

In rice fields in northern Greece, the larvae are associated with *An. sacharovi* and *Cx. modestus*. From the coastal plains of Turkey, Postiglione et al. (1973) reported the presence of larvae in association with those of *An. maculipennis s.l.*, *An. superpictus* and *An. algeriensis*. At a temperature of about 20°C the larval development of *An. hyrcanus* lasts 14–16 days (Senevet and Andarelli 1956). The adults occur in small numbers in late April and May but the size of the population increases towards autumn. *An. hyrcanus* produces 2–4 generations/year. The biting behaviour of the adult females is predominantly exophilic (outdoors), but the degree of exophily is known to differ between places. Usually they rest outdoors in bushes and other dense vegetation during the day time. They rarely enter houses, but are common in cattle sheds or shelters, from where they return into the open after blood feeding. Livestock, or when these are not available as hosts, humans are readily attacked in the open field at dusk or in the night. Occasionally, feeding is observed during the day time in shaded situations. Autogenous populations have been reported from Kirgisia (Rioux et al. 1975).

Distribution: In Europe *An. hyrcanus* is widely distributed throughout the northern Mediterranean countries, from Spain, southern France, Italy, and Greece to Turkey. In central Europe it is reported from Hungary (Toth 2003), Slovakia (Halgos and Benkova 2004) and the Czech Republic (Votypka et al. 2008; Sebesta et al. 2009). It is distributed in the Ukraine, southern Russia, southern Kazakhstan, the Caucasus and middle Asia. Together with closely related species it is common in southeast Asia and its eastern distribution range includes China, Japan and Korea.

Medical importance: Because of its exophilic behaviour, *An. hyrcanus* has never been regarded as a significant vector of malaria in the Mediterranean region. With regard to the probability of changes in human behaviour (e.g. increased mobility of humans or increase in the number of seasonal workers in the rice and cotton fields), its role as a potential malaria vector should not be ignored.

Note on systematics: *An. hyrcanus* is one of the most widespread and common species of the genus *Anopheles*, and certainly one of the most variable. It has an enormous distribution range throughout the Palaearctic from the Atlantic in the west to the Pacific in the East and the Oriental region in the south. Due to its variability, a number of different forms have been

described from different localities as variations or subspecies of the nominative form. Some of these forms from south-east Asia are now considered as distinct species within the *An. hyrcanus* sibling species group, e.g. *An. sinensis* Wiedemann, *An. nigerrimus* Giles, *An. paeditaeniatus* (Leicester) and others (Reid 1953; Harrison 1972). The Palaearctic variations include the form *mesopotamiae* from western Asia with a lighter, diffused colour pattern of the wing caused by more pale scales intermixed on the wing veins and a western Palaearctic form *pseudopictus* with tarsomere IV of the hind legs entirely white, found mainly in southern and southeastern Europe. Glick (1992) treated *An. pseudopictus* as a distinct species being clearly separated from *An. hyrcanus* by its sympatric distribution throughout Turkey, Iran and Afghanistan with apparently no evidence of hybridization. However, Gutsevich (1976) reported a wide variation of *An. hyrcanus* in the extent of the pale ringing on the hind legs and found intermediate forms. Because Glick's judgment was solely based on one character of female morphology and no investigations on the morphology, of the male genitalia, the developing stages or cross-mating experiments have been carried out since, the opinion not to treat the European form *pseudopictus* as a distinct species nor a subspecies of *An. hyrcanus* is favored here.

Anopheles Maculipennis Complex

The mosquitoes of the *Anopheles Maculipennis* Complex are the classical example of a “species complex”, comprising various sibling species. Before 1925, it was reported that malaria was transmitted by the malaria mosquito “*An. maculipennis*”. Further research on the distribution and ecology of this mosquito uncovered considerable irregularities. It was found that the distribution of malaria and the distribution of *An. maculipennis* were not closely correlated. In some areas where individuals of *An. maculipennis* were abundant, the incidence of malaria was low or absent. This situation was characterized as anophelism without malaria (Bates 1940). Furthermore, differences in the biology and behaviour of various populations were discovered. In some regions the larvae were restricted to fresh water, in others, to brackish water; the adult females preferred to feed on humans in some areas and

elsewhere they mainly fed on livestock. Exceptional differences in the swarming and mating behaviour (stenogamy, eurygamy) between certain populations were also observed. The first evidence for the existence of a complex of sibling species was brought up by Falleroni (1926), who described distinct morphological differences of the chorion pattern of the eggs in populations with a different biology. Unfortunately, Falleroni's observations lay dormant for five years and were not rediscovered until 1931. Research of Martini et al. (1931), Van Thiel (1933) and Hackett and Missiroli (1935) gave more evidence of the presence of a complex and by the end of the 1930s, most of the present species of the complex were identified (Bates 1940). Studies using cross-breeding experiments, cytotoxic methods or enzyme electrophoresis confirmed the existence of the different species within the complex (Stegnii and Kabanova 1976; Bullini and Coluzzi 1978; Suzzoni-Blatger et al. 1990). The complex comprises of at least a dozen reproductively isolated but morphologically similar species in the northern hemisphere (White 1978) and it can be expected that more members will be found with the use of the above men-

tioned advanced techniques of identification, e.g. *An. daciae* (Nicolescu et al. 2004).

Once the existence of a complex of sibling species was established, some morphological differences between adults and larvae were also found. The females may be separated by the form and shape of scales on certain wing veins, the males differ in the form and length of the spines on the outer claspette lobe and larvae may be distinguished by the overall number of branches of the four antepalpal setae on abdominal segments IV and V (2-IV and 2-V, four setae together). Unfortunately, there exists an intra-specific variation of the characters and overlapping between the species is not uncommon. Therefore, it is necessary to investigate a series of specimens with statistical analysis of the results, which is not applicable for individual identification. Thus the morphological identification is still based largely on characters of the egg (markings of the dorsal exochorion, presence of floats and their size, position and texture). Engorged females can be directly sampled into vials, e.g. from animal shelters, and allowed to lay their eggs for individual identification.

Identification key for European species of the *Anopheles Maculipennis* Complex based on characters of the eggs (after White 1978):

- 1 Egg without floats (but rudimentary floats may develop at low temperatures), egg surface uniformly pale (frosty-white) from pole to pole (Fig. 9.4a) *sacharovi*
Egg with fully developed floats, egg surface dark, barred or mottled..... 2
- 2 (1) Intercostal membranes of floats (surface of the air cells) smooth..... 3
Intercostal membranes of floats rough (finely corrugated) 5
- 3 (2) Surface of egg entirely dark (Fig. 9.4b) *melanoon*
Surface of egg otherwise, barred or mottled 4
- 4 (3) Surface of egg softly patterned with wedge-shaped black marks on a pale background, pale almost at the tip (Fig. 9.4c) *atroparvus*
Surface of egg with pattern of 2 transverse dark bars near the ends of floats, poles dark and remainder of the surface irregularly mottled (Fig. 9.4d) *subalpinus*
- 5 (2) Surface of egg richly patterned with wedge-shaped dark marks on frosted pale background, tip of poles dark and narrow (Fig. 9.4e) *labranchiae*
Surface of egg marked with 2 transverse dark bars near the end of the floats, with or without other pattern (mottled background)..... 6
- 6 (5) Transverse dark bars on surface forming part of diffuse mottled pattern (Fig. 9.4f) *messeae*
Transverse dark bars on upper surface sharply contrasted with unmottled pale background colour..... 7
- 7 (6) Tips of eggs less acutely pointed, chorion of upper egg surface relatively rough, width of egg between floats about 17% of egg length (Fig. 9.4g)..... *maculipennis s.s.*
Tips of eggs more acutely pointed, chorion of upper egg surface relatively smooth, width of egg between floats about 12% of egg length (Fig. 9.4h)..... *beklemishevi*

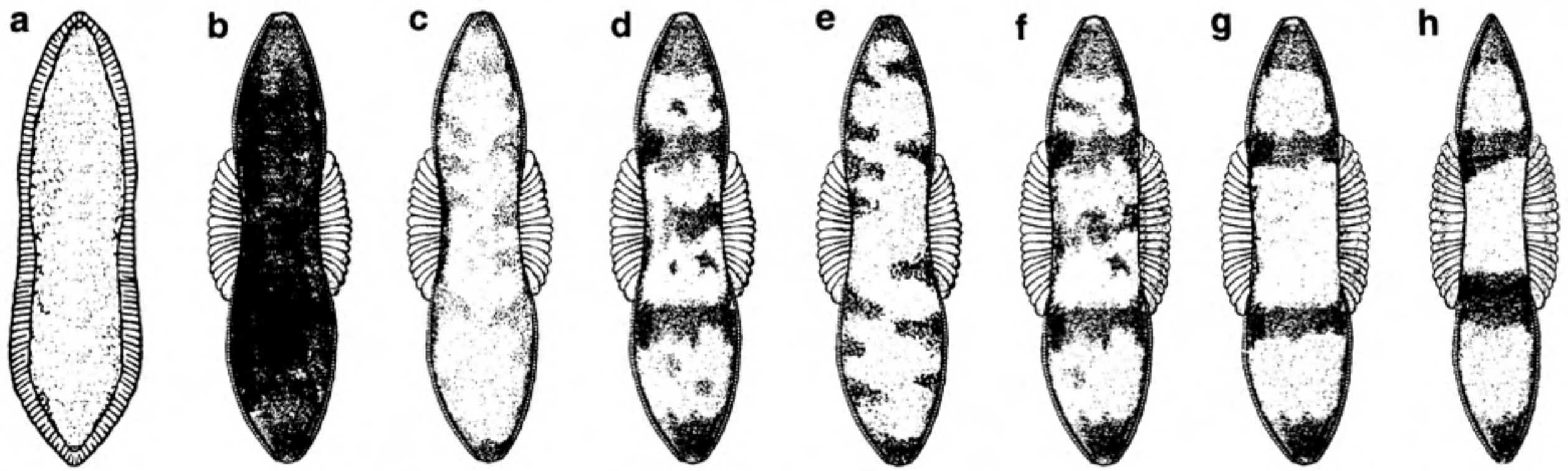


Fig. 9.4 Eggs of *Anopheles Maculipennis* Complex:

(a) *sacharovi*, (b) *melanoon*, (c) *atroparvus*, (d) *subalpinus*, (e) *labranchiae*, (f) *messeae*, (g) *maculipennis s.s.*, (h) *beklemishevi*

Morphological characters of adults and larvae shared between the members of the complex:

Female: Dark or medium brown in colour, although there is a wide variation in colouration and size. Individuals of southern origin are usually lighter and smaller. The characteristic features, which differ from all other European *Anopheles* species, are the wings with an aggregation of dark scales forming several distinct spots (Fig. 6.8a). The proboscis is dark brown, and the palps are nearly as long as the proboscis, and of the same colour. The antennae are brown coloured. The vertex has a tuft of long, whitish, anteriorly directed narrow scales and setae. The occiput has erect dark brown scales. The scutum has a broad greyish median stripe tapering anteriorly and usually 2-3 indistinct brownish stripes on its anterior half. The lateral parts of the scutum are brown anteriorly and blackish brown posteriorly (for different colouration patterns of *An. sacharovi* see under species description). The antechrostichal patch is made of pale, long and thin scales. The scutellum is brown with golden narrow scales, the postnotum is pale brown, and the pleurites are dark brown. The femora are dark brown on the dorsal side and pale brown on the ventral side, the tibiae are brown but slightly paler at their apices, and the tarsi are dark brown. The wings have dark scales unevenly distributed forming several dark spots close to the cross veins, base of R_s and furcations of R_{2+3} and M. The furcations of R_{2+3} and M are situated at about the same distance from the wing base. The fringe of the wing has a patch of pale scales at the apex of the wing (Fig. 6.9b). *An. sacharovi* has dark spots on the wing veins which are more indistinct and the fringe of the wing is uniformly dark (Fig. 6.9a). The abdomen is brown or

blackish brown with long, golden brown, narrow scales.

Male (Fig. 9.5): The gonocoxite has 2 single parbasal setae positioned on small, but distinct tubercles, and the setae are unequal in length, with the outer one being longer. The internal seta is inserted near the middle of the gonocoxite. The gonostylus is well developed, and is longer than the gonocoxite, with a short apical spine. The claspette lobes are not well defined, and have spine-like setae of variable length and shape situated close together but not fused. The aedeagus is long and narrow with leaflets at the apex.

Larva: Very variable in pigmentation and size according to their different habitats. Larvae from the northern parts of Europe are usually larger with a darker head capsule. The head is longer than the width, and the antenna is nearly straight, sparsely spiculate, and about 2/3 as long as the head. The antennal seta (1-A) is small, with 4-6 branches arising at the basal



Fig. 9.5 Hypopygium of *An. maculipennis s.l.*

1/3 to 1/4 of the antennal shaft (Fig. 8.13b). The inner clypeal setae (2-C) are situated close together, with long apical branches. The outer clypeal seta (3-C) is dendriform (Fig. 8.9b). The frontal setae (5-C to 7-C) are long and plumose. The palmate setae on abdominal segments I and II are rudimentary, but well developed on segments III–VII, with 16–24 leaflets which are slightly wider in the middle. The terminal filament is nearly 1/3 as long as the leaflet.

***Anopheles (Anopheles) atroparvus* Van Thiel 1927**

Biology: The larvae can be found in a variety of stagnant, semi-permanent or permanent, clean breeding sites, both in saline and fresh water, but they show a slight preference for brackish water. They may occur in canals, ditches, marshes in coastal areas, river margins, pools in river beds, rice fields, and are sometimes even found in septic tanks. The water bodies are usually exposed to the sun and carry a considerable amount of filamentous green algae and other floating and submerged vegetation. In their southern distribution range, the larvae are often found together with those of *Cx. theileri*, *Cx. impudicus*, and *Cx. p. pipiens* (Ramos et al. 1978). *An. atroparvus* hibernates as the adult female and usually shows incomplete diapause. After seeking shelter in stables or dwellings in autumn, the females remain active during winter time and may irregularly take blood-meals without subsequent oviposition. This habit mainly contributed to the indoor transmission of winter malaria in Great Britain, Netherlands, and other parts of Europe at the beginning of the twentieth century. The problem disappeared in the late 1940s, due to improved socio-economic conditions. The duration of the diapause depends on the length of day and also on temperature and thus varies with the latitude of the distribution of *An. atroparvus*. It may last from September–April in northern Europe or from November–February in southern Europe. The females are mainly zoophilic and prefer different domestic animals according to their availability in different areas, but also readily feed on humans. They usually rest indoors, predominantly in stables and man-made shelters. Although swarming of the males before mating has been observed on several occasions, it is considered to be a vestigial characteristic, playing only a minor part in the actual mating behaviour of the sexes (Cambournac and Hill 1940). Usually the adults of *An. atroparvus* do not swarm before mating (stenogamy)

and mate almost entirely indoors. Flight ranges of *An. atroparvus* females of at least 3 km have been reported (Cambournac and Hill 1938).

Distribution: It is a largely a littoral species occurring from southeastern Sweden to Portugal along the coasts of the Atlantic, Baltic, and Mediterranean Sea. In southern and southeastern Europe it has a patchy distribution, e.g. from northern Italy and inland areas of central Italy through southwestern parts of Russia and the coastal area of the Black Sea. In Serbia and Macedonia it is widespread in the lowlands, but conspicuously dominant only in areas with saline/alkaline soils in the Pannonean Plain (Adamovic 1980). In Portugal it is the most common, most abundant, and most widely distributed *Anopheles* species throughout the country (Ribeiro et al. 1988). *An. atroparvus* was more common in the past, but has a diminished distribution today. In the Netherlands, industrial pollution is considered to be one reason for the decrease of the species abundance (Jetten and Takken 1994).

***Anopheles (Anopheles) beklemishevi* Stegnii and Kabanova 1976**

Biology: The larvae may occur in breeding sites quite typical for *An. messeae*. Stegnii and Kabanova (1976) reported a finding of larvae in a pond that was heavily polluted with organic material and aquatic vegetation was absent. The larvae could be found only along the edges of the pond. They seem to be more tolerant of the cold than larvae of *An. messeae*, and thus show an adaptation to the continental climate. So far little is known about the biology and the biting behaviour of the females. Many characteristics of this species seem to be similar to those of *An. maculipennis s.s.*, e.g. its zoophily (preference for feeding on animals). The adults usually rest indoors. In northern Sweden, Jaenson et al. (1986a) collected females from livestock shelters. *An. beklemishevi* was found resting in cattle sheds, horse stables, and pigsties without showing a preference for a particular species of domestic animal. It is an eurygamous species, e.g. swarming occurs before mating and the species undergoes complete winter diapause of a duration that is inversely correlated with the photoperiod (White 1978).

Distribution: *An. beklemishevi* is endemic to the cooler highlands and northern latitudes of Russia and the Baltics (White 1978). It can be found in Siberia,