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Bulinus on Aldabra and the Subfamily Bulininae in the Indian Ocean Area

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Bulinus on Aldabra and the subfamily Bulininae in the Indian Ocean area

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[Plates 20 to 23]

Contents

1. Introduction

The molluscan family Planorbidae is widely distributed throughout the temperate and tropical regions of the world. The subfamily Bulininae includes two genera only, *Bulinus* which is confined to the Ethiopian zoogeographical region, the Mediterranean area, the Middle East and some islands in the Western Indian Ocean, and *Indoplanorbis* which is common throughout India and Southeast Asia and also occurs on Socotra. These snails have been the subject of particularly intense study because of their importance as intermediate hosts for blood-flukes of the genus *Schistosoma* parasitic in man and domestic animals. The presence of a species of *Bulinus* on Aldabra is interesting because of the relative rarity of freshwater molluscs on atolls and also because it has served as a focus for drawing together the results of recent investigations into the distribution, relationships and intermediate host capacity of bulinids in the Indian Ocean area. This area has presented a number of problems in the interpretation of patterns of schistosomiasis transmission and most of these problems stem from misunderstandings about the taxonomy of the host snails and their parasites. Many of the misunderstandings have arisen from the paucity and unreliable nature of morphological criteria for taxonomic studies in basommatophoran snails and these have now been supplemented by cytogenetic, biochemical and immunological information. The methods used include paper chromatography of body-surface mucus (Wright 1964), electrophoresis of egg proteins on cellulose acetate (Wright & Ross 1965, 1966), starch-gel electrophoresis of digestive-gland enzymes (Wright, File & Ross 1966; Wright & File 1968), Ouchterlony plate gel diffusion and agar-gel immuno-electrophoresis of egg proteins using antisera prepared in rabbits, and colcimid blocking of mitotic metaphase chromosome figures in developing embryos.

2. The subfamily Bulininae

Superficially there is little resemblance between *Indoplanorbis* and *Bulinus*. *Indoplanorbis* has a discoidal, ram's-horn shell similar to that of many other planorbids but resembling most closely the North American genus *Helisoma* while *Bulinus* has a spired, sinistral shell like that in the Physidae and most bulinid species were originally referred to *Physa*. The characters which unite the two genera are a discoidal prostate and an eversible male copulatory organ, termed a pseudopenis, a structure which is unknown in other snails. At no point are the geographical ranges of the two genera known to coincide, the eastern limit of *Bulinus* is in Khuzestan Province of Iran and the western limit of *Indoplanorbis* on the mainland is probably in Pakistan.

Indoplanorbis Annandale & Prashad

Several species of *Indoplanorbis* have been named but the genus is now generally regarded as monotypic.

Indoplanorbis exustus (Deshayes)

1832. *Planorbis exustus* Deshayes, G.P., in Belanger, C. *Voy. Indes Orient.* (6), 417.
1920. *Indoplanorbis exustus* (Deshayes), Annandale & Prashad, *J. Med. Res.* **8**, 112.

The anatomy of *I. exustus* is so well known (Larambergue 1939; Baker 1945) that it is unnecessary to describe it further. Indeed, there would be no call to mention the species here if it did not occur as one of the dominant freshwater molluscs on the island of Socotra. Despite the proximity of Socotra to the African mainland and to South Arabia the freshwater snail fauna of the island is entirely Oriental in character with no Ethiopian elements. Throughout South-east Asia *I. exustus* is the intermediate host for *Schistosoma spindale* and *S. nasalis*, both parasites of domestic ungulates, but laboratory bred specimens of Socotran origin failed to become infected when exposed to miracidia of a strain of *S. spindale* from Malaysia. Repeated attempts to infect *I. exustus* from Socotra with several strains of *Schistosoma haematobium*, *S. bovis* and *S. mattheei*, all of which normally develop in *Bulinus*, have also failed.

Preliminary immunological tests have shown that *I. exustus* is not more closely related to any one of the species groups in *Bulinus* than it is to the others (figure 6*h*, plate 23).

Bulinus O. F. Müller

More than 120 species have been named in *Bulinus*. At the present time only about thirty of these are recognized but not all authorities agree on the status of some forms. Several subgenera have been created but due to incorrect assessment of the affinities of the type species of the genus the nominate subdivision was wrongly allocated. The reshuffling of names which would be needed to remedy this situation would be likely to cause so much confusion among workers in various disciplines connected with the control of schistosomiasis that the subgenera are now rarely used and in their place four species groups have been designated (Mandahl-Barth 1957) and these have the merit of greater flexibility in their definition.

The species groups were originally characterized by features of the shell, radular teeth, male copulatory organ and ridges on the roof of the mantle cavity and it is necessary briefly to review these definitions here. The *africanus* group have medium to low-spired shells without pronounced ribs but ornamented with fine spiral striae which intersect the growth lines to give a finely nodular or ripple-like pattern, the columella is usually sharply truncated and there is a ridge on the ventral surface of the kidney. This group is confined to Africa south of the Sahara where its members are the principle intermediate hosts for *Schistosoma haematobium*. The *tropicus* and *truncatus* groups also have medium to low-spired shells, usually ribbed and with the columella straight and gradually merging with the basal aperture margin. In both groups there is no ridge on the kidney and the intermediate ridge which lies between the kidney and the rectum is short, less than half the length of the kidney. The two groups differ in that the male copulatory organ frequently fails to develop in the *truncatus* groups but is always present in the *tropicus* complex and the central cusp (mesocone) of the first lateral radular tooth is arrow-head shaped in the *truncatus* group and simply triangular in *tropicus*. The *tropicus* group has a similar geographical distribution to the *africanus* complex but none of its members is known to act as a host for *S. haematobium*. The *truncatus* group is distributed throughout the Mediterranean region, the Middle East and West Africa and extends south to the Great Lakes in East Africa. Its members are the hosts for *S. haematobium* in North Africa and the Middle East. The *B. forskali* group have high-spired shells, often strongly ribbed on the upper whorls and the intermediate mantle ridge is as long as the kidney which has no ridge. The group is pan-African in distribution but its members act as hosts for *S. haematobium* only in a limited area of West Africa and in the Indian Ocean region. Since the original criteria for separating the species groups were defined the validity of some has been questioned, particularly the characters of the radula in separating the *tropicus* and *truncatus* groups. However, it is now known that the haploid chromosome number of the *truncatus* group is normally 36 (some populations with 54 and 72 have been found in Ethiopia and South Arabia), while that of the other three groups is 18 (Burch 1960). A further distinctive character of the *truncatus* group is that the major component of the egg proteins when separated by electrophoresis (figure 4*h*, plate 22) consists of three minor fractions while in the other groups there is only a single large fraction (Wright & Ross 1965).

On Aldabra *Bulinus* is represented by a member of the *forskali* group and this group is the principle one throughout the Indian Ocean area. The east coast of the African mainland is dominated by the *africanus* group and members of the *truncatus* complex occur in the Mesopotamian region at the head of the Persian Gulf and in the highland areas of South Arabia.

The *Bulinus forskali* group (= *Pyrgophysa* Crosse)

Bulinus mariei (Crosse)

1879 *Pyrgophysa mariei* Crosse, *J. Conch., Paris* **27**, 208.
1967 *Bulinus forskalii* (Ehrenberg) in part Mandahl-Barth, *Bull. Wld Hlth Org.* **17**, 34.

Described originally from the island of Nossi Bé off the northwestern tip of Madagascar and designated the type of the genus *Pyrgophysa*, *B. mariei* is probably widely distributed throughout Madagascar. The high-spired shell is strongly ribbed and shouldered on the upper whorls and in some populations the spire is exceptionally slender (shells 16 to 20 in figure 2, plate 20). The male copulatory organ has the penis-sheath a little longer than the preputium (no. 15 figure 1) and the cusps of the first lateral radular teeth are well separated from one another (figure 3*f*, plate 21). The paper chromatogram of the body-surface mucus shows a single fast-moving fraction (R_F about 0.85) which fluoresces pale blue in ultraviolet light (major component 365 nm) and exposure of the chromatogram to ammonia fumes merely enhances the brightness of the single fraction and does not reveal any other pattern. The morphological characters suggest that *B. mariei* is the Madagascan representative of the pan-African *B. forskali* and this is borne out by immunological comparison of the egg proteins of the two species. Laboratory experiments have so far failed to infect *B. mariei* with *Schistosoma haematobium* or with *S. bovis* or *S. mattheei*.

Bulinus bavayi (Dautzenberg)

1894 *Pyrgophysa bavayi* Dautzenberg, *J. Conch., Paris* **42**, 103.
1925 *Isidora forskalii* Ehrenberg, Connolly *J. Conch., Lond.* **17**, 265.
1957 *Bulinus forskalii* (Ehrenberg) in part, Mandahl-Barth, *Bull. Wld. Hlth Org.* **17**, 34.
1965 *Bulinus bavayi* (Dautzenberg) Mandahl-Barth *Bull. Wld Hlth Org.* **33**, 42.
1967 *Bulinus* sp. Wright, *Proc. R. Instn Gt Br.* **41**, 655.

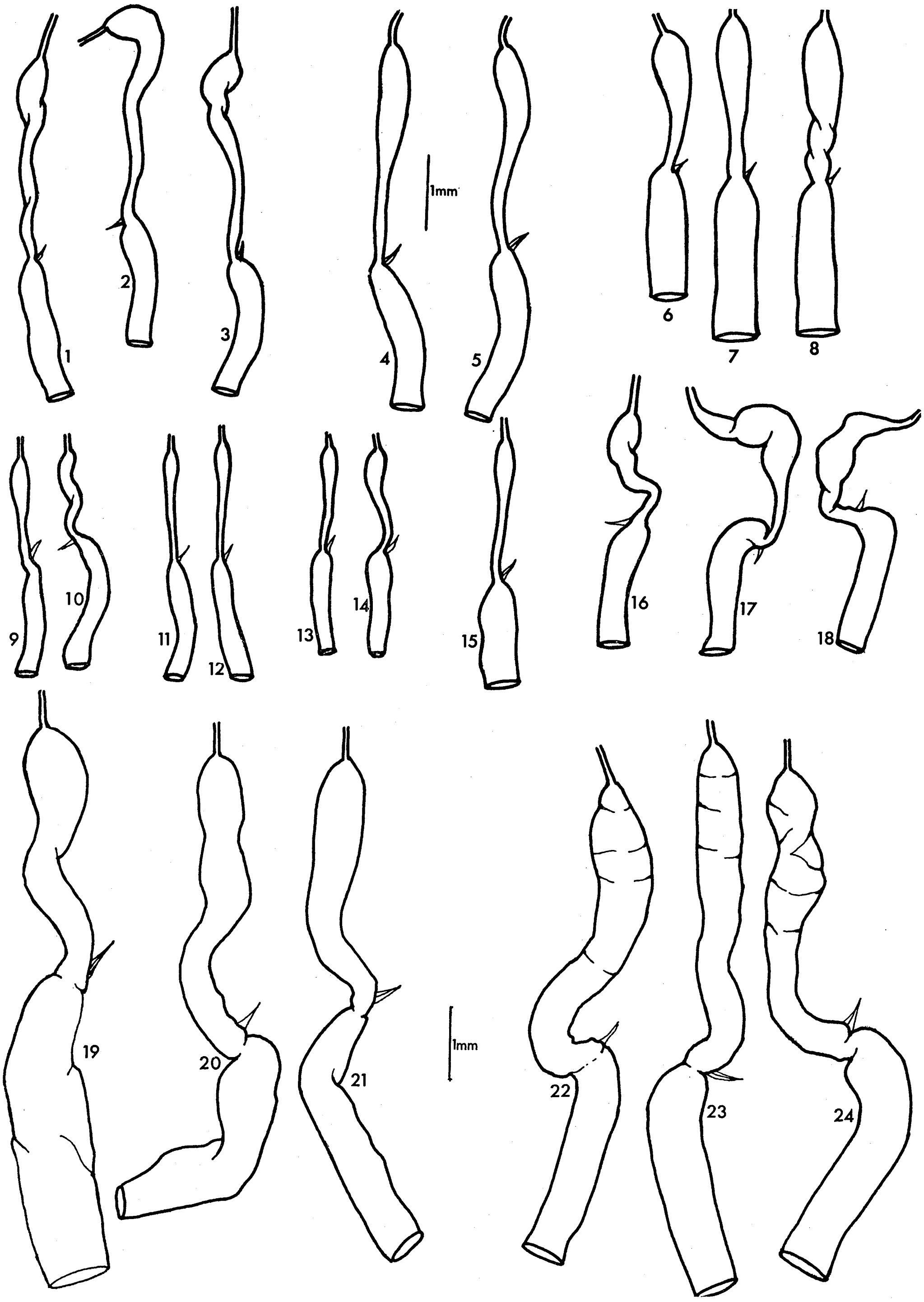

Figure 1. Male copulatory organs in *Bulinus*. 1 to 3, *Bulinus cernicus*, Cité le Cure, Mauritius; 4, 5, *B. cernicus* Clemencia, Mauritius (laboratory-bred); 6 to 8, *B. reticulatus* Kisumu, Kenya; 9, 10, *B. bavayi* Aldabra; 11, 12, *B. bavayi* Madagascar; 13, 14, *B. beccarii* Dirgag, Aden; 15, *B. mariei* Madagascar; 16 to 18, *B. wrighti* Samh-ba-Rooh, South Arabia; 19 to 21, *B. obtusispira* Madagascar; 22 to 24, *B. liratus* Madagascar.

Following its original description from the neighbourhood of Diego Suarez at the extreme northern tip of Madagascar *B. bavayi* received very little further attention and was generally included with *B. mariei* in the synonomy of *B. forskali*. Because of this confusion its distribution on Madagascar is not fully known but material used for the present study originated from Tanandava on the Mangoky River in the southwestern part of the island and the map showing the general distribution of *B. forskali* on Madagascar suggests that the species is widespread (Brygoo 1965). On Aldabra, from which the species is now recorded with certainty for the first time, *B. bavayi* is found in shallow freshwater pools, many of them temporary in nature, at the eastern end of South Island. Dead shells were particularly abundant in the area around Frigate Pool, also at Takamaka, but the living material from which a strong laboratory colony was founded originated from the Cinq Cases area.

The shell in *B. bavayi* is wider than that in *B. mariei* the whorls are more rounded, less strongly ribbed and lack a shoulder (shells 1–6 in figure 2, plate 20). The male copulatory organ is smaller than that of *B. mariei* at equivalent shell length and the penis sheath is either a little shorter than or equal to the preputium in length (nos. 9 to 12, figure 1). The first lateral radular teeth have the cusps well separated, the mesocone is relatively long and lanceolate and there are interstitial cusps on all of the specimens examined (figure 3*a*, plate 21). The chromatogram of the body surface mucus is typical for the *forskali* group and similar to that described for *B. mariei*. The egg proteins separated by electrophoresis show two secondary peaks following the main fraction and this is a feature not previously seen in any member of the *forskali* group (figure 4*a*, *b*, plate 22); there are some minor differences between the Aldabran and Madagascan samples in the slow-running protein fractions but these differences are of the order frequently found between quite closely adjacent populations of other bulinid species. The digestive gland aromatic esterases of the Aldabran and Madagascan samples differ only in a single slow-running fraction (figure 5*a*, *b*, plate 22). Immunological tests using an antiserum to egg proteins of the Aldabran population indicate that *B. bavayi* is closer to *B. cernicus* than to any of the other species against which it was tested (figure 6*a*, plate 23). Laboratory bred specimens of *B. bavayi* from Aldabra have proved to be highly susceptible to infection by the Mauritian strain of *Schistosoma haematobium* and the Iranian strain of *S. bovis*, very slightly susceptible to South African strains of *S. haematobium* and *S. mattheei* and completely resistant to the Iranian strain of *S. haematobium* (Wright & Knowles in prep.).

Bulinus beccarii (Paladilhe)

1872	*Physa beccarii* Paladilhe, *Annali Mus. civ. Stor. nat. Giacomo Doria* **3**, 12–16.
1956	*Bulinus forskali* (Ehrenberg) Azim & Gisman, *Bull. Wld Hlth Org.* **14**, 436.
1957	*Bulinus cernicus* (Morelet) in part Mandahl-Barth, *Bull. Wld Hlth Org.* **17**, 32.
1963	*Bulinus beccarii* (Paladilhe) Wright, *Bull. Br. Mus. nat. Hist. Zool.* **10** (4), 267.

Described from shells found in a dry stream bed near Aden, *B. beccarii* was for many years overlooked as a distinct species until it was found to be a natural host for *Schistosoma haematobium* in South Arabia (Wright 1963*a*, *b*).

The shell is similar to that of *B. bavayi* but smaller and with the body-whorl slightly less obese (shells 13 to 15 in figure 2, plate 20). In a few specimens there is a weakly defined carination forming a slight shoulder on the upper whorls but even these whorls lack transverse ribs. The male copulatory organ is very similar to that in *B. bavayi* and has the penis sheath and preputium of approximately equal length (nos. 13 and 14, figure 1). The radula too is similar to that in *B. bavayi* in that the endo- and mesocones of the laterals are well separated but the endocone in *B. beccarii* is slightly longer relative to the mesocone. The chromatogram of the body-surface mucus is similar to that in the two preceding species with a single blue fluorescent band of high R_F. The electrophoretic pattern of the egg proteins is quite distinct from that of *B. bavayi* but resembles that of several other species in the *forskali* group (figure 4*f*, plate 22). Material of this species has not been available for immunological or enzyme studies.

Bulinus cernicus (Morelet)

1867 *Physa cernica* Morelet, *J. Conch., Paris* **15**, 440.
1921 *Bulinus forskalii* (Ehrenberg) Germaine, *Faune Malacologique des Isles Mascareignes* Angers, p. 237.
1956 *Bulinus cernicus* (Morelet) Wright, *Nature, Lond.* **177**, 43.

Known only from Mauritius this species, in common with the others so far mentioned, was for a long time considered to be no more than an isolated race of the pan-African *B. forskali*. *B. cernicus* is quite widely distributed on Mauritius, particularly in the low-lying eastern and southern parts of the island where it is commonly associated with the extensive watercress beds.

The shell is high-spired but a characteristic of many of the populations is the uniform loss of the upper whorls. Specimens from three populations are illustrated (shells 7 to 12 in figure 2, plate 20) and despite the discrepancy in size of the individuals in the samples all of them were reproductively mature. When bred in the laboratory the strain from Clemencia (shells 7 and 8, figure 2) exceeds in size the specimens illustrated from Cité le Cure (shells 9 and 10, figure 2) and the upper whorls are not lost. The male copulatory organ is distinctive by its large size and by having the penis sheath 1.5 to 2.0 times as long as the preputium (nos. 1 to 5, figure 1). In its general appearance and proportions the copulatory organ of *B. cernicus* is most like that of the central African *B. scalaris* but it lacks the very long and convoluted epiphallus coiled within the dilated part of the sheath which is characteristic of the second species. The radular teeth of *B. cernicus* were remarked upon by Mandahl-Barth (1957) as the most characteristic feature of the species because of the greatly increased size of the mesocones and the incomplete separation of these cusps from the endocones. While this is certainly true of some populations it is not of others as is shown in figure 3*b* to 3*d*, plate 21.

The chromatographic pattern of the mucus of *B. cernicus* is the same as that of the preceding species. Electrophoretic separations of the egg proteins of three populations are illustrated in figure 4*c* to 4*e*, plate 22, and it can be seen that the pattern in this species is more complex than in the three preceding forms, also that there are quite marked differences between the three samples. Only one sample was available for enzyme studies and although this showed a single acid phosphatase fraction similar to that in *B. bavayi* the aromatic esterases of the digestive gland are quite different in the two species (figure 5*d*, plate 22). *B. cernicus* not only has a greater number of esterase fractions than are present in *B. bavayi* but it also has two quite well-marked fractions which move in the cathodal direction. Immunological tests suggest that despite the apparent differences in the electrophoretic patterns of the egg-proteins between *B. cernicus* and *B. bavayi* the two species are quite closely related (figure 6*a*, plate 23).

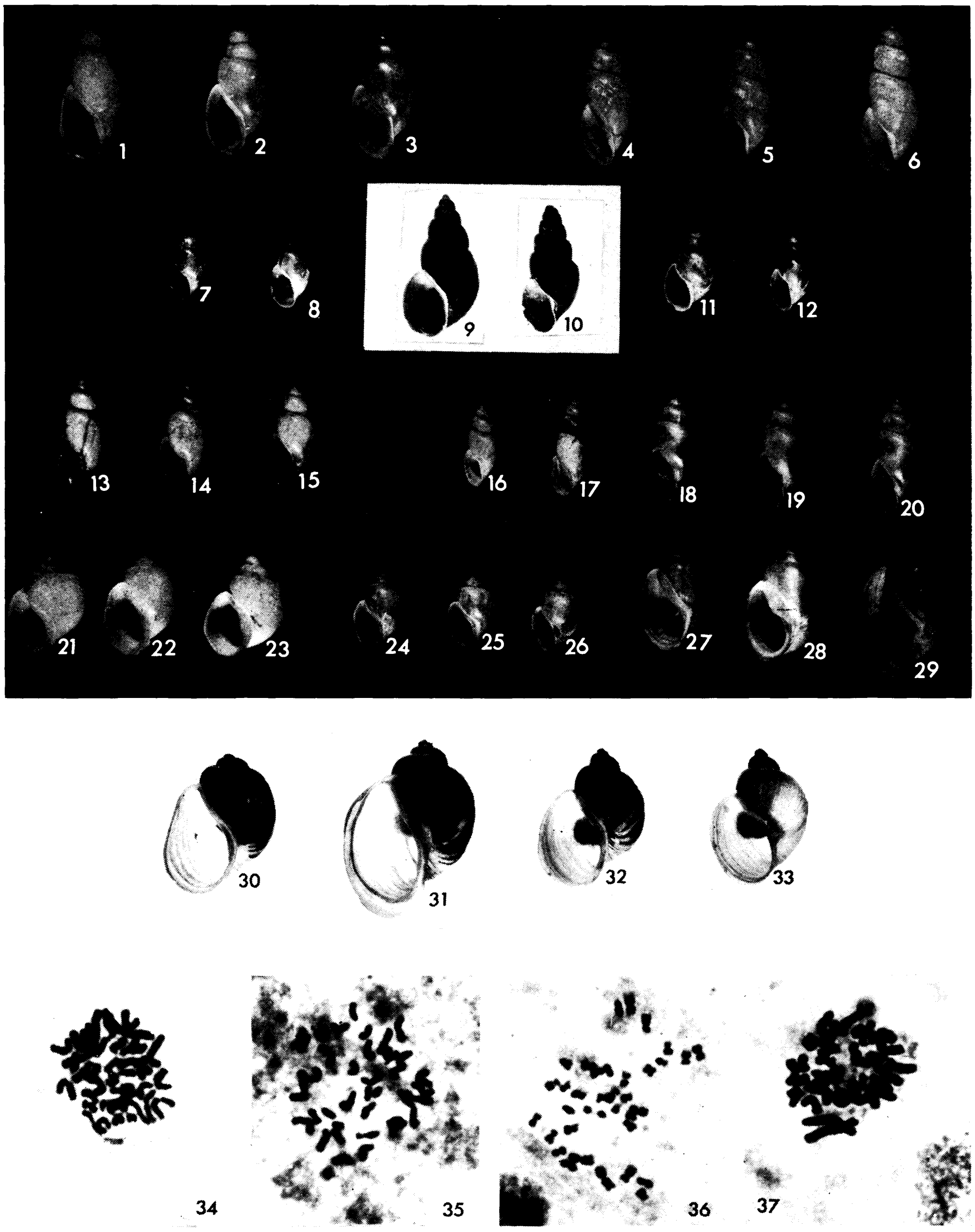

FIGURE 2. Shells and chromosomes of *Bulinus*. 1 to 3, *B. bavayi* Aldabra; 4 to 6, *B. bavayi* Madagascar; 7, 8, *B. cernicus* Clemencia, Mauritius; 9, 10, *B. cernicus* Cité le Cure, Mauritius; 11, 12, *B. cernicus* Carreau Acacias, Mauritius; 13 to 15, *B. beccarii* Dirgag, Aden; 16 to 20, *B. mariei* Madagascar; 21 to 23, *B. wrighti* Samh-ba-Rooh, South Arabia; 24 to 26, *B. reticulatus* Kisumu, Kenya; 27 to 29, *B. obtusispira* Madagascar; 30 to 33, *B. liratus* Madagascar. (1 to 33 all magn × 2.)

34, mitotic metaphase chromosomes of *B. bavayi*; 35, mitotic metaphase chromosomes of *B. wrighti*; 36, mitotic metaphase chromosomes of *B. obtusispira*; 37, mitotic metaphase chromosomes of *B. globosus*. (34 to 37 magn about × 1300.)

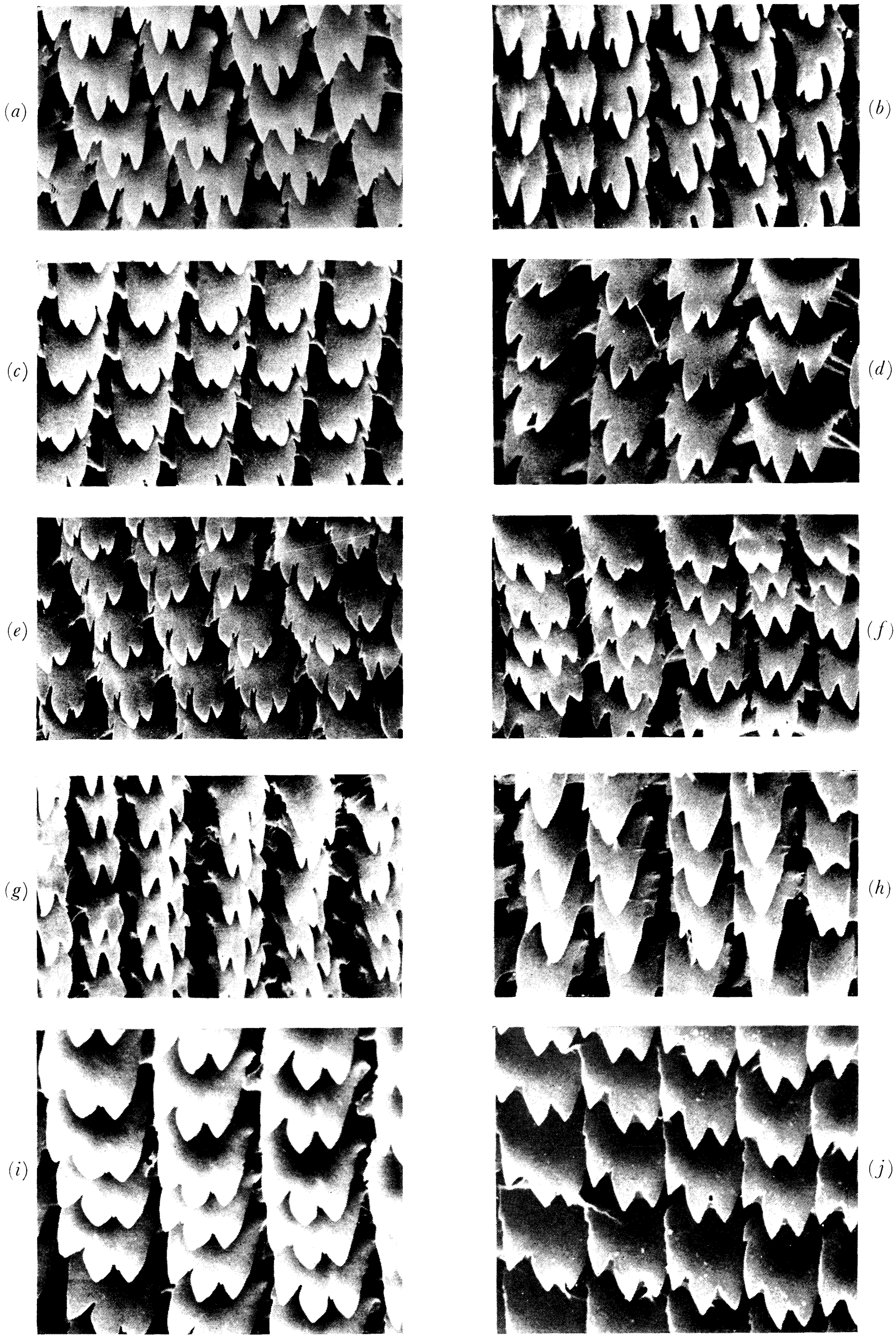

FIGURE 3. Stereoscan pictures of *Bulinus* radulae. *a*, *B. bavayi* Madagascar; *b*, *B. cernicus* Carreau Acacias, Mauritius; *c*, *B. cernicus* Clemencia, Mauritius; *d*, *B. cernicus* Cité le Cure, Mauritius; *e*, *B. beccarii* Dirgag, Aden; *f*, *B. mariei* Madagascar; *g*, *B. reticulatus* Kisumu, Kenya; *h*, *B. wrighti* Samh-ba-Rooh, South Arabia; *i*, *B. liratus* Madagascar; *j*, *B. obtusispira* Madagascar. (All figures magn. about × 1000.)

The *Bulinus tropicus* Group

Bulinus liratus (Tristram)

1863	*Physa lirata* Tristram, *Proc. zool. Soc. Lond.* 1863, p. 60.
1882	*P. madagascariensis* Angas, *Proc. zool. Soc. Lond.* 1877, p. 258
1882	*P. lamellata* Smith, *Proc. zool. Soc. Lond.* 1882, p. 386.
1886	*P. hildebrandti* Clessin, *in* Martini & Chemnitz, *Systematisches Conchylien-Cabinet* **1** (17), 351.
1936	*Bulinus* (*Diastropha*) *contortus bullaceous* Haas, *Abh. senckenb. naturforsch. Ges.* (431), p. 31.
1957	*B. liratus* (Tristram) Mandahl-Barth, *Bull. Wld Hlth Org.* **17**, 23.

The list of synonyms for *B. liratus* is an indication of the considerable variation in shell shape which is encountered in the *B. tropicus* group. It is possible that some of these forms may eventually prove to be specifically distinct but experience with this group on the African mainland suggests that this is unlikely. Specimens raised in aquaria at the Institut Pasteur in Tananarive had the depressed spire and very strong costulation characteristics for the species but their progeny reared in aquaria in London had less well-developed ribs and more exserted spires, both characteristics of some of the 'species' listed in the synonymy. The type locality of *B. liratus* was given as two days' journey west of Tananarive but the means of transport was not specified. According to the surveys made by Brygoo (1965) and his colleagues *B. liratus* is most common in the central and southeastern areas of the island but it has also been recorded from the northwestern region.

The globose, strongly ribbed shell with a depressed or only slightly exserted spire (shells 30 to 33 in figure 2) serves to define this species on Madagascar and suggests its strong affinities with the *B. tropicus* complex in southern Africa. These affinities are further indicated by the large male copulatory organ in which the ratio of the length of the penis sheath to the preputium is about 3/2 and in which the dilated proximal part of the sheath is marked by narrow transverse bands of pigment (nos. 20 to 24, figure 1). The large radular teeth have the lateral mesocones strongly arrow-head shaped (figure 3*i*, plate 21), a character which was at one time thought to have suggested affinities with the *B. truncatus* group but which is now known to occur also in *B. tropicus*. The haploid chromosome number of *B. liratus* is 18.

The chromatographic pattern of the body-surface mucus of *B. liratus* shows three quite strong fluorescent bands between R_F 0.3 and 0.5, the inner band is bright blue, the middle one light blue and the outer is more diffuse and yellowish in colour. This type of pattern with variations in the presence or absence of one or other of the bands is found throughout the *tropicus* and *truncatus* groups but when the chromatogram of *B. liratus* is exposed to ammonia fumes a wide, diffuse light blue band appears between R_F 0.8 and 0.95. This additional band had not previously been seen in either the *tropicus* or *truncatus* groups but subsequent examination of certain populations of *B. tropicus* from the neighbourhood of Potchefstroom in South Africa has revealed its presence. The electrophoretic pattern of the egg-proteins of *B. liratus* it closely similar to that of the South African *B. tropicus* (figure 4*i*, *j*, plate 22) and on Ouchterlony immunodiffusion plates the egg proteins of both species give identical reactions to an antiserum prepared to the South African material (figure 6*d*, plate 23). The acid phosphatases of the digestive gland in *B. liratus* show a complex pattern of four fractions unlike that of any of the other Madagascan species and the aromatic esterases show a characteristic very large cathodal fraction (figure 5*e*, plate 22). *B. liratus*, like other members of the *tropicus* complex has not proved to be susceptible to any strain of *S. haematobium* to which it has been exposed.

Species of uncertain affinities

Bulinus obtusispira (Smith)

1882 *Physa obtusispira* Smith, *Proc. zool. Soc. Lond.* 1882, p. 386.
1957 *Bulinus liratus* (Tristram) in part Mandahl-Barth, *Bull. Wld Hlth Org.* **17**, 23.
1966 *B. obtusispira* (E. A. Smith) Brygoo & Moreau, *Bull. Soc. Path. exot.* **59** (5), 835–839.

After its initial description from a locality about 32 km from Tananarive *B. obtusispira* remained an obscure species, generally considered to be no more than a variant of *B. liratus*, until in 1965 a large number of specimens thought to be *B. liratus* were collected at Majunga in northwest Madagascar and were found to be shedding the cercariae of *Schistosoma haematobium* (Brygoo & Moreau 1966). These specimens were submitted to Dr Mandahl-Barth who referred them to Smith's species. The material which has been available to me for examination consists of two laboratory-bred colonies, one derived from the strain maintained at the Institute Pasteur in Tananarive and the other from Basybasy in southwestern Madagascar.

Smith's description of the shell (light brown in colour, somewhat glossy, narrowly rimate with indistinct spiral striations, longitudinally plicately ridged and strongly lirated) fits the present material quite well with the exception of the last character (shells 27 to 29 in figure 2). The holotype of *B. obtusispira* (B.M. (N.H.) col. no. 92.3.5.10) has got well-marked ribs on the upper whorls but these are scarcely developed or absent on the laboratory-bred specimens. On a few individuals the spiral striations are sufficiently well-developed to give, by intersection of the growth lines, a fine ripple-like microsculpture on the body whorl. The ridges on the underside of the mantle are similar to those in the *tropicus* and *truncatus* groups, the intermediate ridge is about half the length of the kidney and a renal ridge is lacking. The male copulatory organ is large for the size of the animal and the penis sheath and preputium are approximately equal in length and maximum diameter (nos. 19 to 21, figure 1). The radular teeth are large and the mesocone of the laterals is roughly triangular in shape (figure 3*j*, plate 21).

The chromatogram of the body-surface mucus shows no more than a trace of fluorescence around R_F 0.4 to 0.5 but this is so indistinct that in many specimens it cannot be seen at all. The egg proteins separated by electrophoresis show a fairly complex pattern with some particularly strongly developed slow-running fractions (figure 4*k*, plate 22). Antisera to these egg proteins give a strong cross-reaction only with members of the *B. africanus* group (figure 6*e*, plate 23). The aromatic esterases of the digestive gland are similar to those of *B. bavayi* but the acid phosphatase fraction is slower running and more diffuse than that in *B. bavayi*. The haploid chromosome number of *B. obtusispira* is 18 but the chromosomes are remarkable for their very small size. Colcimid-blocked mitotic metaphase figures for developing embryos are shown in figure 2 (no. 36) with figures from *B. bavayi*, *B. wrighti* and *B. globosus* for comparison (nos. 34, 35 and 37 respectively).

It was the presence of a high infection rate with *Schistosoma haematobium* which drew attention to *B. obtusispira* as a distinct species. In the laboratory it has proved to be highly susceptible to the Mauritian strain of the parasite, very slightly susceptible to the Iranian strain normally carried by *B. truncatus* and totally resistant to strains from South Africa and Ghana (both of these strains have *africanus* group snails for their normal hosts) and to *S. bovis* from Iran and *S. mattheei* from South Africa.

Bulinus wrighti Mandahl-Barth

1941 *B. truncatus* (var.) Connolly, *British Museum* (*N.H.*) *Expedition to South-west Arabia* 1937–8 **1** (4), 37.

1963 *B. reticulatus* Mandahl-Barth, Wright, *Bull. Br. Mus. nat. Hist. Zool.* **10** (4), 265.

1965 *B. reticulatus wrighti* Mandahl-Barth, *Bull. Wld Hlth Org.* **33** (1), 41.

Apart from Connolly's single sample of shells which he considered to be no more than an extreme variant of *B. truncatus* this is the most recently discovered species of *Bulinus*. Collected in 1962 from several localities in South Arabia the material was originally referred to *B. reticulatus*, another recently discovered species from East Africa, but Mandahl-Barth (1965) considered that the morphological differences between the South Arabian and East African forms were sufficiently great to justify the naming of the Arabian snails as a subspecies. More recent work has emphasized the differences and the two forms are now treated as specifically distinct. Connolly's specimens came from Al Bahr in the Hadhramaut region of southwest Arabia and my original material (Wright 1963*a*) was collected from two localities in Upper Aulaqui in the eastern part of what was then the Western Aden Protectorate. Since then material has been obtained from two localities near Dhala on the Yemen border north of Aden (Dhala and Tafwa) and from Samh-ba-Rooh in the Raidal al Sa-ar area north of the Wadi Hadhramaut. Dr Mandahl-Barth informs me that he has received two samples from the Central Province of Saudi Arabia (Mansuriah Oasis in the Anaiwa District and Hayil) and these records extend the range far northward suggesting that *B. wrighti* may be widely distributed in the Arabian peninsula.

The shell is characterized by its globose form with a short but acute spire, wide open umbilicus and reticulate microsculpture. *B. wrighti* grows to a greater size than *B. reticulatus* and shells of both species at comparable states of maturity are illustrated (shells 21 to 26, figure 2). Both species lack a renal ridge in the mantle cavity and the intermediate ridge is shorter than the kidney. The male copulatory organ in *B. wrighti* has the penis sheath a little longer than the preputium and the maximum dilatation of the sheath occurs at about one-third of its length from the proximal end (nos. 16 to 18, figure 1), while in *B. reticulatus* the preputium is usually a little longer than the sheath and the dilatation is more nearly proximal (nos. 6 to 8, figure 1). The radular teeth of the two species are strikingly different, those in *B. reticulatus* being small with well divided lanceolate cusps, while in *B. wrighti* the teeth are large and the endocones of the laterals appear as small points on the inner margins of the spatulate mesocones (figure 3*g*, *h*, plate 21).

Chromatograms of the body-surface mucus of *B. wrighti* show no trace of any fluorescent material. Electrophoretic separation of the egg proteins of the three populations available for study shows a relatively simple pattern with a few minor differences between the populations (figure 4*n* to 4*p*, plate 22). In contrast the egg proteins of *B. reticulatus* give a complex pattern unlike that of any other bulinid. Figure 4*m* illustrates the pattern from a population collected from near to the type locality at Kisumu in Kenya and this pattern differs from that given by a Rhodesian population (Wright & Ross 1966) which in turn is similar to samples from Masingire (Mozambique) and Potchefstroom (South Africa). An antiserum to the egg proteins of *B. wrighti* gives poor cross-reactions with species belonging to all four of the bulinid groups (figure 6*g*, plate 23) but *B. wrighti* antigen tested against antisera to members of each of the groups shows slightly greater affinity for the *B. tropicus* complex (figure 6*g*). The aromatic esterase patterns of *B. wrighti* have proved difficult to resolve (figure 5*f* to 5*h*, plate 22) but in their lack of any particular dominant fraction they resemble the pattern given by *B. cernicus*. The acid phosphatases consist of at least two poorly resolved fractions with mobility similar to the fastest fraction in *B. liratus*.

B. wrighti is a natural host for *Schistosoma haematobium* in South Arabia and in the laboratory it has proved to be susceptible in varying degree to every strain of *S. haematobium* to which it has been exposed, also to several strains of *S. bovis* and *S. mattheei*. This range of susceptibility is not known in any other bulinid species and *B. wrighti* is a better laboratory host for some strains of schistosome than are their natural hosts, giving higher infection rates, more prolonged cercarial shedding and shorter incubation periods.

3. Discussion

It is scarcely surprising that of the various species of *Bulinus* in the Indian Ocean area the one which occurs on Aldabra is from the nearest adjacent land mass, Madagascar. The reasons why *B. bavayi* rather than any of the other Madagascan species has become established on the island must await the results of further work on Madagascar. Two main possibilities suggest themselves, either that there is not equal opportunity of transport for all the species, or that the ecological conditions on Aldabra can only be tolerated by *B. bavayi*. It is difficult to assess equality of opportunity of transport without certain knowledge of the means by which snails reach the island. The classical vehicle of wading birds seems to be the most likely and if this is the case there is no obvious reason as to why *B. bavayi* should travel more easily than the other species. The question of adult size is irrelevant because hatching sizes of most bulinids are similar and young individuals of all species could be carried with equal ease. It is possible that *B. liratus* does not occur at the northern end of Madagascar and is not therefore so readily available for transport but both *B. mariei* and *B. obtusispira* are known to occur in the northwest part of the island. Perhaps *B. bavayi* is more abundant in habitats frequented by waders or its seasonal breeding cycle is such that peak numbers of young occur at the time of maximum bird movement to Aldabra or it may be that the habits of *B. bavayi* make it more readily picked up by waders. All of these points and probably many others need investigation but two factors of the Aldabran environment which could be responsible for the selective survival of *B. bavayi* are the temporary nature of the freshwater pools and their high calcium content. *B. bavayi* belongs to a species complex of which other members are known to have a greater tolerance of high-water temperatures and desiccation than have most bulinids, and it is therefore probable that *B. bavayi* would be less affected by the exposed nature and seasonal fluctuations of the Aldabran pools. Nothing is known of the precise chemical requirements of any of the Madagascan species but water chemistry, particularly calcium bicarbonate concentrations, has recently been found to play a

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Description of plate 22

Figure 4. Electrophoretic patterns of *Bulinus* egg proteins. *a*, *B. bavayi* Aldabra; *b*, *B. bavayi* Madagascar; *c*, *B. cernicus* Clemencia, Mauritius; *d*, *B. cernicus* Carreau Enouf, Mauritius; *e*, *B. cernicus* Cressonville, Mauritius; *f*, *B. beccarii* Dirgag, Aden; *g*, *B. mariei* Madagascar; *h*, *B. truncatus* Dezful, Iran; *i*, *B. liratus* Madagascar; *j*, *B. tropicus* Potchefstroom, South Africa; *k*, *B. obtusispira* Madagascar; *l*, *B. globosus* Nigeria; *m*, *B. reticulatus* Kisumu, Kenya; *n*, *B. wrighti* Rassais, South Arabia; *o*, *B. wrighti* Tafwa, South Arabia; *p*, *B. wrighti* Marbum, South Arabia.

Figure 5. Starch-gel electrophoretic patterns of digestive gland isoenzymes in *Bulinus*. Aromatic esterases on the left, acid phosphatases on the right. *a*, *B. bavayi* Aldabra; *b*, *B. bavayi* Madagascar; *c*, *B. cernicus* Clemencia, Mauritius; *d*, *B. obtusispira* Madagascar; *e*, *B. liratus* Madagascar; *f*, *B. wrighti* Rassais, South Arabia; *g*, *B. wrighti* Tafwa, South Arabia; *h*, *B. wrighti* Marbum, South Arabia.

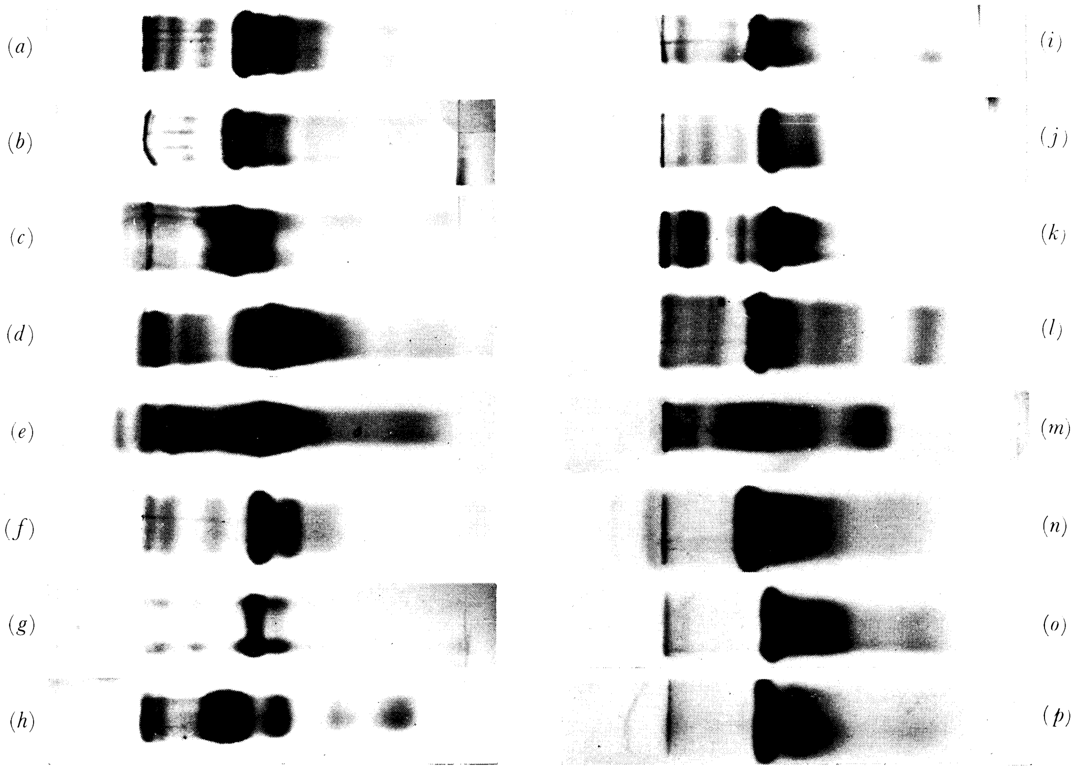

FIGURE 4. For legend see facing page.

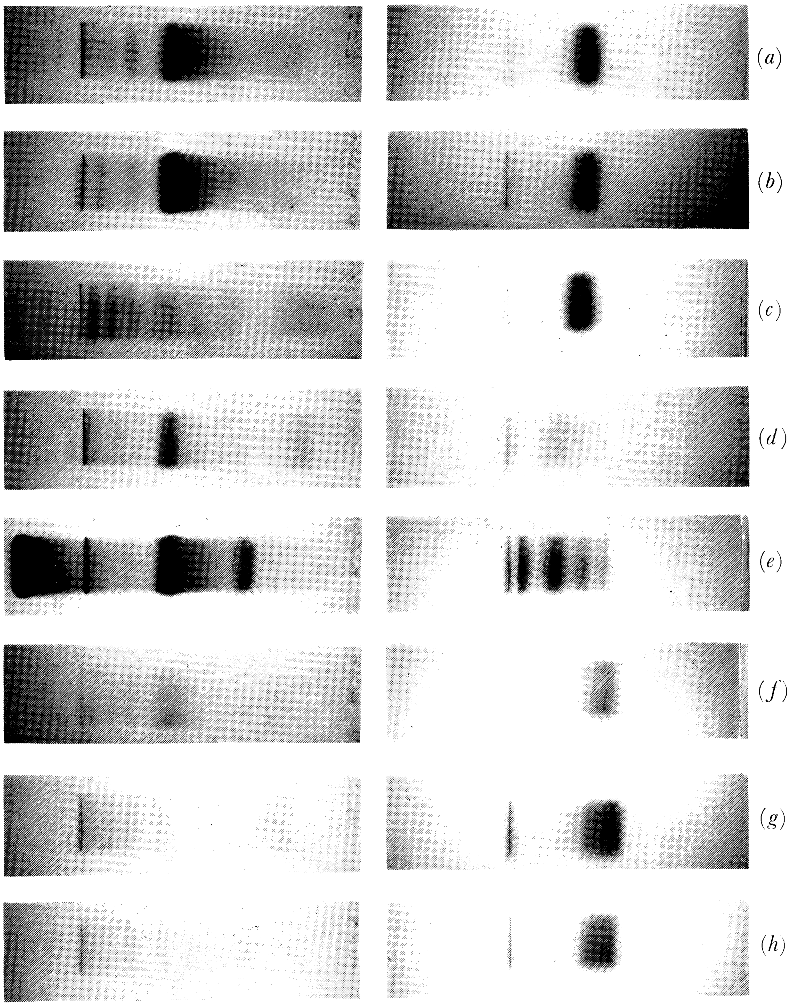

FIGURE 5. For legend see facing page.

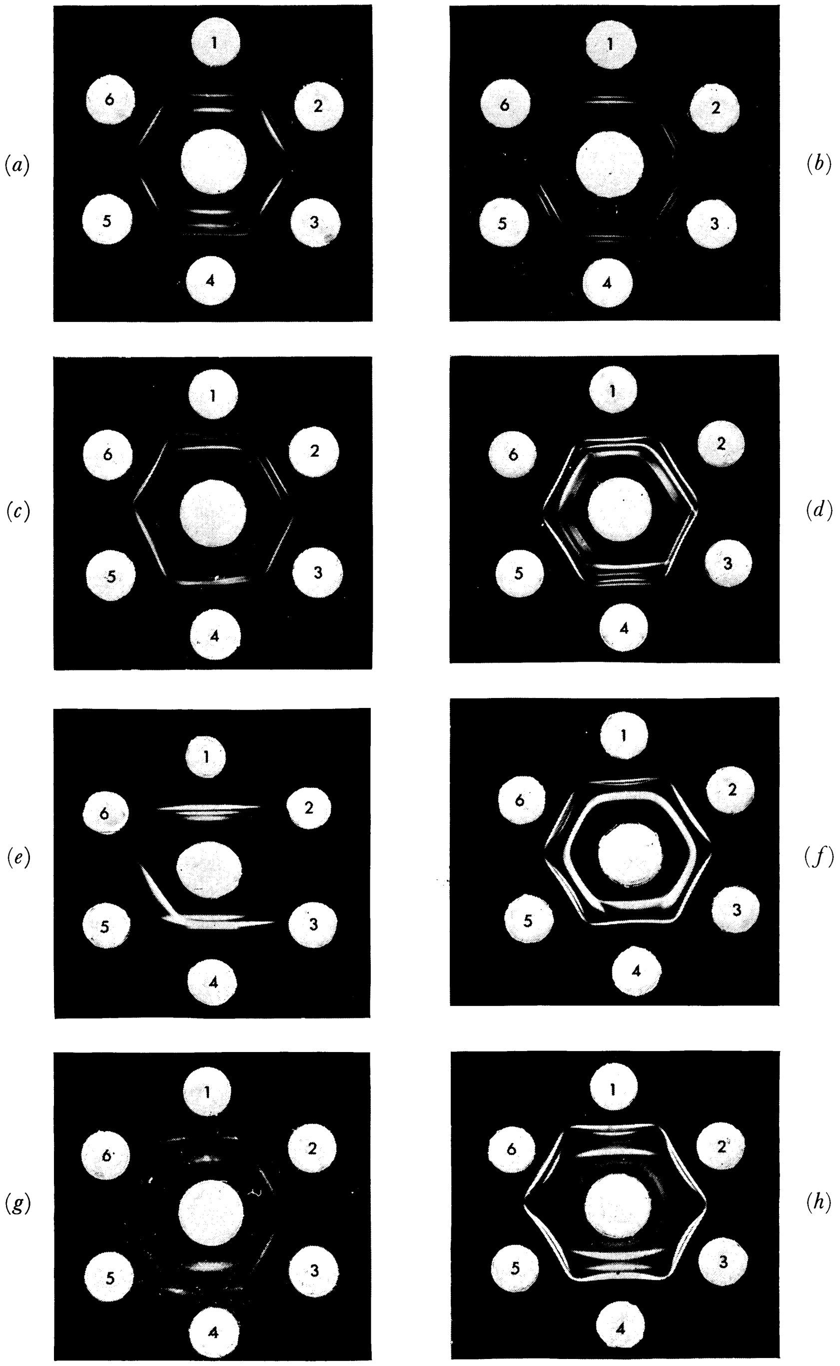

FIGURE 6. Ouchterlony immunodiffusion plates with egg proteins of *Bulinus*. Antisera in centre wells, antigens in peripheral wells. (*a*) Antiserum to *B. bavayi*: 1, *B. bavayi*; 2, *B. cernicus*; 3, *B. scalaris*; 4, *B. bavayi*; 5, *B. mariei*; 6, *B. wrighti*.

(*b*) Antiserum to *B. scalaris*: 1, *B. scalaris*; 2, *B. bavayi*; 3, *B. cernicus*; 4, *B. scalaris*; 5, *B. forskali*; 6, *B. wrighti*.

(*c*) Antiserum to *B. tropicus*: 1, *B. tropicus*; 2, *B. wrighti*; 3, *B. forskali*; 4, *B. tropicus*; 5, *B. scalaris*; 6, *B. cernicus*.

(*d*) Antiserum to *B. tropicus*: 1, *B. tropicus*; 2, *B. liratus*; 3, *B. obtusispira*; 4, *B. tropicus*; 5, *B. bavayi*; 6, *B. wrighti*.

(*e*) Antiserum to *B. obtusispira*: 1, *B. obtusispira*; 2, *B. truncatus*; 3, *B. tropicus*; 4, *B. obtusispira*; 5, *B. globosus*; 6, *B. bavayi*.

(*f*) Antiserum to *B. ugandae*: 1, *B. ugandae*; 2, *B. globosus* (Sudan); 3, *B. globosus* (Zambia); 4, *B. obtusispira*; 5, *B. africanus*; 6, *B. globosus* (Ghana).

(*g*) Antiserum to *B. wrighti*: 1, *B. wrighti*; 2, *B. tropicus*; 3, *B. truncatus*; 4, *B. wrighti*; 5, *B. africanus*; 6, *B. forskali*.

(*h*) Antiserum to *Indoplanorbis exustus*: 1, *I. exustus*; 2, *B. truncatus*; 3, *B. tropicus*; 4, *I. exustus*; 5, *B. globosus*; 6, *B. bavayi*.

very critical rôle in the distribution of certain African planorbids. It is therefore quite possible that the high calcium content of the temporary pools on Aldabra (evidence of which is given by the areas of re-deposited limestone surrounding the pools in the dry season) may be beyond the limits tolerated by *B. mariei*, *B. liratus* and *B. obtusispira*. The shells of *B. obtusispira* maintained in the relatively hard London tap-water tend to be more dense than those of specimens recently arrived from Madagascar and a high proportion of them develop deformities due to overgrowths of the aperture lip.

Whether *B. bavayi* is a truly permanent element of the Aldabran fauna must also be questioned. The abundance of shells in the area of the freshwater pools leaves no doubt that the species can thrive on the island when conditions are favourable but the meagre meteorological data which are available suggest that the rainfall on the island is somewhat irregular. Consecutive failures of the wet season would result in conditions unsuitable for the survival of freshwater snails but if immigration is easy they could become re-established when favourable circumstances return. The very close similarity between the Aldabran and Madagascan samples of *B. bavayi* both in their egg proteins and in their digestive gland esterases, suggests that the Aldabran population has not long been isolated and may even be augmented annually by recruitment from Madagascar. The closeness of these two populations is emphasized by the differences which can be demonstrated between different populations of *B. cernicus* from the geographically very limited area of Mauritius.

B. bavayi is obviously closely related morphologically to *B. beccarii* from South Arabia and immunodiffusion tests on the egg proteins show a close affinity with *B. cernicus* from Mauritius (figure 6*a*, plate 23). *B. beccarii* also shows morphological similarities to *B. senegalensis*, a West African species confined to the semi-arid Sudanese savannah area (Wright 1959), and there are also similarities between the electrophoretic patterns of the egg proteins of the two species. These four apparently related forms occupy areas to the north and east periphery of the main range of the *forskali* group to which they belong, a restricted distribution which probably represents the remains of a former wide range. All four of these species have certain features in common. As far as is known they all occur in habitats where there are no other bulinids, the status of *B. bavayi* on Madagascar requires investigation but *B. beccarii*, *B. cernicus* and *B. senegalensis* have never been found associated with other species. This suggests that they are all able to tolerate certain conditions which are not acceptable to most other bulinids, particularly high-water temperatures and prolonged periods of desiccation but it also suggests that they may be unable to withstand close ecological competition. All four species are susceptible to infection by certain strains of *Schistosoma haematobium* and, in the case of *B. senegalensis* and *B. bavayi* by *S. bovis*. In this susceptibility to infection may lie the explanation of their contemporary restricted range. *B. forskali*, the dominant member of the species group has a pan-African distribution and is not normally susceptible to schistosome parasites. Since such infection has a deleterious effect on the fecundity of snails *B. forskali* would have a selective advantage over any susceptible species with similar ecological requirements and such susceptible species would only be likely to survive in habitats with conditions not acceptable to *B. forskali*. Thus the restriction of the susceptible species in the *forskali* group to peripheral areas and to habitats not shared by other species may be in part attributable to the expansion of the range of the definitive hosts of *S. haematobium*, *S. bovis* and *S. mattheei*, man and his domesticated ungulates. Only schistosome parasites have been mentioned but amphistomes have also been found in *B. cernicus* and *B. senegalensis* and notocotylids in *B. bavayi* from Madagascar, while *B. forskali*, on the other hand, is only rarely found to harbour trematode infections of any kind. Parasite pressure as an agency in natural selection has not received a great deal of attention but it is possibly an important factor in the relationships between molluscs and larval trematodes. Two other species in the *forskali* group are possible hosts for *Schistosoma haematobium*, *B. crystallinus* from the rain-forest escarpment of Angola and *B. camerunensis* from a crater lake in the rain-forest area of Cameroon. *B. crystallinus* is not a proven host but epidemiological evidence points strongly towards it (Wright 1963*c*) and although *B. camerunensis* was not successfully infected in the laboratory (Wright 1965) Dr B. O. L. Duke (private communication) has recently found naturally infected specimens. Neither of these two species appears to be closely related to the *senegalensis–beccarii–bavayi–cernicus* complex but both have very restricted distributions in the rain-forest areas (where few planorbids occur) and it seems possible that these two may also be relics of previously more widely distributed species. Only one other species is known with certainty to be a member of the *forskali* group, *B. scalaris*, which has a patchy distribution in Central and East Africa, frequently being found together with *B. forskali*. Despite a fairly close immunological relationship to the *bavayi* complex (figure 6*a*, *b* plate 23) *B. scalaris* is not susceptible to any known strain of schistosome and is not therefore subject to that particular parasite pressure. The most likely restriction on the wider distribution of *B. scalaris* is suggested by laboratory experiments which indicate that it is the only species in the *forskali* group which is not capable of self-fertilization.

The affinities of two of the other bulinids in the Indian Ocean are easily established. *B. liratus* is simply the Madagascan counterpart of the southern African *B. tropicus* and *B. mariei* is no more than a race of *B. forskali*. In the light of the remarks made above concerning the ecological relationship between *B. forskali* and other members of the *forskali* species group, detailed surveys of the distribution of *B. bavayi* and *B. mariei* on Madagascar might be very rewarding. It is possible that *B. mariei* may be a relatively recent arrival on the island and in time it could supplant *B. bavayi* or it may be that *B. bavayi* occurs in habitats unsuitable for *B. mariei* so that the two species are ecologically isolated from one another as is the case with *B. senegalensis* and *B. forskali* in West Africa. The relationships of the remaining two bulinids in the area, *B. obtusispira* on Madagascar and *B. wrighti* in South Arabia, are less easily determined.

B. obtusispira is singularly lacking in distinctive morphological characters. The general shape of the shell is similar to that in some forms of the *tropicus* group and it is for this reason that the species was previously included in the synonymy of *B. liratus*. The ripple-like microsculpture which is occasionally present suggests affinities with the *africanus* group but the main conchological character of that group, a truncated columella, is lacking. The mantle ridges are similar to those in the *tropicus* and *truncatus* groups but the hind end of the foot in living specimens is very sharply pointed, and this is usually considered to be a distinctive feature of the *africanus* group. The haploid chromosome number of 18 suggests that there is no relationship to the *truncatus* group but the very small size of the chromosomes is a feature unique to *B. obtusispira*. The chromatogram of the body-surface mucus is equivocal in its indications in that a few individuals show the faintest traces of fluorescence in the region where the main pattern of the *tropicus* and *truncatus* groups occurs but the pattern is too weak to interpret and many specimens show no fluorescence at all, a character usually associated with the *africanus* group. The electrophoretic egg protein pattern shows an intense and complex set of slow-running fractions and although no particular conclusions can be drawn from this such a pattern is more often encountered in the *forskali* group. The results of the immunodiffusion experiments are definite and clear, the only species group with which antisera to *B. obtusispira* react strongly is the *africanus* group (figure 6*e*, plate 23). However, if *B. obtusispira* antigen is tested against an antiserum to any other member of the *africanus* group as in figure 6*f* (antiserum to *B. ugandae*) it always reacts less strongly than other species in the group suggesting that it is somewhat apart from its nearest relatives. If a number of *africanus*-group antigens (including *B. obtusispira*) are tested against an antiserum to *B. tropicus* there is a slightly stronger cross-reaction with the *B. obtusispira* antigen and similar results are obtained if the tests are made with an antiserum to a member of the *forskali* group. These data suggest that the ancestral stock of *B. obtusispira* became isolated on Madagascar at a time when the *africanus* group had relatively recently diverged from the rest of the bulinid line and the species has retained more of the antigens common to the other groups than have the *africanus* group species on the continent of Africa. There remains, however, the remarkable problem of the susceptibility of *B. obtusispira* to infection by *Schistosoma haematobium*. This parasite is strongly host-specific in its relationships with the genus *Bulinus* and each of the three species groups of snails which act as hosts (*africanus*, *truncatus* and *forskali*) have their own main strain of parasite which will develop almost exclusively in snails of that group and not the others (Wright 1966). It is surprising therfore that while immunological tests show *B. obtusispira* to be a member of the *africanus* complex it appears to be totally resistant to the schistosomes which normally parasitize that group but it is highly susceptible to a strain which develops in the *forskali* group (the Mauritian strain in *B. cernicus*) and slightly susceptible to the *B. truncatus*-borne strain from Iran. The physiological factors which restrict the host–parasite relationships between bulinid snails and their schistosome parasites have not yet been discovered but when they are it seems likely that *B. obtusispira* will be found to have much in common with the *senegalensis–beccarii–bavayi–cernicus* complex of the *forskali* group.

B. reticulatus was originally included by Mandahl-Barth (1957) in the *forskali* group, largely because of the small size of its radular teeth, but he did mention that it 'could just as well be referred to the *truncatus* group'. The discovery of *B. wrighti* (obviously a close relative of *B. reticulatus*) has provided a great deal of information with which a reassessment of the relationships of these two species can be made. The globose form of the shell with its short, acute spire bears some resemblance to certain small forms of *B. tropicus* or *B. truncatus* but the wide open umbillicus is peculiar to *B. reticulatus* and *B. wrighti*. The mantle ridges are of the *tropicus/truncatus* type but the complete lack of fluorescent materials in the body-surface mucus is usually a character of the *africanus* group. The marked differences in the egg proteins of *B. reticulatus* and *B. wrighti* has already been emphasized but only those of *B. wrighti* have been available in sufficient quantity for immunological experiments. In tests against antisera to *forskali*-group species (figure 6*a*, antiserum to *B. bavayi* and figure 6*b*, antiserum to *B. scalaris*) *B. wrighti* reacted less strongly than other members of the *forskali* group and, when tested against a very weak antiserum to *B. forskali* itself, *B. wrighti* gave no reaction at all while *B. bavayi*, *B. cernicus* and *B. scalaris* all cross-reacted. When tested together with a variety of *forskali*-group antigens against an antiserum to *B. tropicus* (figure 6*c*) *B. wrighti* reacted more strongly than any of the other species. Finally, a weak antiserum to *B. wrighti* (figure 6*g*) gives similar reactions to *B. tropicus* , *B. truncatus* and *B. africanus* but slightly less to *B. forskali*. The indications from these tests are that *B. wrighti* is a very distinctive species whose affinities, if any, are with the *tropicus* group and definitely not with the *forskali* group. Again the problem of susceptibility to infection with *Schistosoma* spp. arises to confuse the issue although this case is very different from that of *B. obtusispira* in that *B. wrighti* has proved to be susceptible in varying degree to every strain of *S. haematobium*, *S. bovis* and *S. mattheei* to which it has been exposed. *B. reticulatus* has been exposed in small numbers to several strains of *S. haematobium* without success, although on one occasion a few sporocysts and cercariae were found in a moribund specimen which had been exposed to the Mauritian strain of parasite. The wide susceptibility of *B. wrighti* to schistosomes cuts right across the close host-restriction which is shown by most bulinids and suggests that this species has failed to develop any physiological defence mechanisms against the parasites. This lack of 'immunity' may well account for the restricted distribution and isolated habitats of the species in South Arabia. Taking all of the available evidence into consideration it seems that *B. wrighti* and *B. reticulatus* are rather unspecialized bulinids without very close affinities to any other existing species and that they should be regarded as forming a separate complex, the *B. reticulatus* group. The evidence of the egg proteins suggests that *B. reticulatus* itself may be a composite species with a distinct form occupying the southern part of Africa.

The general conclusion to be drawn from this study is that the bulinid fauna of the Indian Ocean area is made up in part by the remains of species formerly more widely distributed, possibly forced into peripheral isolation by the joint pressures of parasitism and competition from non-susceptible species (*B. bavayi*, *B. beccarii*, *B. cernicus* and *B. wrighti*) with a single 'relic' species (*B. obtusispira*) and two more recent arrivals from the African mainland (*B. mariei* and *B. liratus*) on Madagascar.

I am grateful to the members of the Royal Society Expedition to Aldabra who sent back living specimens of *B. bavayi* and to Dr E. R. Brygoo of the Institut Pasteur, Tananarive, for sending living material of the Madagascan species. I am also grateful to Dr R. Elsdon-Dew of Durban, Dr F. Arfaa of Teheran and Mr C. N. Courtois of Candos for the collection and despatch of *Schistosoma haematobium* eggs from South Africa, Iran and Mauritius respectively and I wish to acknowledge the technical assistance of Mrs J. Horton, Miss J. R. L. Lines, Mr G. C. Ross, Mr D. Claugher and Mr R. J. Knowles.

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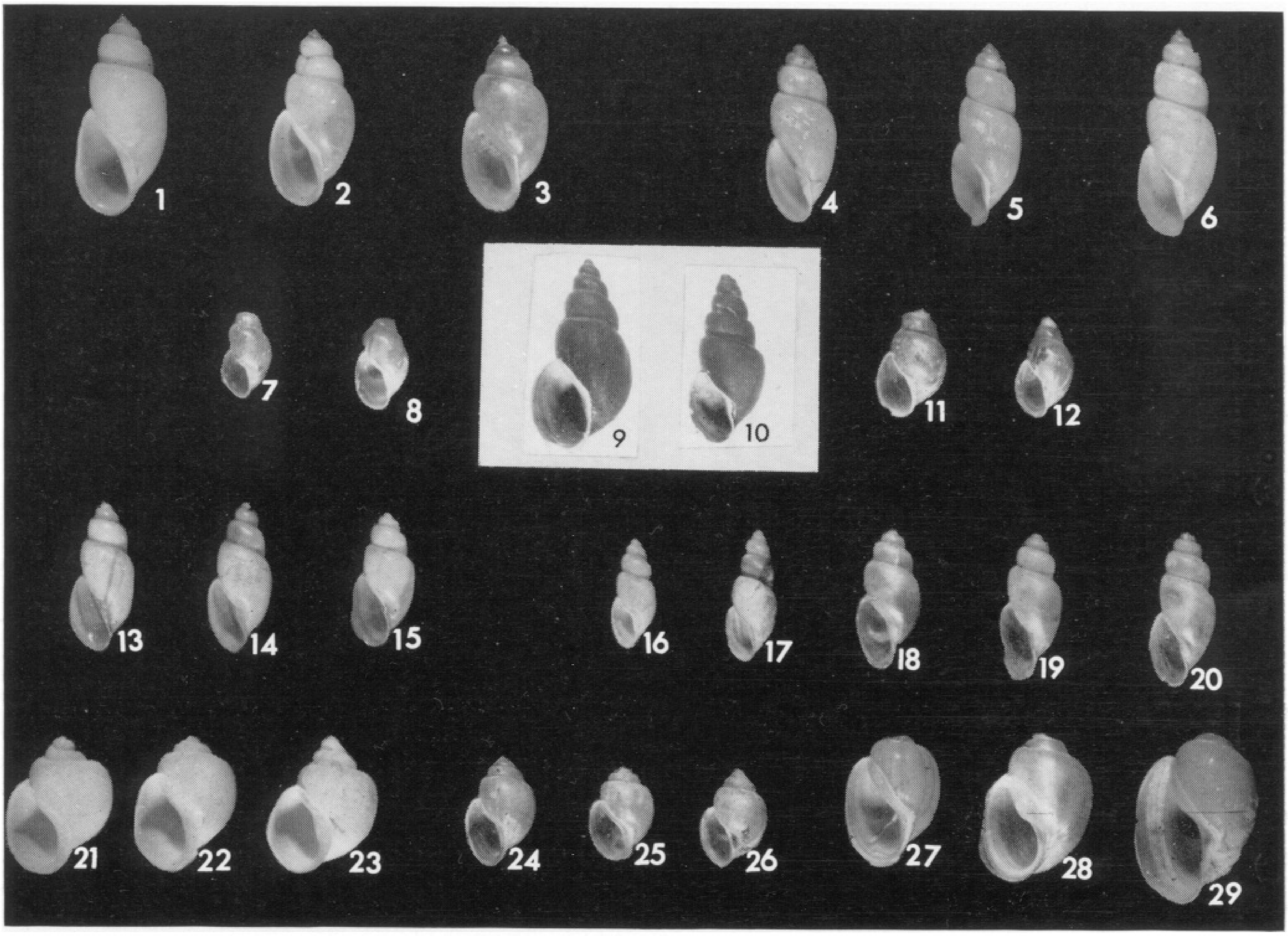

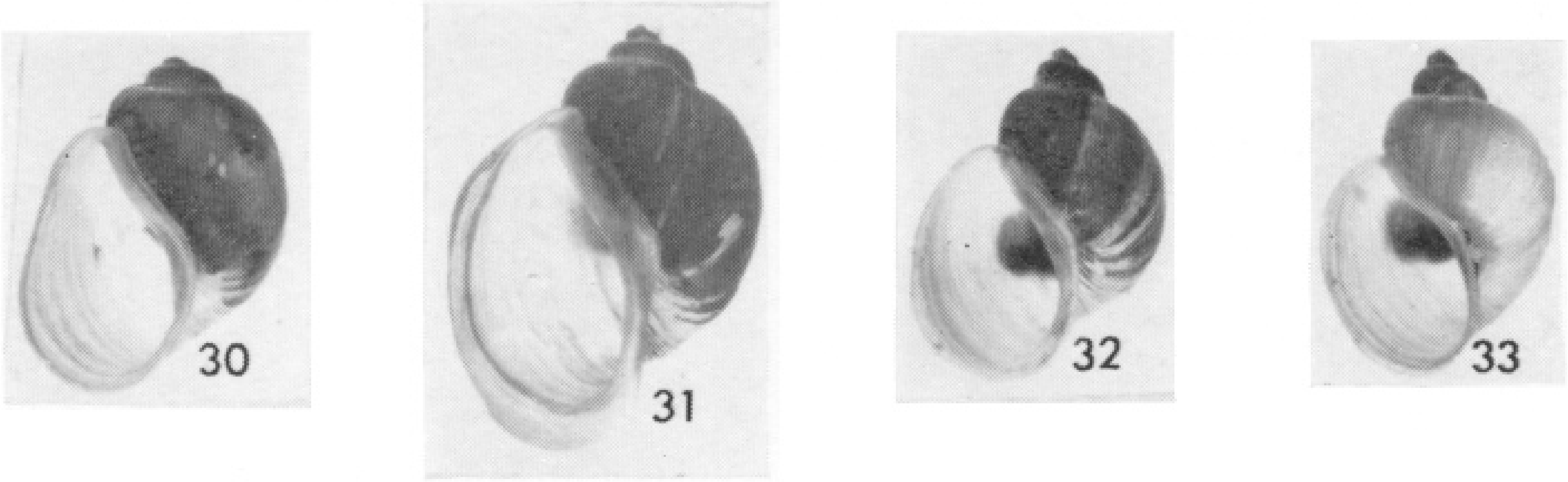

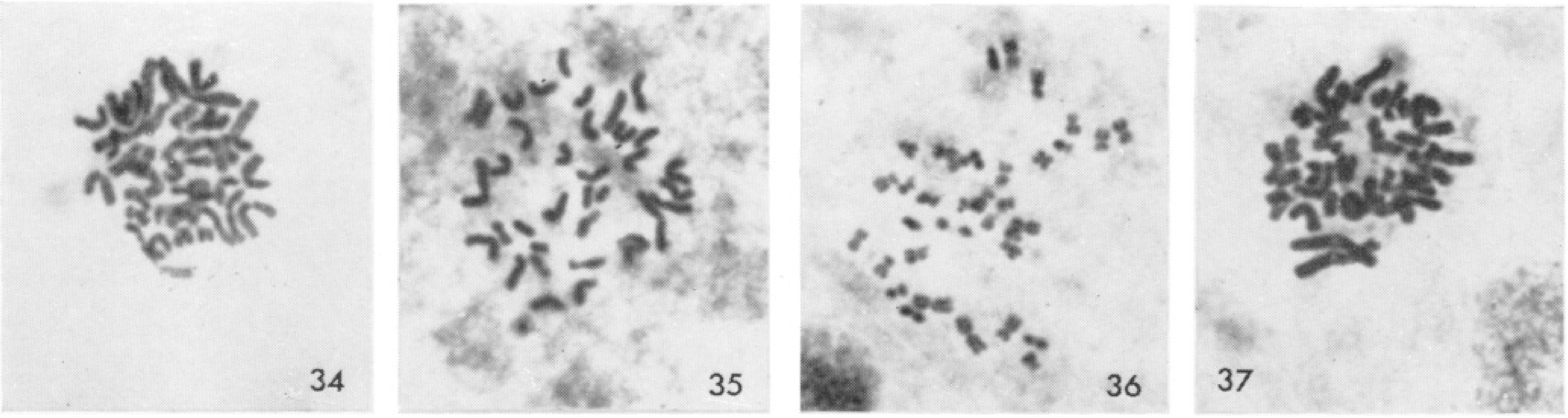

IGURE 2. Shells and chromosomes of *Bulinus*. 1 to 3, *B. bavayi* Aldabra; 4 to 6, *B. bavayi* Madagascar; 7, 8, *B. cernicus* Clemencia, Mauritius; 9, 10, *B. cernicus* Cité le Cure, Mauritius; 11, 12, *B. cernicus* Carreau Acacias, Mauritius; 13 to 15, *B. beccarii* Dirgag, Aden; 16 to 20, *B. mariei* Madagascar; 21 to 23, *B. wrighti* Samh-ba-Rooh, South Arabia; 24 to 26, *B. reticulatus* Kisumu, Kenya; 27 to 29, *B. obtusispira* Madagascar; 30 to 33, *B. liratus* Madagascar. (1 to 33 all magn ×2.)

34, mitotic metaphase chromosomes of *B. bavayi*; 35, mitotic metaphase chromosomes of *B. wrighti*; 36, mitotic metaphase chromosomes of *B. obtusispira*; 37, mitotic metaphase chromosomes of *B. globosus*. (34 to 37 magn about ×1300.)

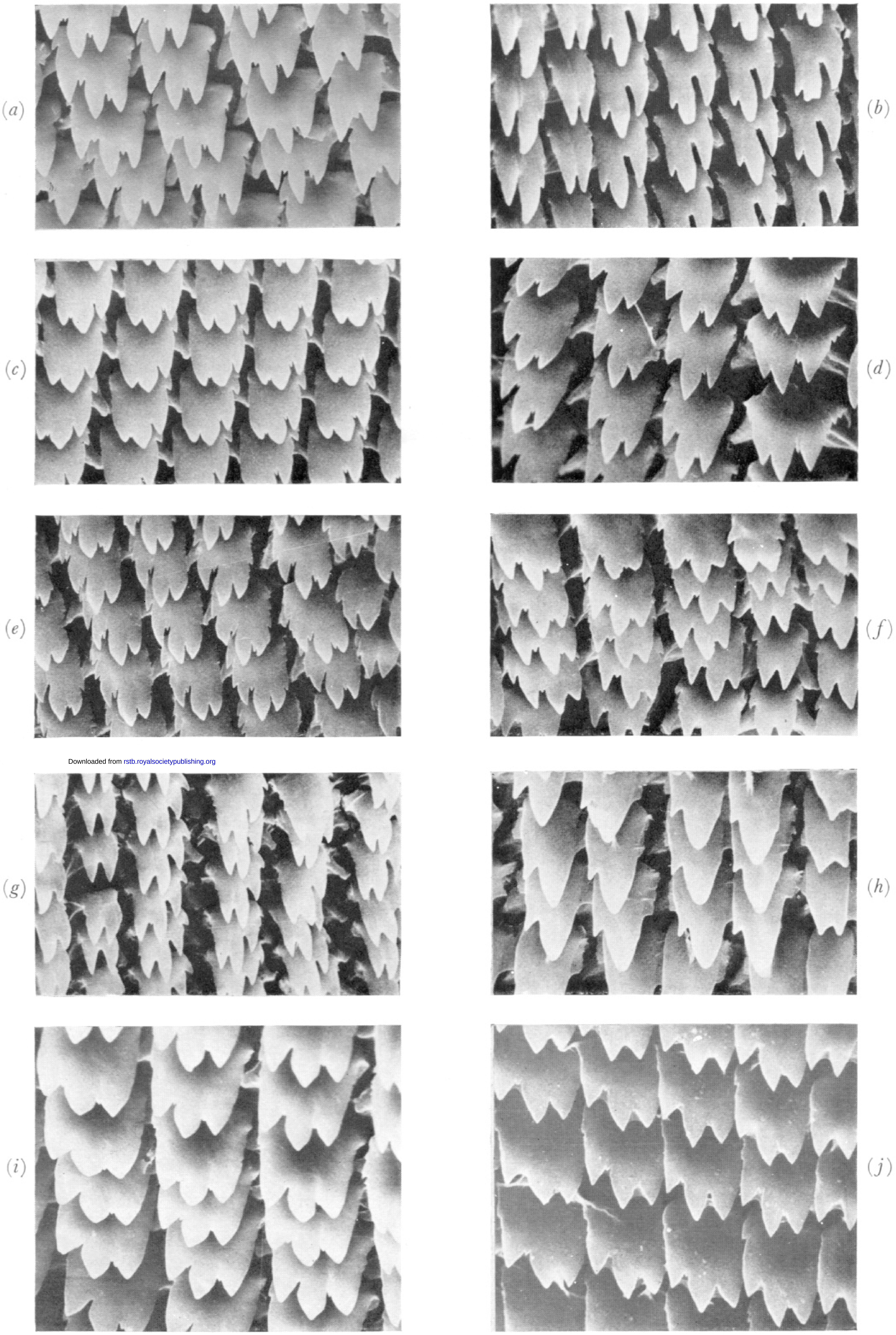

FIGURE 3. Stereoscan pictures of *Bulinus* radulae. *a*, *B. bavayi* Madagascar; *b*, *B. cernicus* Carreau Acacias, Mauritius; *c*, *B. cernicus* Clemencia, Mauritius; *d*, *B. cernicus* Cité le Cure, Mauritius; *e*, *B. beccarii* Dirgag, Aden; *f*, *B. mariei* Madagascar; *g*, *B. reticulatus* Kisumu, Kenya; *h*, *B. wrighti* Samh-ba-Rooh, South Arabia; *i*, *B. liratus* Madagascar; *j*, *B. obtusispira* Madagascar. (All figures magn. about × 1000.)

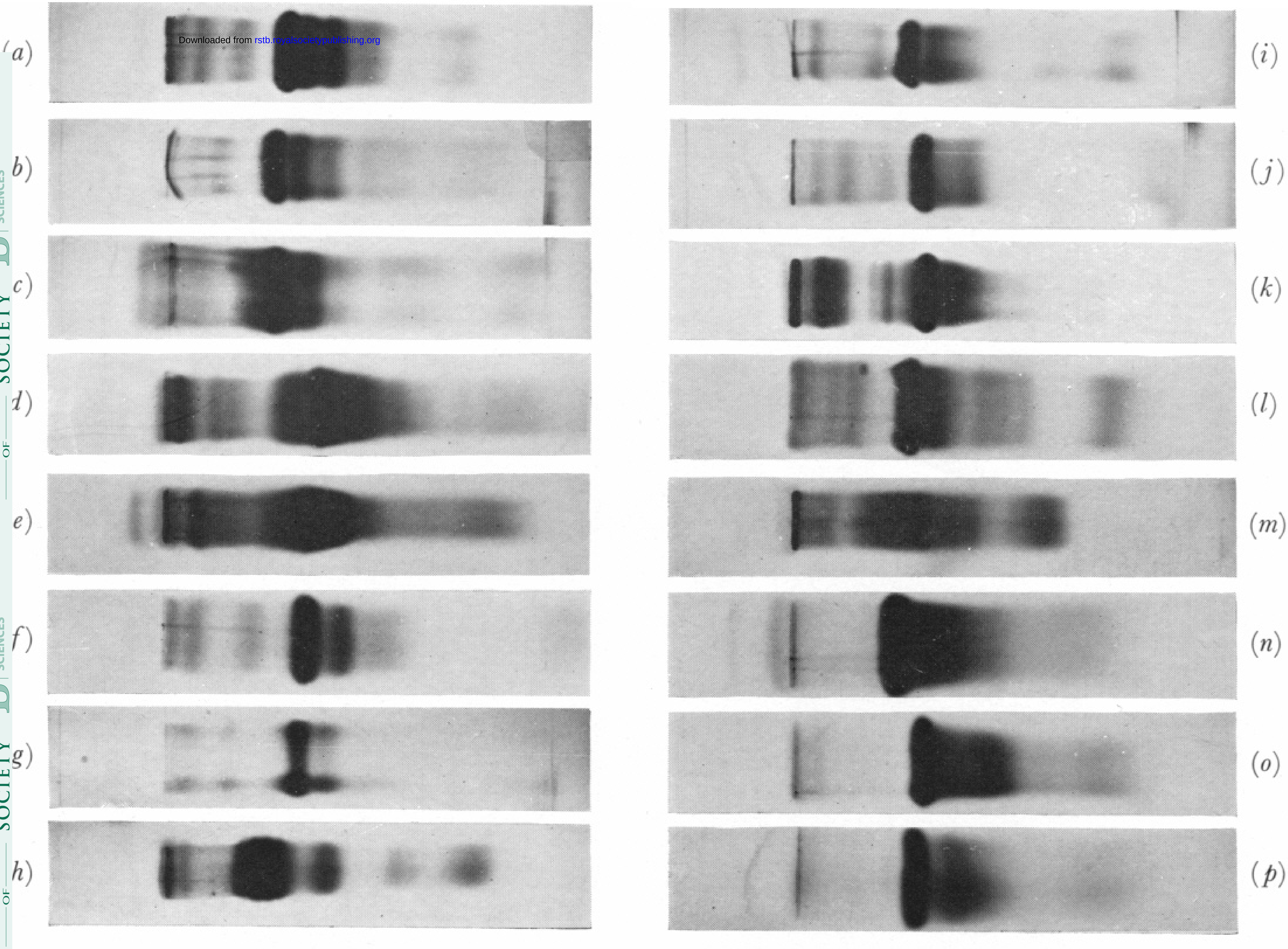

FIGURE 4. For legend see facing page.

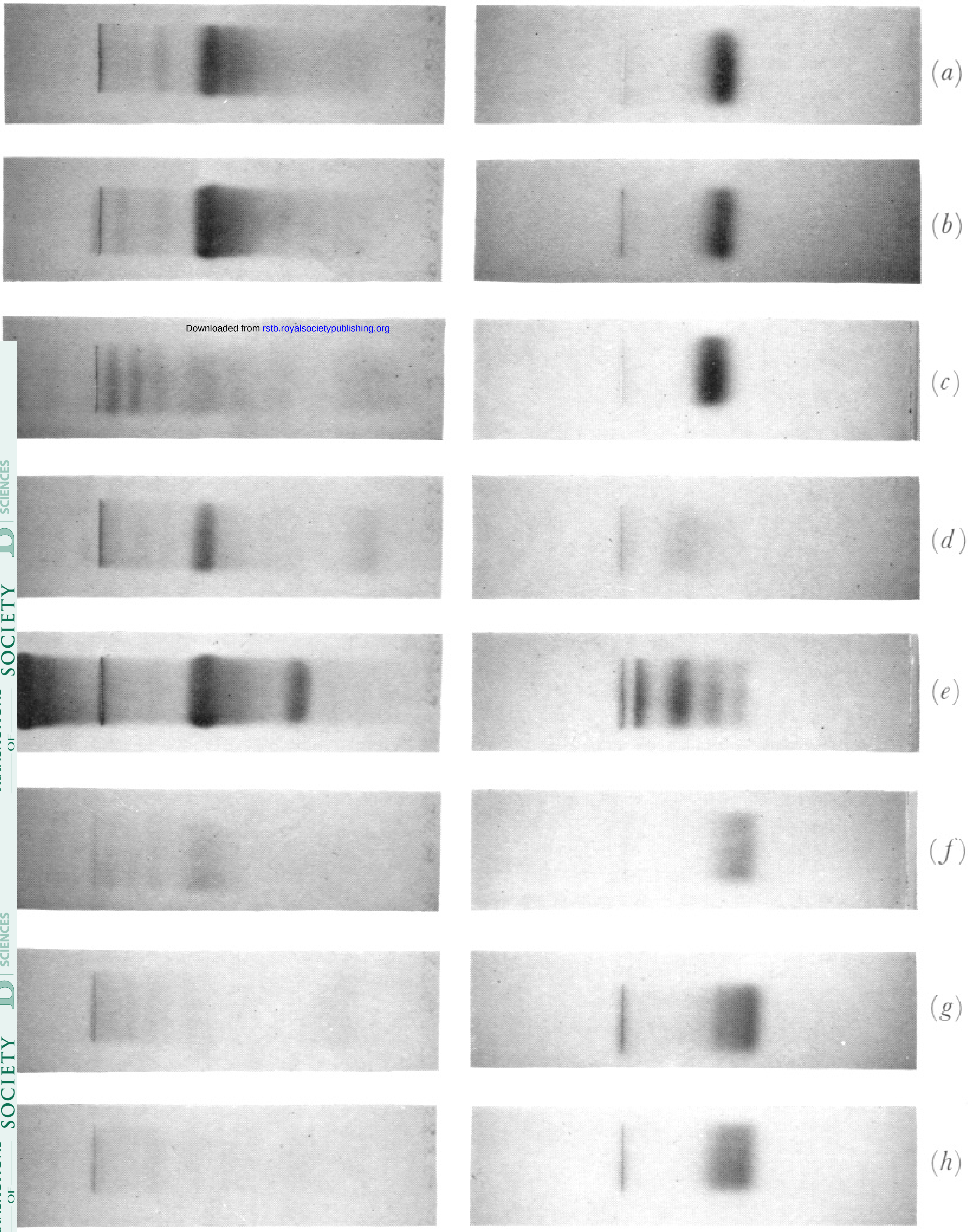

FIGURE 5. For legend see facing page.

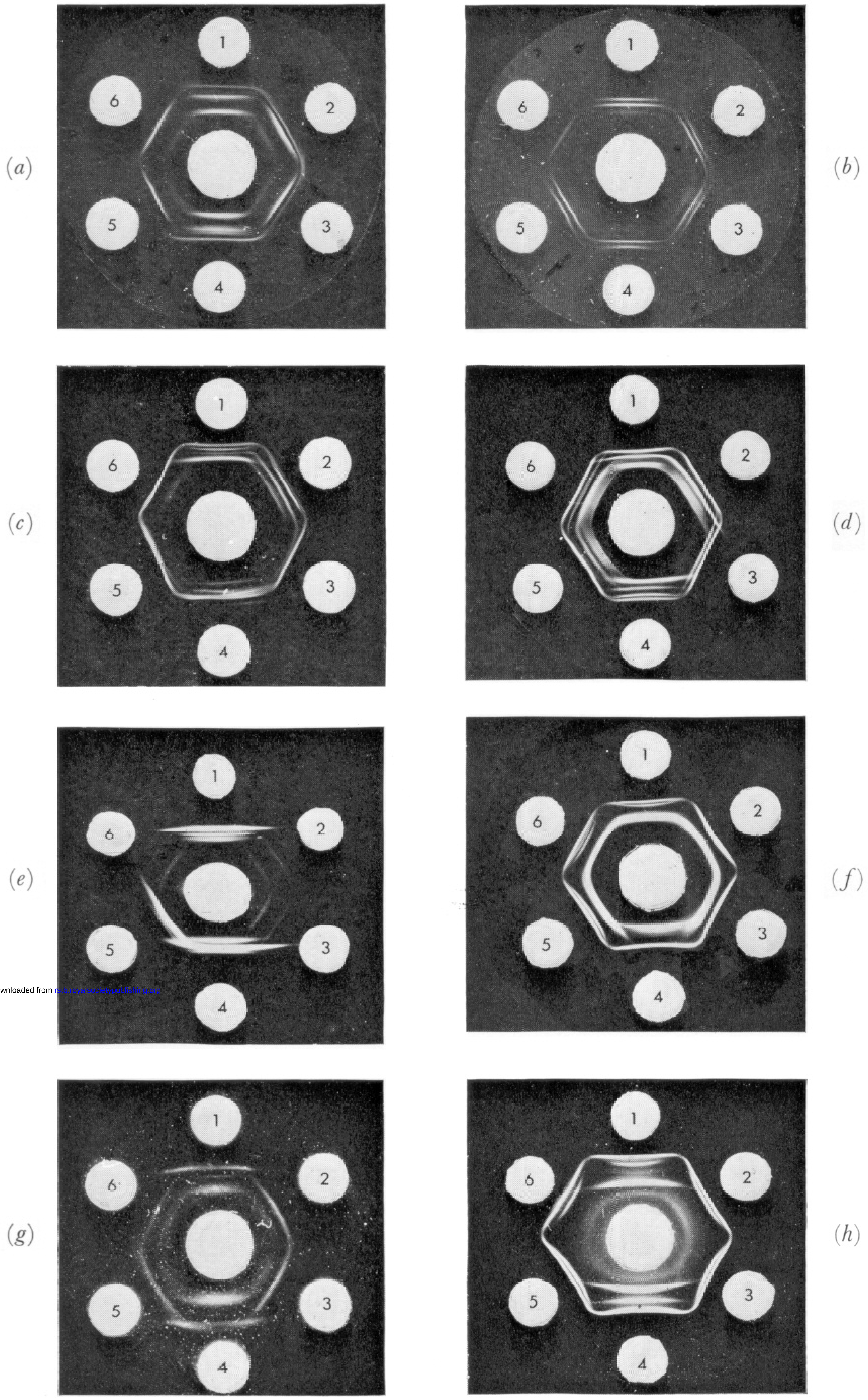

FIGURE 6. Ouchterlony immunodiffusion plates with egg proteins of *Bulinus*. Antisera in centre wells, antigens in peripheral wells. (*a*) Antiserum to *B. bavayi*: 1, *B. bavayi*; 2, *B. cernicus*; 3, *B. scalaris*; 4, *B. bavayi*; 5, *B. mariei*; 6, *B. wrighti*.

b) Antiserum to *B. scalaris*: 1, *B. scalaris*; 2, *B. bavayi*; 3, *B. cernicus*; 4, *B. scalaris*; 5, *B. forskali*; 6, *B. wrighti*.

c) Antiserum to *B. tropicus*: 1, *B. tropicus*; 2, *B. wrighti*; 3, *B. forskali*; 4, *B. tropicus*; 5, *B. scalaris*; 6, *B. cernicus*.

d) Antiserum to *B. tropicus*: 1, *B. tropicus*; 2, *B. liratus*; 3, *B. obtusispira*; 4, *B. tropicus*; 5, *B. bavayi*; 6, *B. wrighti*.

e) Antiserum to *B. obtusispira*: 1, *B. obtusispira*; 2, *B. truncatus*; 3, *B. tropicus*; 4, *B. obtusispira*; 5, *B. globosus*; 6, *B. bavayi*.

f) Antiserum to *B. ugandae*: 1, *B. ugandae*; 2, *B. globosus* (Sudan); 3, *B. globosus* (Zambia); 4, *B. obtusispira*; 5, *B. africanus*; 6, *B. globosus* (Ghana).

g) Antiserum to *B. wrighti*: 1, *B. wrighti*; 2, *B. tropicus*; 3, *B. truncatus*; 4, *B. wrighti*; 5, *B. africanus*; 6, *B. forskali*.

h) Antiserum to *Indoplanorbis exustus*: 1, *I. exustus*; 2, *B. truncatus*; 3, *B. tropicus*; 4, *I. exustus*; 5, *B. globosus*; 6, *B. bavayi*.