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Review of the Minimus Complex of *Anopheles*, main malaria vector in Southeast Asia: from taxonomic issues to vector control strategies

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Summary

BACKGROUND The Minimus Complex of *Anopheles* subgenus *Cellia* is composed of two sibling species, A and C, on the Southeast Asian mainland, and a third allopatric species E that occurs in the Ryukyu Archipelago (Japan), a malaria-free region. *Anopheles minimus* s.l. is considered to be one of the main malaria vector in the hilly forested regions of Southeast Asia. Despite a large number of studies over its range of distribution, it is difficult to have a global view of the ecological and bionomical characteristics of the individual species as different identification methods were used, generally without specific identification of the sibling species.

OBJECTIVES (1) To review the main malaria studies on *An. minimus* s.l.; (2) to discuss recently published data on the biology and ecology of each sibling species; and (3) to identify gaps in our understanding of the Minimus Complex.

REVIEW RESULTS Major biological and ecological trends are addressed, such as the high plasticity of trophic behaviour and the sympatry of species A and C over the Southeast Asian mainland. Despite the availability of rapid molecular identification methods, we still lack important information concerning the biological characteristics of each sibling species. These gaps must be filled in the future because *An. minimus* species A and C may exhibit different abilities to transmit malaria.

CONCLUSION We expect that entomological surveys will employ molecular methods to clearly identify these two species, and thus elucidate the biological characteristics of each species. As a consequence, current vector control strategies will be improved by targeting the most efficient vectors.

keywords *Anopheles minimus* s.l., sibling species, distribution, bionomics, malaria, Southeast Asia

Introduction

A clear and comprehensive understanding of malaria transmission dynamics is crucially needed in the context of implementation and development of malaria control strategies. This is achievable only through a thorough knowledge of the anopheline vectors. Three main malaria vectors are recognized in Southeast Asia: *Anopheles dirus* sensu lato (Dirus Complex) occurring in forested areas, *An. minimus* s.l. (Minimus Complex), widespread in the hilly forested regions, and *An. sundaicus* s.l. (Sundaicus Complex), a brackish water mosquito present along coastal areas. The Minimus Complex belongs to the Myzomyia Series, Funestus Group,[1] with 24 other species that are distributed in various areas across southern Asia and sub-Saharan Africa (Harbach 2004; Garros *et al.* 2005a,b). The Minimus Complex includes two sibling species on the Southeast Asian mainland, species A and C, and a third species, named E, in the Ryukyu Archipelago of Japan (Green *et al.* 1990; Somboon *et al.* 2001; Harbach 2004).

Current knowledge of the *An. minimus* s.l. vector system is incomplete. Although extensive work on the bionomics or ecology of the complex was carried out in different sites over the range of distribution, it was done at local scales using different identification methods. Moreover, misidentification of species closely related to this complex is not uncommon (Van Bortel *et al.* 2001). Thus, assessment of the biology of the species is imprecise

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[1] Funestus Group: *An. jeyporiensis*; Aconitus Subgroup: *An. aconitus*, *An. filipinae*, *An. mangyanus*, *An. pampanai*, *An. varuna*; Culicifacies Subgroup: *An. culicifacies* A, B, C, D and E; Funestus Subgroup: *An. aruni*, *An. confusus*, *An. funestus*, *An. parensis*, *An. vaneedeni*; Minimus Subgroup: *An. flavirostris*, *An. fluviatilis* T and U, *An. leesoni*, *An. minimus* A, C and E; Rivulorum Subgroup: *An. brucei*, *An. fuscivenosus*, *An. rivulorum*, *An. rivulorum*-like sp.

or misleading because most of the earlier studies undoubtedly refer to other or a mixture of species. Since the discovery of species A and C (Green *et al.* 1990), and despite the development of simple molecular identification methods (Van Bortel *et al.* 2000; Phuc *et al.* 2003; Garros *et al.* 2004a,b), the recent bionomic or ecological studies refer mainly to *An. minimus* s.l. and are based on morphological identifications for which no molecular controls were done (Dev 1996; Overgaard *et al.* 2002; Chareonviriyaphap *et al.* 2003a,b). Few studies employed molecular techniques to distinguish the different sibling species and their biological characteristics (Trung *et al.* 2004; Van Bortel *et al.* 2004). As the sibling species may exhibit different trophic behaviour and vectorial capacities, it is important to know the biological characteristics of each species. In addition to improving our understanding of the complex, precise knowledge of the individual species may considerably improve current vector control approaches.

We reviewed the available information on the Minimus Complex, especially species A and C, which are important malaria vectors compared with species E, which occurs in Japan, a malaria-free region. This review presents (1) up-to-date information on the main malaria studies involving *An. minimus* s.l.; (2) a discussion of recently published data on the distribution, ecology, and bionomics of immature as well as adult stages, and on the vectorial capacity of each sibling species; and (3) it highlights the gaps in our understanding of the Minimus Complex.

Taxonomic information and species identification

The Minimus Complex belongs to the Minimus Subgroup, which is closely related to the Aconitus Subgroup within the Funestus Group (Harbach 2004; Garros *et al.* 2005a,b). Most species of these subgroups occur in sympatry in Southeast Asia, and are difficult to distinguish based on the morphology of the immature and adult stages. The key characters of adults are overlapping and polymorphic, such as ornamentation of the wings and maxillary palpi. *Anopheles minimus* s.l., *An. aconitus* (Harrison 1980), *An. flavirostris* (Harrison 1980; Kirnowardoyo 1985), *An. fluviatilis* s.l. (Harrison 1980), *An. pampanai* (Van Bortel *et al.* 2000), and *An. varuna* (Van Bortel *et al.* 2001) are often misidentified on the basis of morphology.

Three sibling species are recognized within the Minimus Complex: species A, C and E (Harbach 2004), and no morphological characters are currently known to reliably distinguish them. However, based on the observation of Sucharit *et al.* (1988), several authors used the presence or absence of pale spots on the wing costa to distinguish species A from C (Rwegoshora *et al.* 2002). Yet, these characters are unreliable because they vary over time and space (Van Bortel *et al.* 1999; Chen *et al.* 2002).

The existence of two or more morphologically undefined species within *An. minimus* s.l. was first suspected based on differences in egg morphology (Baba 1950) and ecological and behavioural heterogeneities (Suthas *et al.* 1986). Two forms, designated A and B, were described in China (Yu & Li 1984; Yu 1987) based on morphological features in larvae, pupae and adults (Sucharit *et al.* 1988), and form B was subsequently regarded as a species. However, genetic evidence for the existence of two species was first provided for populations in Thailand (Green *et al.* 1990), then confirmed in Vietnam (Van Bortel *et al.* 1999), where sympatric homozygotes at the *Odh* locus (Octanol deshydrogenase) occurred in the absence of heterozygotes. The species were informally named species A and C (Green *et al.* 1990), the latter designated to distinguish it from form B previously described in China (Yu & Li 1984). Recently, Chen *et al.* (2002) showed that forms A and B in China are morphological variants of *An. minimus* A. In Japan, Somboon *et al.* (2001, 2005) provided morphological, cytogenetic, molecular and hybridization evidence for the recognition of another sibling species, designated species E, on the Ishigaki island of the Ryukyu Archipelago. Crossing experiments between species E and either species A or C showed that F1 crosses were sterile (Somboon *et al.* 2001, 2005). Both studies showed hybrid male sterility, which is generally accepted as very clear evidence of specific status. There is now no reason to cast doubt on the specific status of *An. minimus* E (Van Bortel & Coosemans 2003; Walton & Somboon 2004). Therefore to date, three species, designated as A, C and E are formally recognized within the Minimus Complex (Harbach 2004).

However, the complex may include two other species, species D (Baimai 1989) and specimen no. 157 (Sharpe *et al.* 1999) in Thailand. The specific status of these two entities is uncertain and needs further study. It seems that species D is a chromosomal variant of *An. minimus* A (V. Baimai, personal communication). It is difficult to assess the status of specimen no. 157 because only a single individual of this molecular variant (variation of two nucleotides of 313 on D3 domain of the 28S subunit, ribosomal DNA) is known.

Morphological evidence (Harrison 1980; Chen *et al.* 2002) and recently the comparison of nucleotide sequences of *An. fluviatilis* S (AF437880 from India), a member of the Fluviatilis Complex, with sequences of *An. minimus* C from several locations in Vietnam and Thailand (Garros *et al.* 2005b,c) showed that these informally designated species are conspecific, the latter being the senior synonym (Harbach 2004). This finding has important implications for our knowledge on the distribution and bionomics of *An. minimus* C.

Therefore, the problem with the identification of *An. minimus* s.l. is twofold. First, the taxon on the Southeast Asian mainland is a complex of two isomorphic species, and secondly, it is closely related to the sympatric Aconitus Subgroup (Garros *et al.* 2005a,b) based on the morphology of both immature and adult stages. In recent years, several molecular methods were developed to accurately identify sympatric species of the Minimus Complex and related species (Van Bortel *et al.* 2000; Phuc *et al.* 2003; Garros *et al.* 2004a,b; Garros *et al.* 2005d). These reliable molecular techniques need to be used regularly during entomological surveys to avoid misidentifications. It is strongly recommended that future studies routinely use these methods to improve our knowledge of the individual species.

Distribution

Anopheles minimus s.l. has a wide distribution over the Oriental Region (Harrison 1980), probably because of its ability to adapt to temperate climate. The range of *An. minimus* s.l. appears to extend from Uttar Pradesh southward to the north-eastern tip of Andhra Pradesh State in India across the Indochina–Malay peninsular countries and northward across southern China (Table 1). Harrison (1980) suspected that the published records of *An. minimus* s.l. in Sumatra and Java were *An. flavirostris*, which was confirmed in several recent surveys (Somboon *et al.* 2000; Barcus *et al.* 2002). According to Harrison (1980), it may occur to 30°N latitude in India and China and probably does not occur further south than 6°N latitude (Figure 1). The northern limit has been molecularly confirmed by Chen *et al.* (2002) who found species C in southern China up to 32.5°N.

It seems that *An. minimus* s.l. is rare in Taiwan (Lien 1991; Chen *et al.* 2002) and has disappeared from Nepal and several parts of India (Parajuli *et al.* 1981). The species has been reported in the north-eastern states of South Bihar and Assam in India, but it seems to have currently disappeared (Dev 1996; Das *et al.* 2000). Because identifications were not based on molecular methods, these records in the extreme parts of the taxon's distribution are difficult to validate. There are no recent records of *An. minimus* s.l. in Bangladesh (Maheswary *et al.* 1992), north-western India (Gujarat, border with Pakistan) (Bhatt & Kohli 1996), the Malaysian peninsula (Rahman *et al.* 2002), Philippine islands (Wooster & Rivera 1985; Schultz 1989), or Sri Lanka (Amerasinghe *et al.* 1999).

Based on recent application of molecular identification methods, members of the Minimus Complex are known to occur in the following countries (Table 1): Cambodia (Phuc *et al.* 2003); southern China (Chen *et al.* 2002); Japan (Somboon *et al.* 2001); Laos (Phuc *et al.* 2003); Taiwan (Chen *et al.* 2002); Thailand (Kengne *et al.* 2001); and Vietnam (Phuc *et al.* 2003; Trung *et al.* 2004). It has also been found based on morphological identification in India, Assam State (Jana-Kara *et al.* 1995; Dev 1996; Prakash *et al.* 2004), Bihar State (Das *et al.* 2000), and north-eastern states (West Bengal and Arunachal) (Nandi *et al.* 2000; Prakash *et al.* 2000), Hong Kong (Rueda *et al.* 2004), and Myanmar (Lin *et al.* 2000; Soe *et al.* 2001).

Since the majority of the available data on distribution concern *An. minimus* s.l., no complete and accurate information on the distribution of the individual members of the complex is available (Tables 1 and 2; Figure 1). Based on recent molecular studies, *An. minimus* species A is present in Cambodia, southern China, Laos, Thailand, Taiwan, and Vietnam (Van Bortel *et al.* 1999, 2000, 2003; Kengne *et al.* 2001; Chen *et al.* 2002; Phuc *et al.* 2003), and species C is known to occur in southern China (Chen *et al.* 2002), three provinces of Thailand (Sharpe *et al.* 2000; Kengne *et al.* 2001), Laos (Phuc *et al.* 2003), and northern and central Vietnam (Kengne *et al.* 2001; Phuc *et al.* 2003; Garros *et al.* 2005d) (Tables 1 and 2; Figure 1). At the present time, species A and C are known to occur largely in sympatry in a large area that includes northern Vietnam, southern China and northern Laos (Figure 1, shaded circles). Two local sympatric spots occur in central Vietnam and western Thailand (Figure 1, shaded circles). The assumption that species C has a restricted distribution compared with species A is refuted as more molecular studies are being done (Garros *et al.* 2005d). Moreover, synonymy of *An. fluviatilis* S with *An. minimus* C largely extends the distribution of this species to north-western India. On the contrary, *An. minimus* E is only known in the Ishigaki island, Ryukyu Archipelago, Japan (Somboon *et al.* 2001) (Figure 1, black triangle).

Bionomics

Breeding site ecology

The most favourable breeding sites of *An. minimus* s.l. are streams or canals with clear, slow-running water partially shaded by grassy margins (Harrison 1980; Dev 1996) (Figures 2a,b). These habitats are commonly found in foothills. However, *An. minimus* s.l. larvae may also occur in rock pools, ground pools next to streams, seepage pools and rice-field terraces. It is, however, not known whether species A and C occupy different habitats or niches. Water flow is an important factor that decreases the larval density (Overgaard *et al.* 2002). Yet, in the suburbs of Hanoi

Table 1 Overview of the geographical distribution of the Minimus Complex based on literature

	Bangladesh	Cambodia	Southern China	India	Japan	Laos	Malaysia	Myanmar	Nepal	Taiwan	Thailand	Vietnam	References
An. minimus s.l.	X	X	X	X	X	X	X	X	X	X	X	X	Harrison 1980
An. minimus s.l.		X	X	X	X	X		X		X	X	X	*
An. minimus A		X	X			X				X	X	X	See Table 2
An. minimus C			X			X					X	X	See Table 2
An. minimus E					X								See Table 2

* Based on recent confirmed and published records (see the Distribution section).

along the Hong River delta (northern Vietnam), larvae of *An. minimus* s.l. are regularly collected in cisterns containing rainwater or water from wells (Phan 1973; Khai 1975) (Figure 2c). Recently, Van Bortel *et al.* (1999) identified this population as species A. Even though these cisterns may contain a high density of larvae, only a limited number of *An. minimus* A adults were captured during entomological surveys using cattle as bait (Van Bortel *et al.* 2003) and they apparently do not represent a nuisance for humans (H.D. Trung, unpublished data). More studies are needed to understand the low density of adult mosquitoes in this environment. This urban population reflects the ability of this species to adapt to environmental change induced by humans.

Seasonal abundance and flight range

Over its distribution range, *An. minimus* s.l. exhibits peak densities during the dry season. This peak is temporally variable as the start of the rainy season fluctuates. Heavy rains, generally from June to the end of August, increase the turbidity and water flow of breeding sites, leading to a decrease in numbers of larvae. Two peaks were found in Thailand, the first before the rainy season (from March to May) and the second at the end of the rainy season (from July to November) (Harrison 1980; Ratanatham *et al.* 1988; Chareonviriyaphap *et al.* 2003a). It is unknown if these peaks are specific to either species A or C. In northern Vietnam (Hoa Binh Province), the distribution of *An. minimus* A and C based on molecular identifications was not identical from June 1995 until November 1995 (Van Bortel *et al.* 1999): *An. minimus* A showed a decline from June to October (mostly rainy season) and *An. minimus* C was particularly abundant in October (dry season) (W. Van Bortel, unpublished data).

The flight range of *Anopheles* varies generally from 1 to 3 km, with an estimation up to 1 km for species E in the Ryukyu Archipelago (Tsuda *et al.* 1999) and up to 2 km for *An. minimus* s.l. in Thailand (Meek 1995).

Factors influencing the occurrence of the members of the Minimus Complex

It is noteworthy that the species of the Minimus Complex exhibit population fluctuations in relation to human actions on ecosystems. *An. minimus* s.l. became rare or disappeared from Taiwan, Nepal and north-eastern parts of India after the use of insecticides (Parajuli *et al.* 1981; Lien 1991; Chen *et al.* 2002). In central Vietnam (Khanh Hoa Province), *An. minimus* A occurred in high densities (17 bites/man/night) in Lang Nhot village until April 1998, then decreased drastically after November 1998 (Trung *et al.* 2004; Van Bortel *et al.* 2004), whereas an increase of the *An. minimus* C population has been noted in the same village since 2000 (Garros *et al.* 2005d). In Thailand, ecological changes to develop agriculture and tourism decreased the forested areas, as well as breeding sites of the species, leading to reductions in populations of *An. minimus* s.l. Indeed, the density of *An. minimus* s.l. is negatively affected by increased landscape diversity and forest fragmentation (Overgaard *et al.* 2002). Moreover, higher water flow, due to deforestation, along with the absence of vegetation, also have a negative impact on suitable breeding sites (Overgaard *et al.* 2002). The effects of land use on the species have not yet been investigated, but in areas were both species are sympatric the relative abundance may vary from one village to the other (Van Bortel *et al.* 1999) indicating that micro-environmental factors probably influence the occurrence of each species. Moreover, it seems that species C may be linked to altered environments, such as recent agro-ecosystems located in deforested areas, whereas *An. minimus* A occurs in less disturbed forested areas and traditional rice agrosystems (C. Garros and S. Manguin, unpublished data). No environmental data is available for species E.

Behaviour of the members of the species complex

Anopheles minimus s.l. bites throughout the night, but exhibits geographical and seasonal variation in peak biting

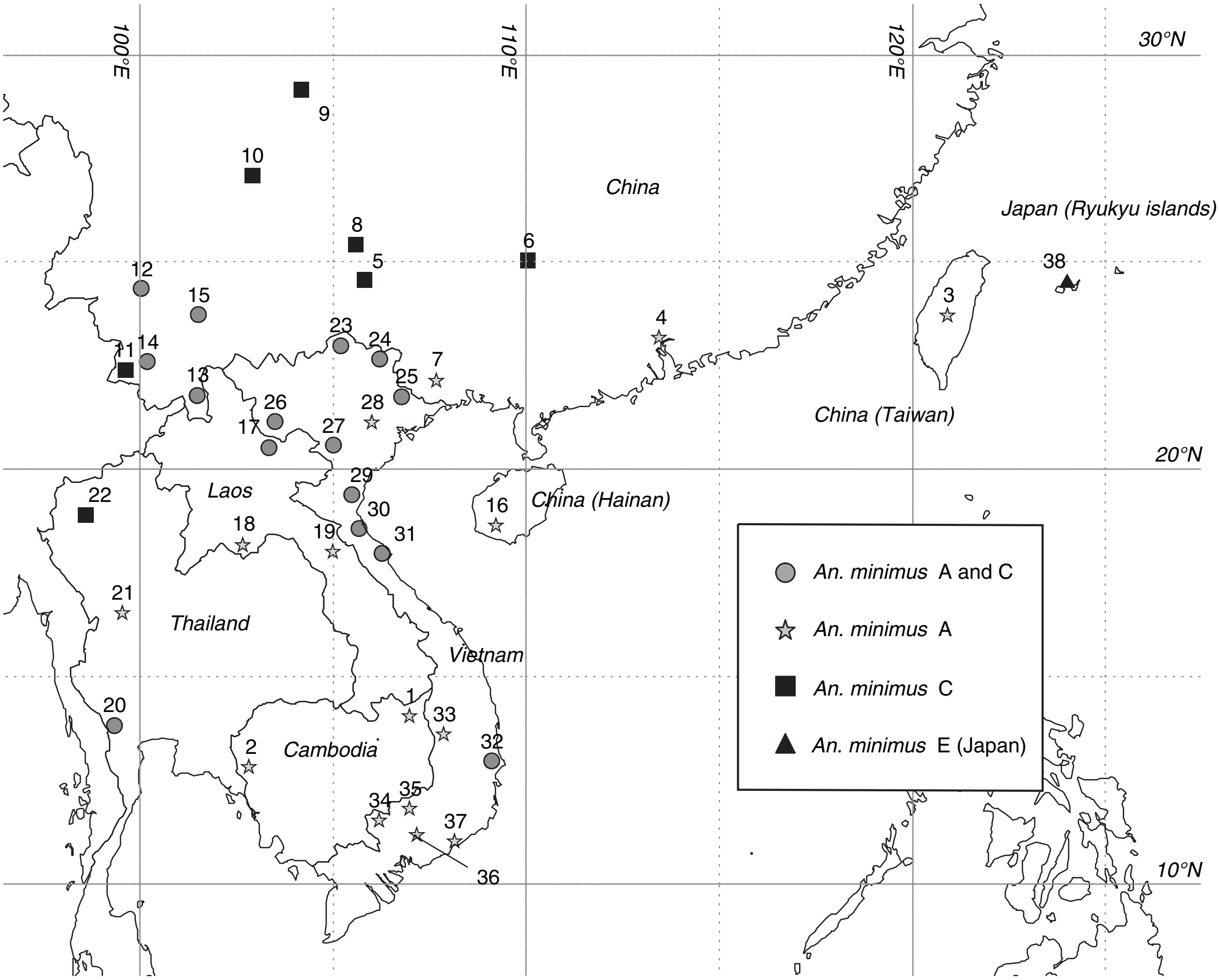

Figure 1 Distribution map of natural populations of species A and C of the Minimus Complex based on published data. Locations are detailed in Table 2.

activity. A large peak from 22.00 hours to 03.00 hours was recorded in Laos (Vythilingam *et al.* 2001), Myanmar (Myo Paing *et al.* 1998), and Vietnam (Phung & Quang 1997). Peak activity was earlier in Thailand from 21.00 hours to 22.00 hours (Harbach *et al.* 1987). Several authors also reported two feeding peaks, regardless of season, one after the sunset and a second before dawn (Ratanatham *et al.* 1988; Rattanarithikul *et al.* 1996; Chareonviriyaphap *et al.* 2003a). Relative risk (RR) of being bitten hourly by *An. minimus* A before 22.00 hours compared with after 22.00 hours ranged from 0.29 (central Vietnam) to 1.00 (Cambodia) (Trung *et al.* 2005). The RR was higher for *An. minimus* C (1.03) than for *An. minimus* A (0.79) in Hoa Binh Province of Vietnam (Trung *et al.* 2005).

Anopheles minimus s.l. is characterized as being principally an anthropophilic mosquito (Harrison 1980). Many data are available on the trophic behaviour of *An. minimus* s.l., but it refers to populations with uncertain species identity. In Bangladesh, India, and Nepal, the taxon was reported to be anthropophilic (with 5.82 bite/person/night *vs.* 0.12 bite/cow/night in India) (Covell 1944; Parajuli *et al.* 1981; Jana-Kara *et al.* 1995; Dev 1996; Dev *et al.* 2003). However, it was observed to be zoophilic in Bihar State (north-eastern India) and in Thailand (Das & Baruah 1985; Ratanatham *et al.* 1988; Das *et al.* 2000), with a ratio of females feeding on humans and cattle of 1.0:2.6 in Thailand. In India, Jana-Kara *et al.* (1995) reported that *An. minimus* s.l. was the main species collected indoors on humans (endophagic behaviour), but in western and

Table 2 Details of sampling sites for *Anopheles minimus* species A, C and E

Locality	Collected species	Date of collection	Reference
Cambodia			
1 Rattanakiry Pr.	*An. minimus* A	1998–2000	Kengne *et al.* (2001); Trung *et al.* (2004)
2 Battambang Pr.	*An. minimus* A	06/1999	Phuc *et al.* (2003)
China			
3 Taiwan Pr.			Chen *et al.* (2002)
Pingtung county	*An. minimus* A	08/2000	
Taichung county	*An. minimus* A	08/2000	
4 Guangdong Pr.			
Huidong county	*An. minimus* A	09/2000, 08/2001	
Taishan county	*An. minimus* A	08/2000	
5 Guangxi Pr.			
Lingzhan county	*An. minimus* C	NA	
Longlin county	*An. minimus* C	08/2000	
6 Guangxi Pr.			
Lonsheng county	*An. minimus* C	08/2001	
7 Guangxi Pr.			
Shangsi county	*An. minimus* A	08/2000, 08/2001	
8 Guizhou Pr.			
Guanling county	*An. minimus* C	08/2000	
Wangmuo county	*An. minimus* C	08/2000	
9 Sichuan Pr.			
Junlian county	*An. minimus* C	07/2000	
Qianwei county	*An. minimus* C	07/2000	
10 Yunnan Pr.			
Daguan county	*An. minimus* C	09/2000	
11 Yunnan Pr.			
Menglian county	*An. minimus* C	09/2000	
12 Yunnan Pr.			
Jingdong county	*An. minimus* A, C	09/2001	
13 Yunnan Pr.			
Mengla county	*An. minimus* A, C	07/2000, 09/2001	
14 Yunnan Pr.			
Simao county	*An. minimus* A, C	08/2000, 09/2001	
15 Yunnan Pr.			
Yuanjiang county	*An. minimus* A, C	07/2000	
16 Hainan Pr.			
Baoting county	*An. minimus* A	08/2000, 08/2001	
Changjiang county	*An. minimus* A	08/2000, 08/2001	
Danzhou couty	*An. minimus* A	08/2001	
Laos			
17 Samneur Pr.	*An. minimus* A, C	12/1999	Phuc *et al.* (2003)
18 Vientiane Pr.	*An. minimus* A	1998–2000	Kengne *et al.* (2001); Trung *et al.* (2004)
19 Bolikhamxay Pr.	*An. minimus* A	02–04/1999	Phuc *et al.* (2003)
Thailand			
20 Kanchanaburi Pr.	*An. minimus* A, C	1998–2000	Kengne *et al.* (2001)
21 Chiang Mai Pr.	*An. minimus* C	NA	Sharpe *et al.* (2000)
22 Tak Pr.	*An. minimus* A	NA	
Vietnam			
23 Ha Giang Pr.	*An. minimus* A, C	03/1999	Phuc *et al.* (2003)
24 Cao Bang Pr.	*An. minimus* A, C	11/1999	
25 Lang Son Pr.	*An. minimus* A, C	04/2000	
26 Son La Pr.	*An. minimus* A, C	10/1999	

Table 2 (Continued)

Locality	Collected species	Date of collection	Reference
27 Hoa Binh Pr.			
Phu Cuong commune	*An. minimus* A, C	1995	Van Bortel *et al.* (1999)
Phu Cuong commune	*An. minimus* A, C	1999	Phuc *et al.* (2003)
Phu Cuong commune	*An. minimus* A, C	1998–2000	Kengne *et al.* (2001); Trung *et al.* (2004)
Quyet Chien commune	*An. minimus* A, C	1999	Phuc *et al.* (2003)
28 Hanoi Pr.	*An. minimus* A	NA	Phuc *et al.* (2003)
Hanoi Pr.	*An. minimus* A	1998–2000	Van Bortel *et al.* (1999)
29 Thanh Hoa Pr.	*An. minimus* A, C	11/1999	Phuc *et al.* (2003)
30 Nghe An Pr.			
Quynh Thang commune	*An. minimus* A	11/1999	
Cam Lam commune	*An. minimus* A, C	11/1999	
31 Ha Tinh Pr.			
Huong Khe commune	*An. minimus* A, C	11/1999	
Ky Thinh commune	*An. minimus* A	11/1999	
32 Khanh Hoa Pr.			
Khanh Phu commune	*An. minimus* A	1993–2002	Garros *et al.* (2005c)
	An. minimus A	1998–2000	Van Bortel *et al.* (2003)
	An. minimus C	2000	Phuc *et al.* (2003)
	An. minimus A, C	2002	Garros *et al.* (2005c)
33 Gia Lai Pr.	*An. minimus* A	06/1999	Phuc *et al.* (2003)
34 Tay Ninh Pr.	*An. minimus* A	05/1999	
35 Binh Phuoc Pr.	*An. minimus* A	1999	
36 Dong Nai Pr.	*An. minimus* A	07/1999	
37 Binh Thuan Pr.			
Binh Thanh commune	*An. minimus* A	1999	Phuc *et al.* (2003)
Suoi Kiet commune	*An. minimus* A	1998–2000	Van Bortel *et al.* (2001)
Japan			
38 Ryukyu Archipelago	*An. minimus* E	08–10/1998	Somboon *et al.* (2001)
Ishigaki island		09/1999	

Pr., province; NA, not available.

upper central Thailand *An. minimus* s.l. was more attracted to humans outdoors (exophagic behaviour, 3.27 mosquitoes/man/night) than indoors (1.15 mosquitoes/man/night) (Ratanatham *et al.* 1988). In Laos, *An. minimus* s.l. was found to bite both indoors and outdoors (densities not provided) (Vythilingam *et al.* 2001). As we do not know precisely which species of the Minimus Complex were studied, it is difficult to infer whether these heterogeneities are due to a mixture of species or to behavioural polymorphism and plasticity of the individual species.

To date, one research programme in Southeast Asia[2] has assessed the specific behaviour of species A and C. Significant intraspecific behavioural differences were observed among populations of *An. minimus* A from Cambodia (one site), Laos (one site) and Vietnam (two sites in northern and central areas). A wide range of anthropophilic behaviour was observed, with a ratio of outdoor human (OH) landing *vs.* cattle (OC) collections ranging from 0.10 (northern Vietnam) to 10.11 (Laos); endophagic behaviour, with a ratio of indoor (IH) *vs.* OH landing collections ranging from 0.62 (Cambodia) to 7.95 (central Vietnam); and indoor resting behaviour (endophilic behaviour), with a ratio of indoor morning collections (IMR) *vs.* combined IH, OH, and cattle collections at night ranging from 0.04 (Laos) to 0.67 (northern Vietnam) (Trung *et al.* 2005). Unfortunately, only one sympatric population of *An. minimus* A and C in northern Vietnam (Hoa Binh Province) was studied, and intraspecific behavioural variation could not be assessed. Zoophilic behaviour was pronounced for both species A and C (ratio OH *vs.* OC of 0.10 and 0.09, respectively), but species C was more exophagic than species A (ratio IH *vs.* OH of 0.35 and 0.93, respectively), as well as more exophilic than species A (ratio IMR *vs.* IH–OH–OC of 0.15 and 0.67, respectively) (Trung *et al.* 2005).

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[2] INCO-project n°ERBIC18.CT.970211.

Figure 2 Habitats of the immature stages of *An. minimus* s.l. (a) Kanchanaburi Province (western Thailand); (b) Hoa Binh Province (northern Vietnam); (c) suburbs of Hanoi (northern Vietnam), unusual habitat of species A in cisterns (pictures taken by CG).

From the above mentioned studies, it is clear that *An. minimus* s.l. exhibits behavioural heterogeneities relevant for disease transmission and vector control. However, the recognition of the two species cannot explain all behavioural heterogeneities within this taxon (Green *et al.* 1990). It appears that both species exhibit variable trophic behaviour depending on the environment. Some behavioural differences are also linked to the abundance of cattle in localities in northern Vietnam and Cambodia (Van Bortel *et al.* 2004) as well as differences in housing construction. For instance, *An. minimus* A in central Vietnam (Khanh Hoa Province) exhibits a high anthropophilic (ratio of 8.10) and endophagic (ratio of 7.95) behaviour most likely influenced by the largely open houses with incomplete walls that allow mosquitoes to easily detect human stimuli and enter into houses. On the contrary, in northern Vietnam (Hoa Binh Province) where cattle are quite abundant and are kept under the stilt houses, *An. minimus* A exhibits a low degree of anthropophily (0.10) and endophagy (0.93), and a high degree of endophily (0.67) (Van Bortel *et al.* 2004; Trung *et al.* 2005). Therefore, it is clear that intraspecific behavioural differences in members of the Minimus Complex occur due to the high behavioural plasticity. Unfortunately, to date, no information on the trophic behaviour of *An. minimus* E is available.

Role of *An. minimus* s.l. in malaria transmission

Anopheles minimus s.l. has been incriminated as a major malaria vector in all areas where it occurs (Harrison 1980; Chareonviriyaphap *et al.* 2000; Chen *et al.* 2002). In 1990, sporozoite infections were recorded all year round for *An. minimus* s.l. in India (Assam), except during August and September. The infection rate was lowest in March (0.7%) and highest in October (8.5%) (Dev 1996). In southern China, Yunnan Province, *An. minimus* s.l. was regarded as the main vector with an average sporozoite rate of 7.05% (Dong 1997). In Gia Lay Province of central Vietnam, the sporozoite rate was 2.58% in 1994 (Phan 1998). *Anopheles minimus* s.l. also has been incriminated as the main malaria vector in Myanmar (Oo *et al.* 2004).

In Cambodia and central Vietnam, *An. minimus* A was found positive in ELISA tests for the detection of circumsporozoite protein (CS) of *Plasmodium falciparum* and *P. vivax* 210 and 247 (Trung *et al.* 2004). *Anopheles minimus* C has been regarded as a less competent malaria vector than species A based only on its more exophagic behaviour (Van Bortel *et al.* 1999). However, the vectorial status of species C is still questionable and needs to be clearly determined, especially as it is conspecific with *An. fluviatilis* S recognized as an efficient malaria vector in India (Dev 1996; Dev *et al.* 2003). *Anopheles minimus* C is suspected to be responsible for malaria transmission in areas of southern China (Chen *et al.* 2002) and in Thailand (T. Chareonviriyaphap, personal communication), where it is the main species of the complex. *Anopheles minimus* specie E has not been incriminated as malaria vector because the Ryukyu Archipelago is malaria-free.

Control strategies

Vector control is largely based on the use of insecticides in residual house spraying and bed net impregnation. Residual indoor spraying programmes were organized throughout the Indochina peninsula during the 1960s with good results for the control of *An. minimus* s.l. (Meek 1995). The use of insecticides in Nepal eliminated *An. minimus* s.l. (Parajuli *et al.* 1981). In Thailand, after years of DDT application, populations of *An. minimus* s.l. were reduced in the peninsula and central plains (Harrison 1980), but remained abundant in hilly forested areas. *An. minimus* s.l. is still reported as susceptible to DDT and permethrin in Thailand, except in some areas where a slight increase of tolerance was observed (Somboon *et al.* 2003). In Vietnam, longitudinal surveys from 1960 to 1980 in 12 northern sites and four central ones did not report resistance to DDT or any other insecticides (Phan 1998). Then, from 1993 to 1997 insecticide resistance tests indicated possible resistance to permethrin (at a discriminating concentration of 0.25%) in populations from Hanoi (northern Vietnam) and Khanh Hoa (central Vietnam) Provinces (Phan 1998). Moreover, possible differences in the resistance status between *An. minimus* A and C are currently under investigation (MALVECASIA project[3]). Furthermore, the Resistant Pest Management database (http://www.pesticideresistance.org) cited Indonesia, Philippines and Thailand as countries where resistance to organochlorides (DDT and cyclodienes) was reported but without further information. These data are doubtful for the first two countries because *An. minimus* s.l. does not occur there. The data may concern *An. flavirostris* or another closely related species.

Biological control attempts have also been made to control larvae of *An. minimus* s.l. in Thailand using larvivorous fish (Tang Am 1993; Meek 1995). The effectiveness of this strategy has not been demonstrated. The typical breeding sites of *An. minimus* s.l. in small streams with slow-running water may not be suitable for the larvivorous fish to develop. Recently in Japan, copepod species, commonly found in rice fields during the summer season, were tested as predators of *An. minimus* s.l. larvae (Dieng *et al.* 2003). The results supported the contention that these copepods have the potential to be used as biological control agents against mosquito immature stages. More recently, a root extract from *Stemona curtisii* (Stemonaceae) showed significant larvicidal activity against *An. minimus* s.l. (Mungkornasawakul *et al.* 2004). However, biological control may be efficient only if included within an integrated approach.

Because of the high behavioural plasticity of *An. minimus* A, and marked differences between species A and C with regard to local environment (housing construction, cattle abundance, landscape), preliminary entomological studies focusing on each species of the Minimus Complex need to be implemented before choosing and applying an appropriate vector control strategy. This is necessary in order to apply vector control strategies targeting specifically malaria vectors species.

Conclusions

For a better and more precise knowledge about the distribution of members of the Minimus Complex, it is essential to use molecular tests in order to avoid morphological confusion with other species of the Funestus Group. The two members of the Minimus Complex on the Southeast Asia mainland seem to show bionomical differences that may be epidemiologically relevant when they occur in sympatry. However, available information indicates that both species exhibit considerable behavioural and ecological plasticity and this high biological heterogeneity is apparent depending on the geographic location, abundance of cattle, and the construction of houses. However, it is not clear to what extend the stated biological and bionomical variation over the range of distribution are explained by inter- or intraspecific differences. Therefore, the relative role of the individual species in malaria transmission, as well as the specific biological variability of their bionomics, should be further analysed over their ranges of distribution. Several questions of importance need to be addressed: (1) How does immature and adult ecology influence the distribution of the members of this species complex? (2) What are the specific differences in seasonal abundance and nocturnal biting patterns? (3) What role does species C play in malaria transmission? (4) What is the status to insecticide resistance of each species in different regions of Southeast Asia? (5) Is there further evidence to support the conspecificity of *An. minimus* C and *An. fluviatilis* S?

Asian countries are in constant development which leads to deforestation and modification of the original environment. Studies are needed on how the different sibling species will adapt and respond to anthropogenic changes and how these adaptations will influence population densities, distributions, and trophic behaviours.

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Révision du Complexe d'anophèle Minimus, vecteur majeur d'agents du paludisme en Asie du sud-est: de la taxonomie aux stratégies de lutte antivectorielle

DONNÉES DE BASE Le Complexe d'anophèle Minimus (sous-genre *Cellia*) est composé de deux espèces jumelles, A et C, réparties sur le continent sud-est asiatique et d'une troisième espèce E allopatrique restreinte à l'Archipel des Ryukyu (Japon), une région exempte de paludisme. *Anopheles minimus.* s.l est considéré comme le vecteur majeur du paludisme dans les régions collinaires boisées d'Asie du sud-est. Malgré un grand nombre d'études menées sur l'ensemble de l'aire de distribution du complexe, il est difficile d'avoir une vue globale des caractéristiques écologiques et bionomiques de chaque espèce car différentes méthodes d'identification ont été utilisées, généralement sans identification spécifique des espèces jumelles.

OBJECTIFS (1) Faire la synthèse des principales études sur *An. minimus.* s.l.; (2) discuter les données récemment publiées sur la biologie et l'écologie de chaque espèce jumelle; et (3) identifier les lacunes dans notre connaissance du Complexe Minimus.

REVISION DES RESULTATS Les tendances biologiques et écologiques majeures sont discutées telles que la forte plasticité du comportement trophique et la sympatrie des espèces A et C sur le continent sud-est asiatique. Malgré la disponibilité de méthodes d'identification moléculaire rapides, d'importantes informations concernant les caractéristiques biologiques de chaque espèce jumelle sont toujours manquantes. Ces lacunes doivent être comblées dans le futur car les espèces *An. minimus* A et C peuvent jouer des rôles différents dans la transmission du paludisme.

CONCLUSION Il faut que dans le futur les enquêtes entomologiques emploient plus largement les méthodes moléculaires pour clairement identifier les espèces jumelles, et ainsi mieux appréhender les caractéristiques bio-écologiques de celles-ci, et par conséquent améliorer les stratégies actuelles de lutte antivectorielle en ciblant les vecteurs plus efficacement.

Mots clés *Anopheles minimus* s.l., espèces jumelles, distribution, bionomie, paludisme, Asie du sud-est

Revisión del Complejo Minimus de *Anopheles*, principal vector en el Suroeste Asiático: de temas taxonómicos a estrategias de control del vector.

ANTECEDENTES: El complejo Minimus de *Anopheles*, subgénero *Cellia* está compuesto por dos especies hermanas, A y C en el Sureste del continente asiático y una tercera especie alopática E que se encuentra en el archipiélago Ryukyu (Japón), una región libre de malaria. *Anopheles minimus* s.l. es considerado el principal vector de la regiones forestales del Sureste Asiático. A pesar de un gran número de estudios sobre su rango y distribución, es difícil tener una visión global de las características ecológicas y bionómicas de las especies individuales, ya que se han utilizado diferentes métodos de clasificación, generalmente sin especificar la identificación de las especies hermanas.

OBJETIVOS: (1) Revisar Los estudios principales sobre *An. minimus* s.l.; (2) Discutir datos publicados recientemente sobre la biología y ecología de cada una de las especies hermanas; y (3) identificar huecos en nuestra compresión sobre el Complejo Minimus.

RESULTADOS: Se estudian las principales tendencias biológicas y ecológicas tales como la alta plasticidad del comportamiento trófico y la simpatría de las especies A y C en el sureste del continente asiático. A pesar de la disponibilidad de métodos rápidos de identificación molecular, aun no tenemos información importante sobre las características biológicas de cada especie. Estos vacíos deben llenarse en el futuro, porque las especies *An. Minimus* pueden exhibir diferentes capacidades para transmitir la malaria.

CONCLUSIÓN: Esperamos que estudios entomológicos utilicen métodos moleculares para identificar claramente estas dos especies, y así aclarar las características biológicas de cada una. Consequentemente, se mejorarán las estrategias de control vectorial actual, enfocándolas al vector más eficiente.

Palabras clave: *Anopheles minimus* s.l., especies hermanas, distribución, bionómica, malaria, Sureste Asiático