriction in this zone. Behind the constriction is a light broad dotted band, two light short bands and two dark bands respectively. The zone 2 begins with light dotted bands followed by one prominent dark band and slight swelling with one dark band in the midle. The remaining part of zone 2 composed of four prominent dark bands, one dotted band and three dark bands. The following zone 3 has one small swelling with one light and four dark bands respectively. Zone 4 has four dark bands, two light dotted bands and three dark bands. The following region is a very large puff in zone 5, composed of a pair of heavily stained broken band placed in the middle of the puff followed by a broad dark band, one dotted band and short dark band. The centromeric end covered by zone 6, has three pairs prominent dark bands, which make this region together with zone 5 is an important landmark for identification of x-chromosome.

2 R Chromosome /arm 2

It is the longest among four autosomal arms, the free end in zone 7 has two dotted bands at the tip and followed by two dark bands. The remaining part of zone 7 has a large puff having five dotted light bands. The following zone 8 marked by a closely located set of four bands, consist of one dark band, two dotted bands and one dark band respectively. A second series of closely located set of six dotted bands which is separated from the first series by a light area. The region behind it covered by 4 pairs of dark bands.

Another characteristic feature for recognizing for the 2R chromosome is the zone 9. It is a long puff consisting of 9 bands in series of two dark bands, six dotted dark bands and one prominent dark band. The zone 10 and 11 consists of several dark bands; two small puff having dotted bands in first part of zone 12, continued by one dark band, two dotted bands, one dark band and six dotted bands placed in series respectively. Zone 13 begins closely located sets of the three dotted bands, continued by two consisting of several dark bands; two small puff having dotted bands in first part of zone 12, continued by one dark band, two dotted bands, one dark band and six dotted bands placed in series respectively. Zone 14 is characterized by two swellings composed of three and two dotted dark bands in each. The succeeding zone 15, 16, 17 and 18 are darker in an appearence due to a number of dark bands closely located. Zone 18 provides a landmark due to six rows of wafy dark bands. Zone 19 or the centromeric zone is characterized by two pairs of dark bands, with a few light dotted bands in between.

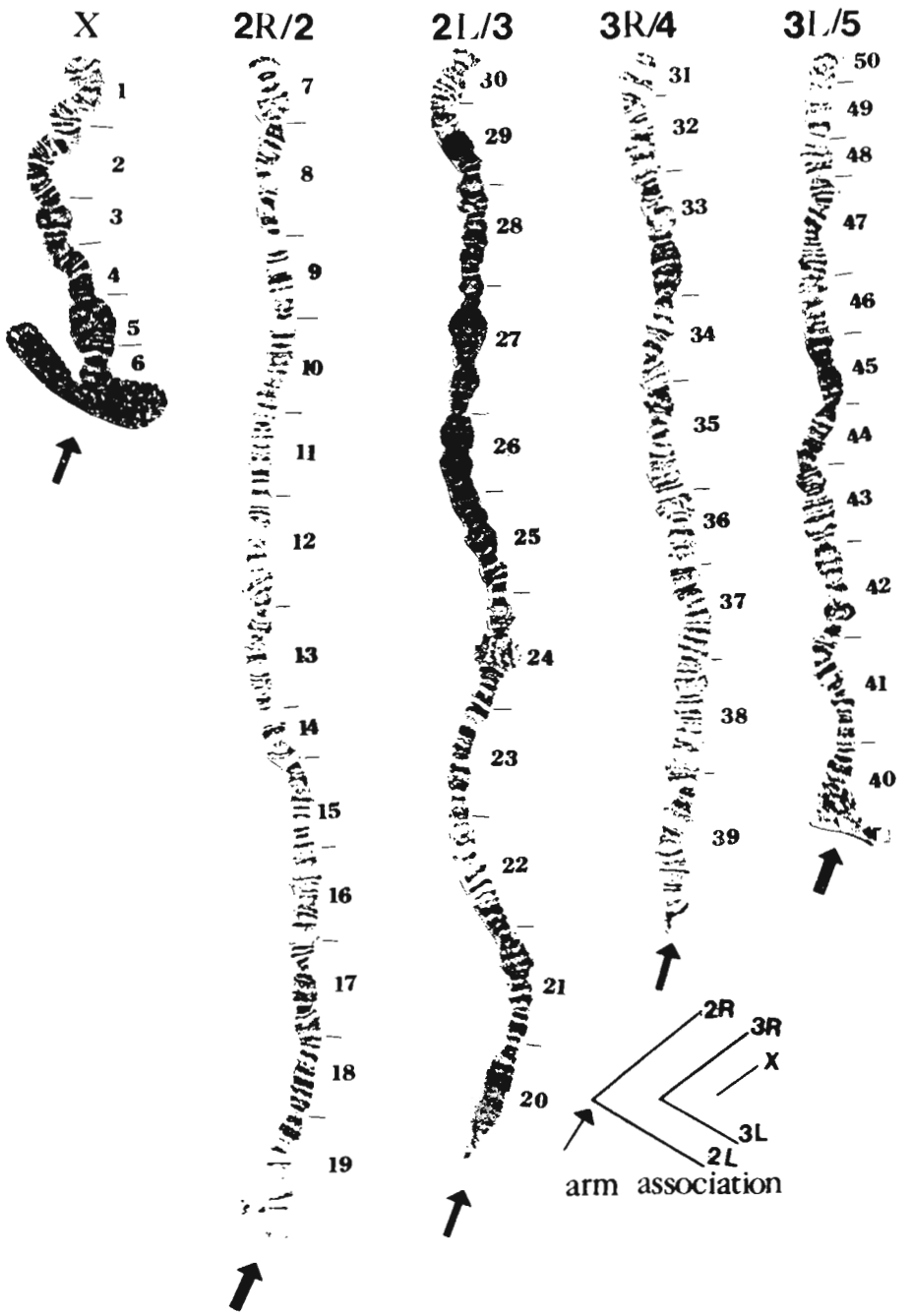

Figure 1. Photo map of the ovarian polytene chromosome arrangement designated as "standard" for the taxon *Anopheles sundaicus* (Rodenwald), 1925.

2L Chromosome /arm 3

Zone 30 marks the free end of 2L, a funnel shaped free end with dotted bands and flare at its tip. It is followed by one dark band, and then one puff consist of six dotted bands arranged in two series, three dotted curve band in each. Zone 29 is composed of one dark band, two curve dotted bands, six bands closely placed in series, and three pairs of prominent dark bands respectively. The following zone 28 is characteristically marked by a pair of slight swelling which have four dark bands in each with the light area in between.

The most conspicuous characters 2 L is moderate swelling in zone 27, its began five prominent short dark curving bands, so its form constriction like a neck, then followed by a moderate swelling composed of two light dotted bands, and one prominent dark band. The rest zone 27 composed of two dotted bands, one dark band, two dotted bands, one dark band and a pair of dark band respectively. The subsequent zone 26 and 25 composed of several small swelling with dark and dotted bands in alternate.

Another characteristic of arm 2 L is the present of a pair of puff in zone 24, one swelling is medium size and the latter is a large in size. The medium size puff has dotted curve banding, and in the large puff composed of diffused banding, and a dark band placed in between the puffs and at the end of the last puff. The subsequent zone 23, 22 and 21 contain numbers of dark bands giving a dense appeareance to this region. At the centromeric end, zone 20 of arm 2L, start with two small dark bands followed by liht pair of dotted faint bands, it reveals a faint area at the centromeric end.

3R Chromosome /arm 4

The free end in zone 31 has a diffused character at its tip, followed by a pair of dotted band and moderate swelling with two dotted bands having apposing curvatures, The next zone 32 starts with three pairs of dark bands, followed by sequence of 3:1:1 dotted bands, one prominent dark band and the rest is a small swelling composed of two curve dotted bands. Two moderate swellings with several dotted bands curving away from each other is characteristic of zone 33, and it begins with a closely placed set of three dark bands. Two slight and two medium swelling alternate in zone 34 containing several straight bands. Zone 35 starts with two prominent dark bands, and two broken dark bands followed by one light band and two dark bands. The rest of zone 35 is a medium swelling consisting of one broken dark band, a pair of dotted dark band, three pairs of dotted light band and a pair of dark band respectively. Zone 36 posseses diffused bands and two dark bands between.

Another important aid in the identification of 3R is provided by a characteristic of an intensity of seven bands closely placed in series in zone 37. This series composed of two dark bands, three dotted wafy bands, one dark band and two dotted wafy band respectively. The rest of bands in zone 37 are either lightly or moderately stained, contains diffuse bands, one broken dark band and a pair of dotted light bands. The following zone is characteristically marked by a pair of medium swelling, which have dotted curve bands in the first part and diffuse bands in the last part. Zone 39 is the last one that covers the centromeric end of 3R chromosome, it is composed of many distinct

thick dark bands. The composition of the bands is as follows: a pair of dark bands, diffuse bands, a pair of broken bands, one dark band, diffuse band in the middle of a puff, a series of dotted light bands, one broad dark band, three light dotted bands, a pair of thick dark band, light faint band, and at the adjacent to centromere is a thick dark band.

3L Chromosome/ arm 5

It is the shortest among the four autosomal arms in the complement but longer then x-chromosome. The free end in zone 50 has flared character at its width tip several light bands, followed by a pair of curved dark dotted bands and several diffused dark bands. Zone 49 starts with one light band, continued by one dotted dark band, two light bands, one dark band, two pairs of dotted light bands, a pair of dark bands, one dotted band and one short dark band respectively. The following zone 48 is characteristically marked by a slight swelling which contains of two pairs of dark bands. Zone 47 consisted of several dark bands, it begins with three closely dotted light bands, continued by one dark band, a pair of dotted dark bands, a pair of prominent dark bands, one light band, one dark band, two short dark bands, and followed by five dotted dark bands. Zone 46 contains many light faint bands, and two dotted bands located at the end of this zone.

Zone 45 is darker in appearance due to a number of dark bands, composed of two prominent dark bands, three light dotted bands, two dark bands, three light dotted bands, and two prominent dark bands respectively. Zone 44 begins with closely placed sets of four dark bands, continued by a pair of light bands, one prominent dark band, one light band, one dark band and six curved dotted bands placed in series. This is preceded by zone 43 which has three moderate swellings with wafy dotted dark bands.

Another characteristic of arm 3L is the presence of a medium puff having diffused dark bands in zone 42. Zone 41 starts with two dotted light bands and one dark band followed by a pair of dotted light bands, a pair of dark bands, a pair of faint light bands, two pairs of dark bands and light bands, one prominent dark band, and three short dark bands closely placed sets in series. The rest of zone 41 consist of several diffused bands. The centrometric end, zone 40 composes of one prominent dark band, a pair of prominent dark bands, two dotted wafy bands and several faint light bands respectively. Zone 40 provides landmark of arm 3L due to diffuse bands at the adjacent to the centromeric end.

Discussion

One of the essential requirements of cytogenetic investigation of mosquitoes is the preparation of "standard" polytene chromosome maps such as having been done i.e. *An. subpictus, An vagus, An. hyrcanus nigerrimus* and *An. barbirostris* [24], *An. albimanus* [25], *An. nili* [26], *An. aconitus* [27], *An. culicifacies* [28], *An. quadrimaculatus* [29]. The difference in the banding pattern and the presence of chromosomal polymorphism or inversion observed among and within population of *An. sundaicus* might be useful as a diagnostic

character in distinguishing cryptic species and chromosomal variability.

The absence of chromosomal polymorphism in these three populations from difference geographical area might be due to no detectable differences in the banding patterns of polytenes (homosequential) in this species. The homosequential banding patterns of polytene chromosome had been reported from some species : eg. *An. atroparvus* and *An. labranchiae, An. funestus* and *An. aruni*, and seven species of the *An. hyrcanus* group [30]. Alternatively, this species might have inversion or polymorphism in polytene chromosome, however it has not been found yet due to the small number of sample being studied. Nevertheless, this standard photomap of polytene chromosome provide a reference point for further studies, where more of natural populations would be carried out together with the necessary taxonomic studies of chromosomally identified materials.

Aknowledgement

The author wish to thank the UNDP/World Bank Special programme for research and training in tropical diseases for the fellowship for Ph.D programme under project of WHO Reasearch Training Grant. This research is part of the Dissertation We are especially indebted and grateful to National Institute of Health Research and Development for it support and facilities for this research. Sincere thanks are extended to all staff and technician of Department of Biology, Faculty of Science, Mahidol University and technicians of Health Ecology Research Centre, National Institute of Health Research and Development, Jakarta for their cooperation.

References

1. Koesoemowinangun, W.R. (1954). Anopheline di Indonesia. Lembaga malaria, Kementerian Kesehatan Republik Indonesia, Jakarta. 191 pp.

2. Sundararaman, S., soeroto, R.M. & Siran, M. (1957). Vector of make malaria in Mid Java. Indian J. Malariol. 11, 4: 321-338.

3. Collins, R.T., Jung, R.K., Hasan Anoer, Soetrisno, R.H & Putut, D. (1979). A study of the coastal malaria vectors, Anopheles sundaicus (Rodenwald) and Anopheles subpictus Grossi in South Sulawesi. WHO/VBC/79.740.

4. Soekirno,M., Bahang, Y.H., Sudomo, M. Tjokorda Putra Pemayun & Flemming, G.A. (1983). Bionomic of Anopheles sundaicus and other anophelines associated with malaria in coastal areas of Bali. Indonesia. WHO/VBC/83.885.

5. Lien, J.C., Koesman,L., Partono, F. Joesoef,A., Kosin E and Cross, J.H. (1975). A brief survey of mosquitoes in North Sumatera, Indonesia. J.Med. Entomol. 12; 2: 223-239.

6. Bruce-Chwatt,L,J, (1985). Essential Malariology. 2nd edn. William Heinemann Medical Books Ltd. London. 45.2 pp.

7. Jica report, (1985). Ecological Aspects and control strategy of Anopheles sundaicus A coastal malaria vector in the Asahan Regency. North Sumatera. July, 1985.

8. Ngadiyo. (1985). Ekologi Anopheles sundaicus (Rodenwald) betina di pantai selatan Kabupaten Kulonprogo, Yogyakarta. Report. Kanwil Departemen Kesehatan. Yogyakarta.

9. Kirnowardoyo, S. (1988). Anopheles malaria vector and control measures applied in Indonesia. Southeast Asian J. Trop. Med. Pub. Hlth. 19, 4:713-716.

10. Bonne-Webster, J., Swellengrebel, N.H. (1953). The anopheline mosquitoes of the Indoe-Australian region. Amsterdam, de Bussy, 506pp.

11. Kirnowardoyo, S. (1985). Pengendalian Anopheles sundaicus Rodenwald di Kampung Laut, Cilacap, Jawa Tengah. Seminar Parasitologi Nasional dan Kongres PAI III, Yogyakarta. 12-24 Desember 1985.

12. White, G.B., Coluzzi, M. and Zahar, A.R. (1975). Review of Cytogenetic studies on Anopheline vectors of malaria. World Health Organization Document. WHO/VBC/75.538.

13. Coluzzi, M. (1968). Chromosomi politinici delle cellule nutrici ovariche nel complesso gambiae del genere Anopheles. Parasitiiogia. 10:179-184.

14. Service, M.W. (1970). Identification of the Anopheles gambiae complex in Nigeria by larval and adult chromosomes. Annal of Tropical Medicine and Parasitology 64:131-136.

15. Green, C.A. (1972). Cytological maps for the practical identification of the three fresh water species of the Anopheles gambiae complex. Annal of Trop. Med. and Parasitology 64:131-136.

16. Hunt, R.H. (1972). A comparison of the X-chromosomes of the ovarian nurse cells of species B and species C of the Anopheles gambiae complex. Soc. of Trop. Med. and Hygiene. 66.1.

17. Green, C.A. and Miles, S.J. (1980). Chromosomal evidence for sibling species of the malaria vector Anopheles (Cellia) culicifacies Giles. J.of Trop. Med. and Hygiene. 83: 75-78.

18. Subbarao, Sarala, K., Vasantha,K., Adak,T. and Sharma, V.P. (1983). Anopheles culicifacies. Evidence for new sibling species, species C. Ann. Entomol. Soc. Amer., 76: 985-988.

19. Green, C.A. and Baimai, V. (1985). Cytogenetic evidence for a complex of species within the taxon Anopheles maculatus (Diptera:Culicidae). Biological Journal of the Linneaus Soc. 24: 321-328.

20. Green, C.A. and Hunt, R.H. (1980). Interpretation of variation in ovarian polytene chromosome of Anopheles funestus Giles, A. parensis Giles and A aruni (?) Genetica, 51: 187-195.

21. Hunt, R.H. (1984). Natural and induced chromosomal arrangements in three members of the Anopheles gambiae complex. A MSC Dissertation submitted to the Faculty of Science University of Withwatersrand, Johanesberg.

22. Stalker, H.D. (1964). Chromosome polymorphism in Drosophila euronotus. Genetics 49:669.

23. Carson, H.L. (1970). Chromosome tracers of the origion of species. Science 168:1414.

24. Chowdaiah,B.N., Avirachan,T.T. and See Tharam P.L. (1967). Salivary gland chromosomes in four species of Tropical Anopheline : A. subpictus, A. vagus, A. hyrcanus nigerrimus and A. barbirostris. Proc. Acad. Sci., Vol 55 (12):181-186.

25. Keppler, W.J., Kitzmiller, J.B. and Rabbani, M.G. (1973). The salivary gland chromosome of Anopheles albimanus. Mosq. News. 33 :42-49.

26. Miles, S.J., Carvevale, P. and Green, C.A. (1984). The ovarian polytene chromosome of the taxon Anopheles (Cellia) nili Theobald. Cah. O.R.S.T.O.M., Ser. Ent. Med. et. Parasitol., Vol. 22, 1:9-11.

27. Sukowati, S. (1987). Species kompleks vektor malaria Anopheles aconitus. Risalah Kongres Ilmu Pengetahuan Nasional IV. Jakarta 447-460.

28. Saifuddin, U.T., Baker, R.H. and Sakai, R.K. (1978). The chromosome of Anopheles culicifacies. Mosq. News. 38: 233-239.

29. Klassen, W., French, W.L., Laven, H and Kitzmiller, J.B. (1965). The salivary chromosome of Anopheles quadrimaculatus. Mosq. News. 25;3:323-334.

30. Green, C.A. (1985). A Critical Review of the current laboratory methods used in species studies and their translation into routine malaria entomology. Working papers presented at an informal consultation of the Scientific working group on applied field research in Malaria. UNDP/ WHO.TDR. Thailand, 29 Ocftober-3 November 1984.