

HOW RELIABLE IS THE HUMERAL PALE SPOT FOR IDENTIFICATION OF CRYPTIC SPECIES OF THE MINIMUS COMPLEX?

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ABSTRACT. The *Anopheles minimus* Complex Theobald (Diptera: Culicidae) is composed of the 3 sibling species A, C, and E. The malaria vectors *An. minimus* A and C are distributed over the Southeast Asian region, whereas species E is restricted to the Ryukyu Japanese islands. Because species A and C can be sympatric and present specific behaviors and have a role in malaria transmission, it is important to differentiate them. The literature mentioned the presence of a presector pale spot on the wing costa of *An. minimus* A, whereas species C may exhibit both presector and humeral pale spots. However, the reliability of their diagnostic power has not been established over large temporal and geographic surveys. From the analyses of 9 populations throughout Southeast Asia, including published data and field populations from 2 sites in Thailand, we showed that the wing patterns present spatial and temporal variations that make these two morphological characters unreliable for the precise identification of *An. minimus* A and C. Therefore, molecular identification remains the most efficient method to obtain an unambiguous differentiation of these 2 species. Correct species identification is essential and mandatory for any relevant study on the Minimus Complex and for the application of successful control strategies.

KEY WORDS *Anopheles minimus* Complex, morphological identification, diagnostic character, malaria, Asia

INTRODUCTION

Anopheles minimus Theobald was described in 1901, and currently the Minimus Complex is composed of the 3 sibling species A, C, and E (Harbach 1994, 2004; Somboon et al. 2001). *Anopheles minimus* species A and C are widespread over the Asian continent (Green et al. 1990, Van Bortel et al. 1999, Chen et al. 2002) and can be sympatric, whereas species E is restricted to the Ryukyu islands in Japan (Somboon et al. 2001, 2005), a malaria-free region. By definition, no morphological characters exist that could clearly identify the 3 species. However, Sucharit et al. (1988) presented a potential diagnostic character that could differentiate the two species. *Anopheles minimus* A may present a wing costa with a presector pale spot (PSP phenotype), whereas *An. minimus* C may exhibit both presector pale and humeral pale spots (HP phenotype) (Fig. 1). *Anopheles minimus* E seems to be distinct from species A and C (presence of both a humeral pale spot and a pale fringe spot at the tip of vein 1A), although there is no unique character or set of characters that are peculiar to it (Somboon et al. 2001). Evidence of morphological differences between eggs of species A and C also was reported (Sucharit et al. 1995), but only colony populations were tested, which raises the question of the validity of this character in natural populations.

Several studies used the two phenotypes to identify both species (Green et al. 1990, Van Bortel et al. 1999, Chen et al. 2002). Moreover, this potential diagnostic character is routinely used in Asia during entomological field surveys when molecular identification is not feasible (Rwegoshora et al. 2002). Recently, several molecular assays were developed to facilitate the identification of both sympatric species (Sharpe et al. 1999; Van Bortel et al. 2000; Phuc et al. 2003; Garros et al. 2004a, 2004b). The reliability of the humeral spot diagnostic power being little tested over large temporal and geographic surveys, we conducted a study to assess and compare the polymorphism of this character over 9 wild populations throughout Southeast Asia. The aim of the present work was to define whether a morphological identification of *An. minimus* A or C based on these characters is reliable.

Several previous studies included both morphological and other identifications (isozymes or DNA-based assays). Green et al. (1990) scored females from western Thailand (Kanchanaburi Province) for the presence or absence of the humeral pale spot and compared the identifications with isozyme assays. These authors found that the majority of *An. minimus* species C had the HP phenotype and that this character may differentiate the 2 species with an error of 37%. In Japan, Somboon et al. (2001) followed the morphological variations of *An. minimus* E during 1 year considering separated males and females. He concluded that seasonal variations existed with a decrease of the presence of the pale spots during the winter. Variations were independent of sex. In northern Vietnam, Van Bortel et al. (1999) evaluated the diagnostic power of

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