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Systematics of the Pomatoschistus Minutus Complex (Teleostei: Gobioidei)

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SYSTEMATICS OF THE *POMATOSCHISTUS MINUTUS* COMPLEX (TELEOSTEI: GOBIOIDEI)

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CONTENTS

Systematic relationships of gobioid fishes of the eastern Atlantic *Pomatoschistus minutus* complex have been investigated by means of morphological, biochemical and karyological criteria. These studies confirm the existence of three taxa, *minutus* (Pallas 1770), *lozanoi* (De Buen 1923) and *norvegicus* (Collett 1903), one of which (*lozanoi*) is morphologically intermediate between, yet genetically distinct from, the other forms. Evidence is presented that *lozanoi* may interbreed with *minutus* or *norvegicus* in the wild, but that backcrossing of resulting hybrids does not occur. It is concluded that *minutus*, *lozanoi* and *norvegicus* all deserve specific status and these species are formally diagnosed.

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1. Introduction

The Gobioidea comprises an extensive group of small acanthopterygian teleosts, best regarded as a suborder of the Percomorphi (Regan 1911; Miller 1973*a*). This primarily marine suborder is centred on the Indo-Pacific region but occurs in all tropical and temperate seas and in many freshwater and estuarine systems (Nelson 1976). Over forty species of goby have been recorded from the Mediterranean and the Atlantic coast of Europe (Wheeler 1969; Miller 1971). The commonest and most widely distributed of these are currently housed in the gobiid genus *Pomatoschistus* Gill, 1864, members of which are abundant and are important small predators in estuarine and coastal ecosystems (see, for example, Miller 1975). Because of their small size and superficial resemblance to each other these present unsolved systematic problems.

Pomatoschistus was founded to contain *Gobius minutus* Pallas, 1770 by Gill (1864) in a brief footnote to a paper on North American gobioid genera. The subsequent complex systematic history and involved synonomy of *Pomatoschistus* and its species has been summarized by Miller (1973*b*) in a checklist of fishes of the northeastern Atlantic and Mediterranean (*Clofnam*). Its generic limits are currently defined by features of the modified lateral-line system (De Buen 1930, 1931; Miller 1968) and Miller (1973*b*) recognizes at least nine species in *Pomatoschistus*. In original binomen these are as follows.

	Clofnam no.
Gobius minutus Pallas, 1770	162.21.1.
Atherina marmorata Risso, 1810	162.21.4.
Gobius microps Krøyer, 1838	162.21.5.
Gobius quagga Heckel, 1840	162.21.8.
Gobius knerii Steindachner, 1861	162.21.3.
Gobius pictus Malm, 1865	162.21.7.
Gobius canestrinii Ninni, 1883	162.21.2.
Gobius norvegicus Collett, 1903	162.21.6.
Pomatoschistus tortonesei Miller, 1968	162.21.9.

Systematic problems involve the type species, *P. minutus* and the related taxa *norvegicus* (Collett 1903) and *lozanoi* (De Buen 1923), which have been included in a '*P. minutus* complex' by Webb & Miller (1975). Members of this complex may be distinguished from congeneric species by the greater number of lateral-line scales, the villose anterior pelvic membrane, the partial separation of the branchiostegal membrane from the isthmus, the occurrence of predorsal scales and the arrangement of the lateral-line system on the head.

The *P. minutus* complex has had a long and complicated systematic history. Pallas (1770) introduced '*G. minuto maris Belgici*' for a species described by Gronovius (1763) under non-binomial nomenclature as '*Gobius albescens*: *pinnis dorsalibus altitudine aequalibus*: *cauda subrotunda*'. Gronovius synonymized this with an entry in Linnaeus's *Systema naturae* that had been given the binomial *Gobius Aphya*. No type specimens of Pallas's *G. minutus*, *G. Aphya* or Gronovius's species exist (Wheeler 1958), but since Linnaeus gave the typical locality of *G. Aphya* as the River Nile, this synonymy is probably incorrect. Boulenger (1911) has also suggested that Gronovius's description was based on more than one species.

By the early nineteenth century, the name *G. minutus* Pallas had become firmly associated with a small inshore goby, found in sandy bays, commonly referred to as the 'spotted goby'.

Pennant (1769) introduced the spotted goby as a synonym of Linnaeus's *Gobius Aphya*, but in a later (post-Pallas) edition of his '*British zoology*' (1812) synonymized it with *G. minutus* Pallas. The specimens from which Pennant made his descriptions were probably caught in the Dee Estuary with shrimp nets, and Miller's (1961*a*) records of *G. minutus* taken in the same way from the same area provide circumstantial evidence that it was fish of this taxon that Pennant had examined.

During the nineteenth century several species closely related to *G. minutus* were described. Many of these were defined on the basis of minor morphological characteristics and the validity of their separation was soon under discussion, so much so that by the early 1900s most of them had been relegated to junior synonyms of *G. minutus*. A notable exception was *G. microps* separated from *G. minutus* by Krøyer in 1838. This species was still recognized (as *G. parnelli*) by Day (1880–4), but the distinctiveness of *microps* and *minutus* was subsequently challenged by Holt & Byrne (1903), who regarded *microps* merely as an estuarine race of *minutus*. Boulenger (1911), using British and Continental material, reviewed the status of *microps* and *minutus* and concluded that they were separate species, a view later confirmed by Fage (1914), Petersen (1919), De Buen (1923) and Hass (1939). Although the distinctiveness of *microps* and *minutus* had been stressed in English works by Boulenger (1911), Lebour (1919, 1920) and Fraser-Brunner (1938), the reproduction, by Jenkins (1925, 1936), of the opinion of Holt & Byrne in the then only modern text on British fishes led to continuing confusion among British workers (Miller 1961*a*).

An important advance in the study of gobioid relationships was made by Sanzo (1911), who found that the nature of the modified lateral-line system provided a useful criterion of affinity in European species. Studies of this system have also figured in the 'post-Sanzo' systematic history of the *P. minutus* complex. Indeed, as early as 1915 Fage demonstrated that *minutus* and *microps* were easily distinguishable by the arrangement of head sensory papillae, a feature that Miller (1961*a*) regarded as providing 'the most clear-cut differences between the two species'. The study of head lateral-line sensory papillae has also been important in the delimitation of the other taxa, *lozanoi* and *norvegicus*, currently included in the *P. minutus* complex.

Collett (1903) was the first to mention a deep-water form of *G. minutus* Pallas (from the fjords of SW Norway), which he regarded as a possible subspecies *norvegicus*, distinguishing it from the better known littoral form on the basis of small size, pale colour and naked throat. Others also recognized, under several names, an offshore deep-water goby similar to *G. minutus*, which they regarded as specifically distinct because of its different pattern of head sensory papillae (Fage 1914, 1915; De Buen 1923). These authors overlooked *norvegicus*, but Webb & Miller (1975) established that Collett's syntypes were indistinguishable from their specimens, and so determined the priority of *norvegicus* (Collett 1903) as the name for the offshore form.

Although Fage (1915) claimed that *norvegicus* resembles young specimens of *minutus*, most modern authors have regarded it as a distinct and easily separable species. However, the situation is complicated by the existence of a third taxon, *lozanoi* (De Buen 1923), intermediate between *minutus* and *norvegicus* in a number of features (Fonds 1973; Webb & Miller 1975). This rather obscure form was originally described from the Spanish Atlantic coast as a subspecies of *G. minutus* (largely on the basis of head sensory papillae arrangement), but specimens have since been observed that are apparently intermediate between *minutus* and *lozanoi* or *lozanoi* and *norvegicus* (Iljin 1930; Swedmark 1968; Fonds 1973; Webb & Miller 1975) and its status is unclear. Miller (see Fonds 1973) suggested that *lozanoi* might represent a hybrid population between *minutus* and *norvegicus*, while Fonds (1973) regarded it as a valid species. In sharp

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contrast to the latter view, Swedmark (1968) maintained that *lozanoi* was unworthy of even subspecific status and that individuals attributed to it ought to be included in *minutus*.

The systematic relationships of the *P. minutus* complex, which include and to a large extent depend on the true status of *lozanoi*, are thus in need of clarification. Their reinvestigation forms the subject of this communication. Previous systematic investigations have been almost entirely based on the examination of morphological features in preserved material. Latterly other types of character have become available to systematists and biochemical and karyological criteria are used here to supplement conventional morphological characters.

2. Morphological studies

2.1 *Introduction*

The nature of the modified lateral-line system is of special significance in the systematics of the *P. minutus* complex since it provides the main means by which its constituent taxa are currently recognized. Among the Gobioidea, *Rhyacichthys*, a monotypic genus from tropical hill streams of the Indo-Australian archipelago, seems unique in preserving an extensive lateral-line system (Miller 1973*a*). Other gobioids have this system reduced to head canals, at best in merely oculoscapular and preopercular series, with exposure of sensory papillae (neuromast organs) in rows over the head and body (Sanzo 1911; Aurich 1939; Takagi 1957). Comparisons of head sensory papillae, particularly the suborbital series, have proved most useful in the delimitation and grouping of genera and species, not least within the *minutus* complex, taxa of which are identified largely on the arrangement of suborbital papillae.

The first descriptions of sensory papillae patterns in *minutus*, *lozanoi* and *norvegicus* were made by Fage (1914), De Buen (1923) and Fage (1914, 1915), respectively. The suborbital arrangements illustrated by these authors are reproduced in figure 1 together with a diagram, after Webb & Miller (1975), of the pattern in one of Collett's syntypes of *norvegicus*. Subsequent descriptions have been given by various authors (see: Miller 1973*b*; Webb & Miller 1975). Important features of patterns described as characteristic of the three main forms of the complex are listed below.

(*a*) *minutus*

(i) Usually a long longitudinal row *b*, terminating anteriorly under the anterior half of the orbit, although Georgiev (1966) reported specimens with a row *b* slightly shorter than in European examples.

(ii) A high number (9–12) of transverse *c* rows.

(iii) Usually only the last transverse *c* row (*cp*) penetrates below the level of the horizontal section of longitudinal row *d*, though Fonds (1971, 1973) and Lee (1974) have recorded specimens with one of the more posterior transverse *c* rows penetrating a short distance below row *d*.

(*b*) *norvegicus*

(i) A shorter row *b* than in *minutus*, terminating anteriorly under the posterior half or third of the orbit.

(ii) Fewer (7/8) transverse *c* rows.

(iii) The second, fourth and last transverse *c* rows all penetrate well below the level of row *d*.

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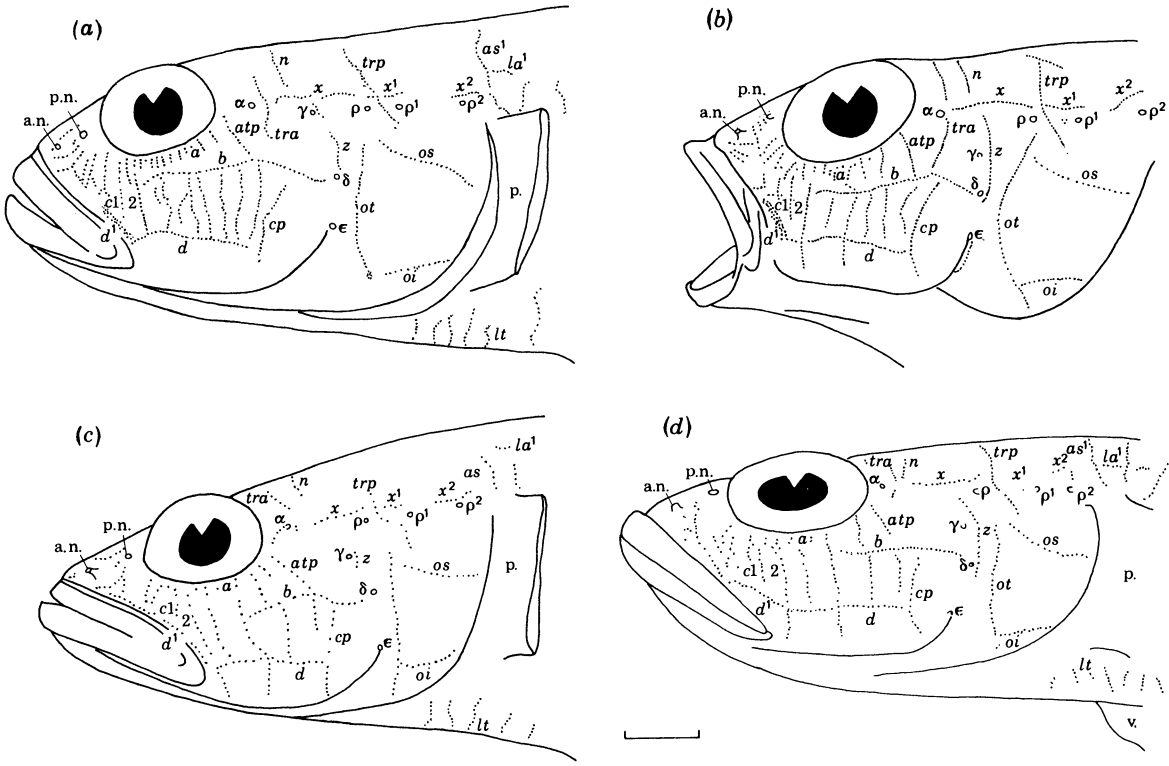

FIGURE 1. Lateral view of head sensory papillae and canal pores in: (*a*) *minutus* (after Fage (1914), as *Gobius minutus*, Pallas), no scale; (*b*) *lozanoi* (after De Buen (1923), as *G. minutus lozanoi*, De Buen), no scale; (*c*) *norvegicus*, (after Fage (1915), as *Gobius elongatus* Canestrini), no scale; (*d*) *norvegicus*, after Webb & Miller (1975), scale bar 2 mm. Terminology of lateral-line system after Sanzo (1911): rows of papillae are labelled with italic and pores with Greek letters. Abbreviations; a.n., p.n., anterior and posterior nostrils; p. pectoral fin; v. ventral fin.

(*c*) *lozanoi*

(i) A long row *b*, terminating anteriorly under the anterior half of the orbit.

(ii) Nine to ten transverse *c* rows.

(iii) The second, fourth and last transverse *c* rows all penetrate well below the level of row *d*.

Fage (1915) claimed that the pattern of suborbital papillae in young *minutus* (defined as individuals 33 mm or less in standard length) is similar to that found in *norvegicus* and that only older (i.e. larger) individuals possess distinctive patterns. However, this sort of age- or size-dependent variation has not been detected in more recent investigations (see, for example: Swedmark 1968; Fonds 1973).

Representatives of the *minutus* complex have also been observed with suborbital patterns, or combinations of patterns, apparently intermediate between those of *minutus* and *lozanoi*, or *lozanoi* and *norvegicus* (Iljin 1930; Swedmark 1968; Webb & Miller 1975). However, the frequency with which such specimens occur is not known, for although the arrangement of suborbital papillae is of primary diagnostic importance in the *minutus* complex it has been scarcely studied. An investigation of the variability of suborbital patterns has therefore been undertaken.

In systematic ichthyology meristic characters have always played an important part in the description and definition of lower taxonomic categories. Various authors have suggested that

members of the *minutus* complex display meristic and other morphological differences (table 1), although Hesthagen's (1974) contention that *norvegicus* can be identified by an absence of villi on the anterior pelvic membrane was not supported by Webb & Miller (1975). As with suborbital sensory papillae, however, no serious attempt has been made to investigate the variability of morphological characters. It is true that meristic variation in *minutus* was studied extensively by Boulenger (1911), Hass (1936), Johnsen (1936) and Swedmark (1968), but these

TABLE 1. DIFFERENCES IN SOME MORPHOLOGICAL ATTRIBUTES OF *MINUTUS*, *LOZANOI*, AND *NORVEGICUS*

(Data from Fage (1914, 1915), Lebour (1920), De Buen (1923, 1931), Dunker (1928), Wheeler (1969), Miller (1971), Hesthagen (1974) and Webb & Miller (1975).)

characters	*minutus*	*lozanoi*	*norvegicus*
meristic			
vertebrae	33 (32–34)	32 (30–33)	32 (30–33)
first dorsal fin rays	6 (6–7)	6	6 (6–7)
second dorsal fin rays	I/10–12	I/10–12	I/8–10
anal fin rays	I/9–12	I/10–12	I/8–11
pectoral fin rays	18–21	18–21	16–18
lateral-line scales	55–75	57–65	55–60
coloration			
body background	sandy or grey, with fine reticulation and ferruginous specks	brown, marbled, with fine reticulation and irregularly dispersed orange spots	pale fawn or translucent, with faint reticulation and scattered orange dots
first dorsal fin spot	present in males and females	absent in females, present only in maturing males	present in maturing males and females
male nupital	4 main dark lateral bars	7–9 lateral stripes	10–12 mostly thin vertical striae
general			
maximum size/mm	110	80	63
breast scales	present	present	absent
attachment of branchiostegal membrane	great	?	slight
lateral line			
anterior section of row *i*	in double series	in double series	in single series
supralabial section of row *d*	in double series	in double series	in single series
number of anterior–dorsal longitudinal rows	3	3	2

authors did not use lateral-line features as a systematic character and the validity of their identifications is in doubt. Indeed, Swedmark (1968) included in *minutus* individuals with suborbital papillae patterns characteristic of *lozanoi*, while Fonds (1971, 1973) suggested that a proportion of the sample of *minutus* studied by Hass (1936) belonged to *lozanoi*. The second part of this section deals with the variation of selected morphological features in individuals for which information on the arrangement of suborbital papillae is available.

2.2. *Material and methods*

The head sensory papillae of 1668 specimens of the *minutus* complex were examined. Of these the 857 that possessed undamaged suborbital arrangements on both sides were studied (appendix 1).

Morphological features were examined under a low power binocular microscope. Neuromasts were readily visible without staining if specimens were viewed under liquid with oblique lighting. Three features of the suborbital papilla arrangement (the number of transverse *c* rows, the extension of transverse *c* rows below the level of row *d* and the length and anterior limit of row *b*) were investigated in detail and a standard convention was adopted for the recording and representation of variation in these features in the form of a stylized diagram.

A transverse *c* row is defined as a series of a minimum of three papillae extending for at least one-third of the vertical distance between the longitudinal courses of rows *b* and *d*. A *c* row is regarded as penetrating below *d* if one or more of its papillae occurs beneath the horizontal course of this row. Extensions of transverse *c* rows below row *d* are classified as 'long' if their most ventral papilla occurs at more than half of the vertical distance between the longitudinal course of *d* and a horizontal drawn through the ventral edge of the operculum and as 'short' if their most ventral papilla occurs at half or less of this distance. Transverse *c* rows are counted in an area limited anteriorly by a vertical through the angle of the jaw and posteriorly by a vertical through cephalic pore δ. The first row counted usually approximates in position to a vertical through the junction of *d* and *d'* (and is equivalent to the second *c* row in Sanzo's terminology), while the last, *cp*, always penetrates below *d* and usually extends a maximum of two-thirds to three-quarters of the vertical distance from row *d* to *b*. Extensions of *cp* beyond this limit are regarded as having arisen through fusion of the penultimate *c* row with *cp*. When this is observed the *c* row count is adjusted accordingly. Row *b* extends over the upper part of the cheek, enclosing a number of transverse *c* rows and separating them from segments of the more dorsal row *a*. In all cases, row *b* runs from close to pore δ to terminate, depending on its length, at some point beneath the eye. Row *b* is categorized as ending under the anterior half, the midpoint, or the posterior half of the orbit.

In the construction of a stylized diagram, transverse *c* rows were represented by an equivalent number of equally spaced vertical lines of standard arbitrary length. Long and short penetrations of *c* rows below *d* were indicated by standard extensions of the appropriate vertical line beneath the horizontal line representing row *d*. Row *b* was illustrated by a horizontal line parallel to that indicating *d*, and the point beneath the orbit at which *b* terminated was also shown, symbols ◂, ▾, and ▸ being used respectively to denote a termination under the front half, midpoint and rear half of the orbit.

Counts of vertebrae, including the urostyle, were determined from X-ray photographs. Counts of other skeletal elements were facilitated by staining with alizarine-red-S in KOH (10 g/l) after hardening in formaldehyde (40 g/l) for at least 3 days. The terminal bifid ray of the second dorsal and anal fins was counted as one. A meristic index was calculated for each individual by summing points awarded for the number of vertebrae, rays of the left and right pectoral fins and branched rays of the second dorsal and anal fins. Points were given to each character on a scale from 0 to 20, 0 and 20 being for the minimum and maximum number of elements in the total sample studied. The variability of meristic index between samples was compared by the graphical method of Hubbs & Hubbs (1953).

2.3. *Results*

2.3.1. *Pattern of suborbital papillae*

On the basis of the convention adopted, 103 different types of suborbital papillae pattern were observed among the 1714 arrangements examined. These are illustrated in figure 2, where they

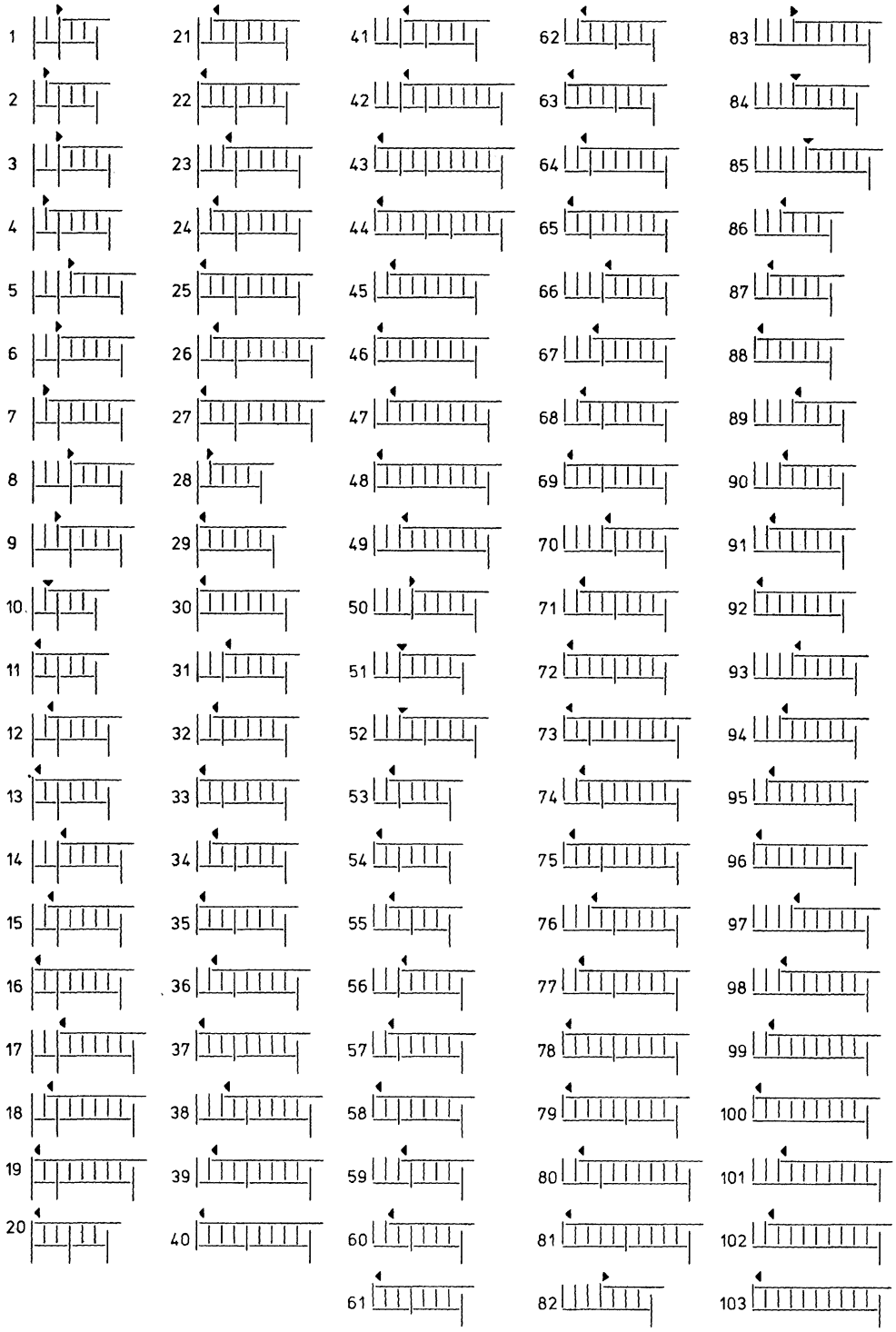

FIGURE 2. Stylized diagrams of suborbital papillae pattern variants encountered in the sample examined.

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have been arranged in an arbitrary sequence and assigned reference numbers. Figure 3 illustrates the number of times that each variant was observed in the sample examined. More than 75 % of the variants were rarely recorded (less than 15 observations) and most arrangements belonged to a few common types, notably 3, 16, 69, 92, 95, 96 and 100.

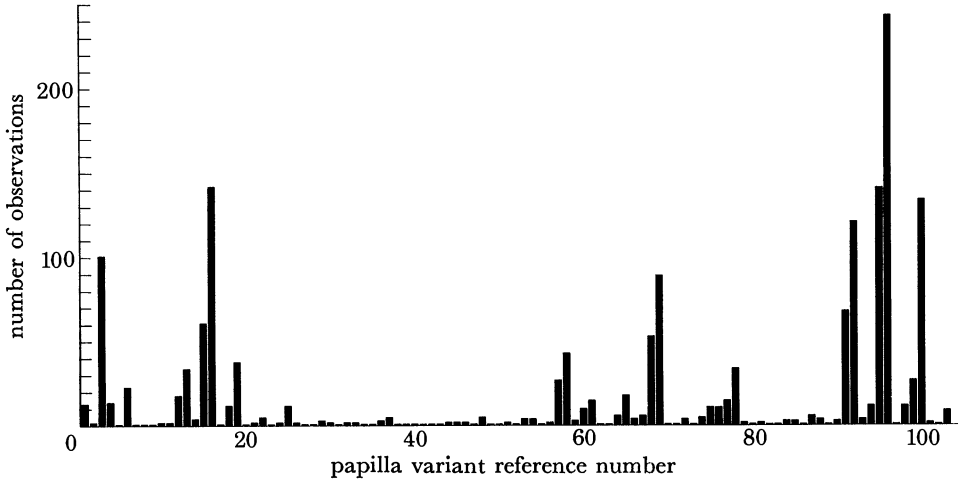

FIGURE 3. Frequency of different suborbital papillae pattern variants.

Many variants possessed the major features of patterns described in the literature as characteristic of *minutus*, *lozanoi* or *norvegicus*. Variants 89–103, for example, all had a long row *b* (reaching to under the anterior half of the orbit) and a high number (9–12) of transverse *c* rows, of which only the last, *cp*, extended below the level of row *d* (figure 2), and thus conform to published accounts of *minutus* arrangements, as do variants 56–81, which differed from 89–103 merely in that one of the more posterior transverse *c* rows (either the fourth, fifth, sixth or seventh) penetrated a short distance below the level of row *d* (figure 2). Variants 1–4, on the other hand, had the essential characters of *norvegicus* patterns (a short row *b*, only reaching to under the posterior third of the orbit, and seven or eight transverse *c* rows, of which the second, fourth and last all extended well below row *d* (figure 2)), while 14–19 possessed the main attributes of *lozanoi* arrangements (a long row *b* and nine or ten transverse *c* rows, the second, fourth and last of which also penetrated well below row *d* (figure 2)).

The remaining variants (5–13, 20–55, 82–88) have been arranged into 15 groups (A–O), as summarized in table 2. While these differed to some extent from patterns previously observed in the main taxa of the complex, variants of groups D, E, F, G, I and J had a *lozanoi*-like arrangement, while group A, B, C and H and group K, L, M, N and O variants had features in common with *norvegicus* and *minutus* patterns respectively.

Each fish had a suborbital pattern on each side of the head. The frequency with which different types occurred together is recorded in table 3, where variants with the main features of patterns described as characteristic of *minutus*, *lozanoi* and *norvegicus* and variants that resembled these are collectively referred to as m, l and n and m′, l′ and n′ arrangements respectively. Most fish (849 specimens) had two m, m′, l, l′, n or n′ variants or an m/m′, l/l′ or n/n′ combination.

In the following section, fishes with two m, l or n patterns are referred to as M, L and N specimens, while individuals with m′/m or m′, l′/l or l′ and n′/n or n′ combinations are grouped as M′, L′ and N′ specimens.

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Table 2. Features of suborbital papillae pattern variants 5–13, 20–55 and 82–88

group	variants	length of row *b*	number of *c* rows	*c* rows below *d*	extent of *c* rows below *d*	number of observations
A	5, 6, 7	short	9	2 & 4	both long	25
B	8, 9	short	9	2 & 5	both long	2
C	10	short	7	2 & 4	both long	2
D	11, 12, 13	long	7/8	2 & 4	both long	54
E	20	long	8	2 & 5	both long	1
F	21, 22, 23, 24, 25	long	9/10	2 & 5	both long	22
G	26, 27	long	11	2 & 5	both long	3
H	28	short	7	2	long	1
I	29	long	7	2	long	3
J	30	long	9	2	long	3
K	31–40	long	9–11	2 & 4/5/6	both short	18
L	41–44	long	9–11	4, 6/6 & 8	both short	5
M	45–49	long	9–11	2	short	10
N	50, 51, 52, 82, 83, 84, 85	short	9–11	4/5/-	short/-	12
O	53, 54, 55, 86, 87, 88	long	8	4/5/-	short/-	20

Table 3. Frequency of specimens with various combinations of m, l, n, m′, l′ and n′ suborbital papillae pattern variants

	m	m′	l	l′	n	n′
m	550	—	—	—	—	—
m′	40	12	—	—	—	—
l	5	—	107	—	—	—
l′	—	1	38	23	—	—
n	—	—	1	1	56	—
n′	—	—	—	—	16	7

2.3.2. *Other morphological characters*

(*a*) *Size*. Lengths and size frequency distributions are illustrated in figure 4 and table 4. It may be noted that 28 of the M specimens were less than 33.0 mm in standard length.

(*b*) *Number of vertebrae*. Vertebral counts are recorded in figure 5 and table 4. There was no significant difference between the mean number of vertebrae in M (32.97) and M′ (32.98) individuals or between the mean numbers of L (32.00), L′ (32.03), N (31.88) and N′ (31.83) specimens, though all these were significantly different from the mean vertebral numbers of M and M′ individuals.

(*c*) *Number of fin rays*. Counts of pectoral, second dorsal branched and anal branched rays are recorded in figure 5 and table 4. There was no significant difference between the mean numbers of pectoral ($\bar{x}_p$), dorsal ($\bar{x}_d$) or anal ($\bar{x}_a$) rays in M ($\bar{x}_p = 19.42$, $\bar{x}_d = 10.44$, $\bar{x}_a = 10.71$) and M′ (19.41, 10.56, 10.86), in L (18.56, 9.82, 10.27) and L′ (18.62, 9.76, 10.09) or in N (16.70, 8.97, 9.06) and N′ (16.89, 9.00, 9.15) individuals. The mean ray numbers of L and L′ specimens were intermediate between, yet significantly different from, those of M or M′ and N or N′ individuals.

(*d*) *Meristic index*. Meristic indices are recorded in figures 5 and 6 and table 4. There was no

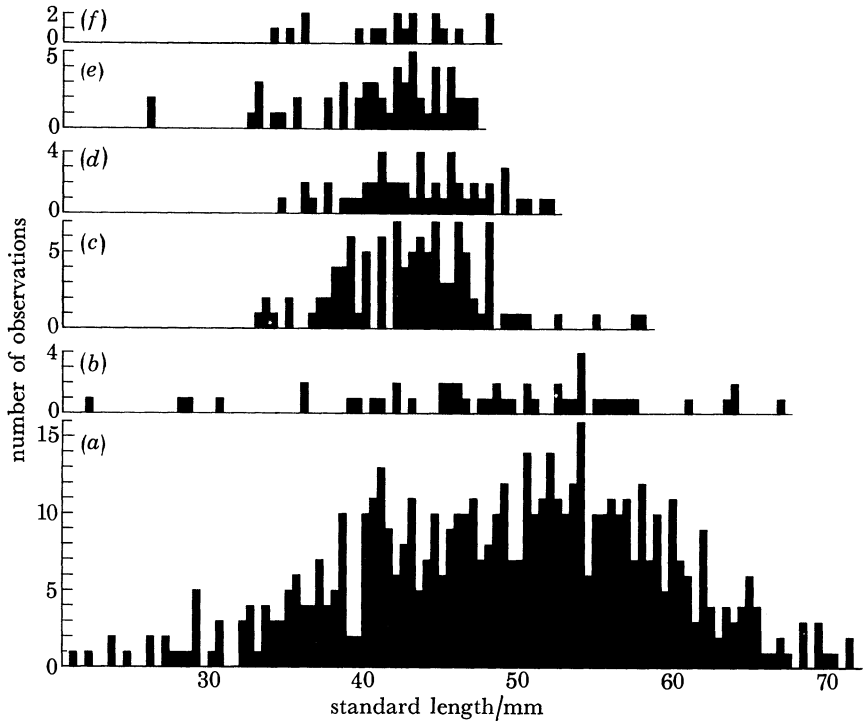

FIGURE 4. Size frequency distribution of (*a*) M, (*b*) M′, (*c*) L, (*d*) L′, (*e*) N and (*f*) N′ specimens.

significant difference between the mean indices of M (65.8) and M′ (66.7), of L (47.7) and L′ (48.5) or of N (23.3) and N′ (23.1) individuals. The mean indices of L and L′ specimens were intermediate between, yet significantly different from, those of M or M′ and N or N′ individuals. There was no obvious relationship between size and meristic index (figure 6).

2.4. *Discussion*

Although the arrangement of suborbital papillae might seem to be rather variable, most individuals have patterns diagnostic of one or other main member of the *minutus* complex. Thus M, L and N specimens can be respectively assigned to *minutus*, *lozanoi* and *norvegicus* on the basis of their suborbital papillae. The remaining individuals had patterns, or combinations of patterns different from those previously observed in these taxa. However, the arrangements in M′, L′ and N′ specimens resembled the patterns characteristic of *minutus*, *lozanoi* and *norvegicus*, and since M′, L′ and N′ and M, L and N individuals were also very similar in meristic characters it seems reasonable to regard them as representatives of *minutus*, *lozanoi* and *norvegicus* with hitherto undescribed patterns of suborbital sensory papillae. In fact only 0.9 % of the sample could not be attributed to *minutus*, *lozanoi* or *norvegicus*. These specimens had characteristics intermediate between those of *minutus* and *lozanoi* or *lozanoi* and *norvegicus*.

The arrangement of suborbital papillae is probably controlled by several genes. Although the number of suborbital neuromasts may vary intraspecifically (see, for example, Miller 1961*b*, 1969), their pattern is usually constant for each gobioid species and it has been the practice to regard taxa with different arrangements of suborbital papillae as being specifically distinct. This practice cannot be automatically adopted with *minutus*, *lozanoi* and *norvegicus*, for, although

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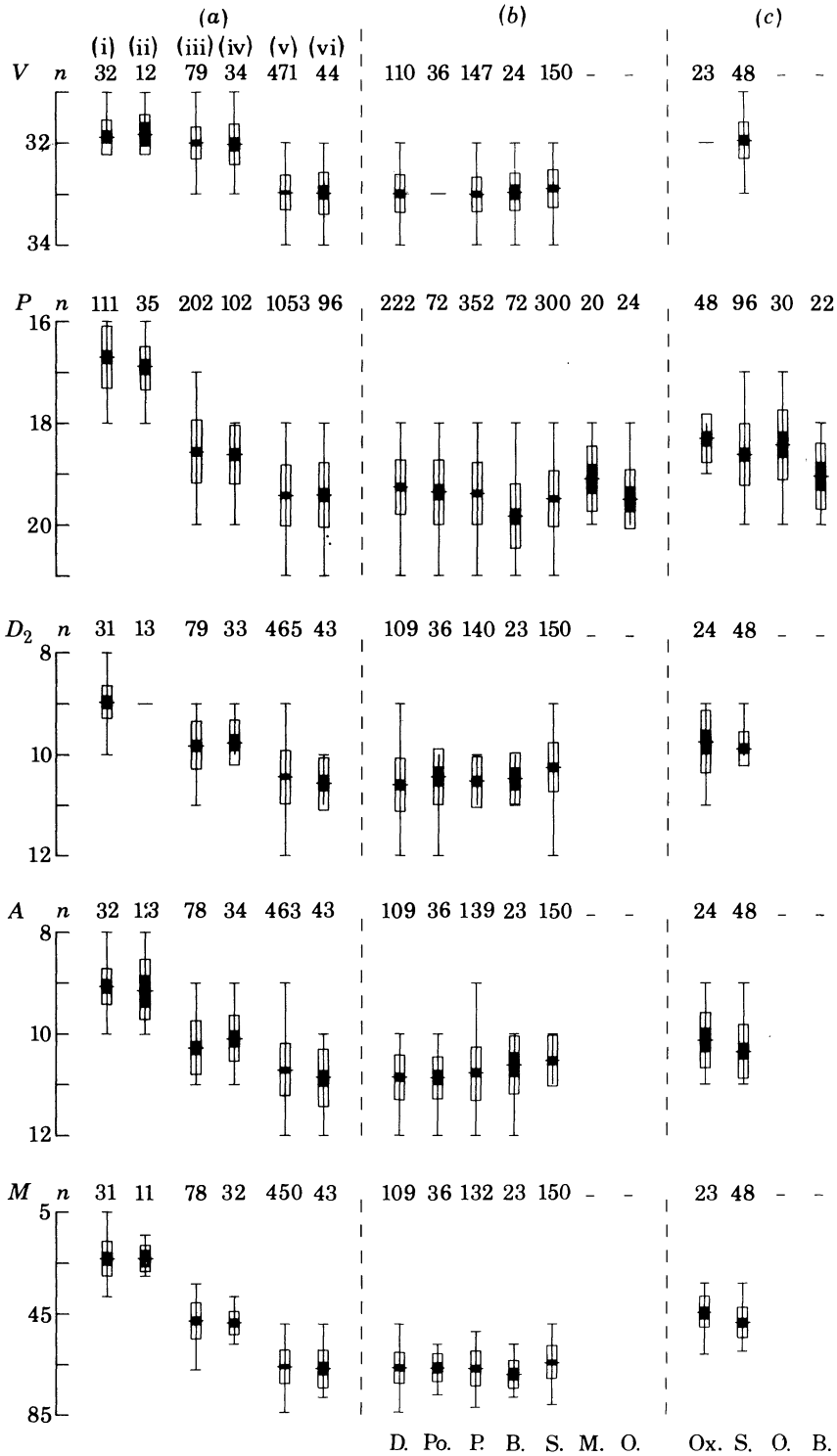

FIGURE 5. (*a*) Meristic variation between N (i), N′ (ii), L (iii), L′ (iv), M (v) and M′ (vi) specimens in number of vertebrae (*V*), pectoral rays (*P*), second dorsal branched rays (D_2), anal branched rays (*A*) and meristic index (*M*). (*b*) Meristic variation between M specimens from different localities. (*c*) Meristic variation between L specimens from different localities. Method of graphical comparison as described by Hubbs & Hubbs (1953). For each sample, range of variation is shown by vertical line, mean by short horizontal line, and, on either side of mean, one standard deviation by limit of open rectangle, and two standard errors by black rectangle; *n*, number of observations. Source of material: D., Dunstaffnage Bay, Oban; M., Millport; Po., Port Erin Bay; Ox., Oxwich Bay; O., Oldbury-on-Severn; P., Plymouth; B., Burnham-on-Crouch; S., Dutch North Sea.

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TABLE 4. SIZE AND MERISTIC FEATURES OF SPECIMENS WITH m/l, m′/l, l/n AND l′/n COMBINATIONS OF SUBORBITAL PAPILLAE PATTERN VARIANTS

variant combination	capture locality	size mm	number of vertebrae	number of pectoral rays	number of branched D_2 rays	number of branched anal rays	meristic index
m/l	S. North Sea	37.0	33	20/20	10	10	65
m/l	S. North Sea	40.0	32	18/19	10	10	47
m/l	S. North Sea	46.7	32	19/19	10	10	51
m/l	S. North Sea	47.0	33	19/19	11	11	67
m/l	Oxwich Bay	44.0	32	18/18	10	10	43
m′/l′	S. North Sea	43.0	32	19/19	9	10	46
l/n	Millport	36.1	33	17/17	9	9	31
l′/n	S. North Sea	44.0	32	18/18	10	10	43

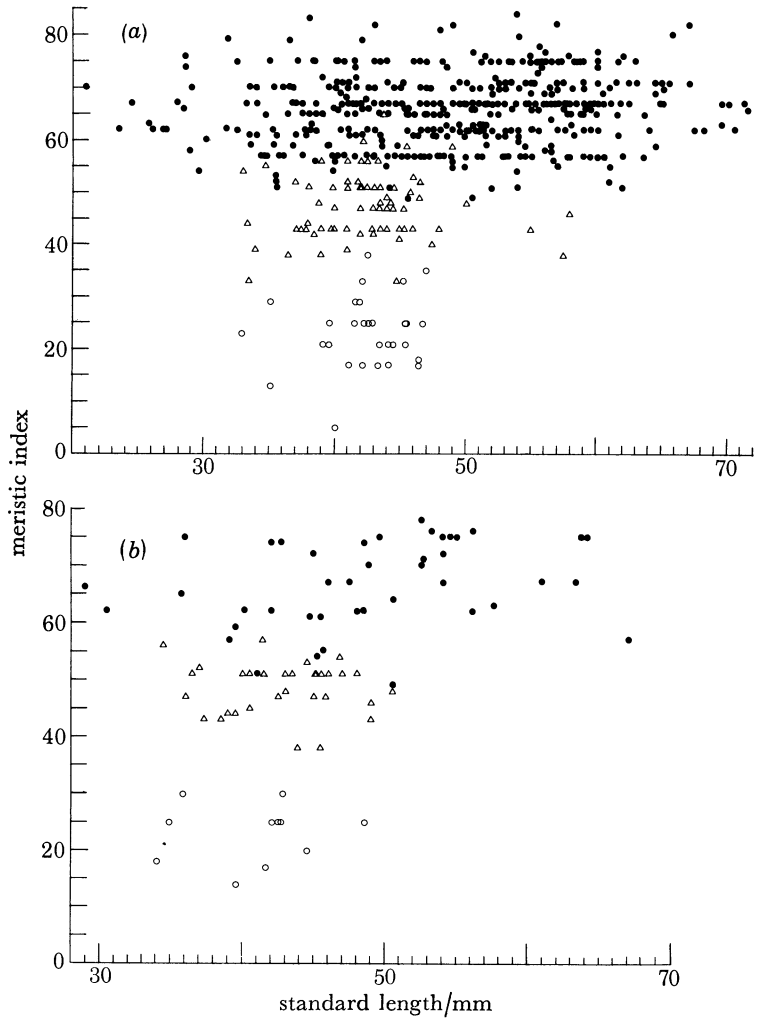

FIGURE 6. Variation of meristic index with size of: (*a*) M (●), L (△) and N (○) specimens and; (*b*) M′ (●), L′ (△) and N′ (○) specimens.

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more easily separated on the basis of their suborbital papillae than many species of *Pomatoschistus* (figure 7), *lozanoi* has arrangements intermediate between the patterns of *minutus* and *norvegicus* and specimens exist with patterns intermediate between those of *minutus* and *lozanoi* or *lozanoi* and *norvegicus*.

In systematic ichthyology it has often been assumed that samples that have significantly different mean numbers of skeletal elements are genetically distinct. However, a substantial amount of observational and experimental evidence has accumulated to indicate that meristic features are only partly determined by heredity and can be influenced by conditions prevailing during early development (see, for example, Garside 1970).

The influence of environmental factors on vertebral number in *minutus* and *lozanoi* was investigated by Fonds (1970, 1971, 1973), who reared fish from eggs under varying regimes of temperature, salinity and oxygen supply. He concluded that vertebral number is not markedly affected by environment and suggested that differences in mean number observed between feral populations of *minutus* and *lozanoi* are indicative of genetic distinctiveness. The current study shows that for both *minutus* and *lozanoi* there is less geographical variation in vertebral number than in the number of fin rays (figure 5). This suggests that, as in the stickleback *Gasterosteus aculeatus* (see Hagen 1967), vertebrae are less subject to environmental modification than are fin rays.

The degree of meristic differentiation within the *minutus* complex is much greater than that between the Atlantic and Mediterranean subspecies of the painted goby *Pomatoschistus pictus* or between boreal and Mediterranean populations of *Gobius niger* (L. 1758) (Miller 1972; Miller & El-Tawil 1974), and is more like that between two nominal species of *Gobius*, *G. couchi* Miller & El-Tawil, 1974 and *G. auratus* Risso (Miller & El-Tawil 1974). However, as with the pattern of suborbital papillae, *lozanoi* is intermediate between *minutus* and *norvegicus* in a number of meristic features.

Natural intermediates between two fish taxa have usually been interpreted as hybrids and the fact that progeny of experimental crosses are usually (though not invariably) intermediate in their features between parental forms supports this conclusion (see, for example, Simon & Noble 1968). Therefore, from the morphological evidence, it might seem reasonable to conclude that *lozanoi* is a hybrid between *minutus* and *norvegicus* and that it can hybridize with both in nature. However, interpretations other than hybrid origin exist for morphological intermediates (Nelson 1968; Aspinwall & Tsuyuki 1968) and in the following section further evidence is sought on the status of *lozanoi* and *minutus–lozanoi* intermediates.

3. Biochemical studies

3.1. *Introduction*

Chemical features of organisms were used by systematists as early as the beginning of this century (see, for example, Nutall 1904). However, only recently has a distinct and rapidly expanding field of biochemical systematics emerged. This employs particularly the large 'information-containing' molecules such as nucleic acids and proteins and has followed from advances made in molecular biology (Alston & Turner 1963; Sneath 1968). For example, it is now apparent that properties of individuals' protein molecules more or less directly reflect the structural organization of their genetic material and that comparison of these molecules can have special significance for the classification of organisms (Sibley 1962).

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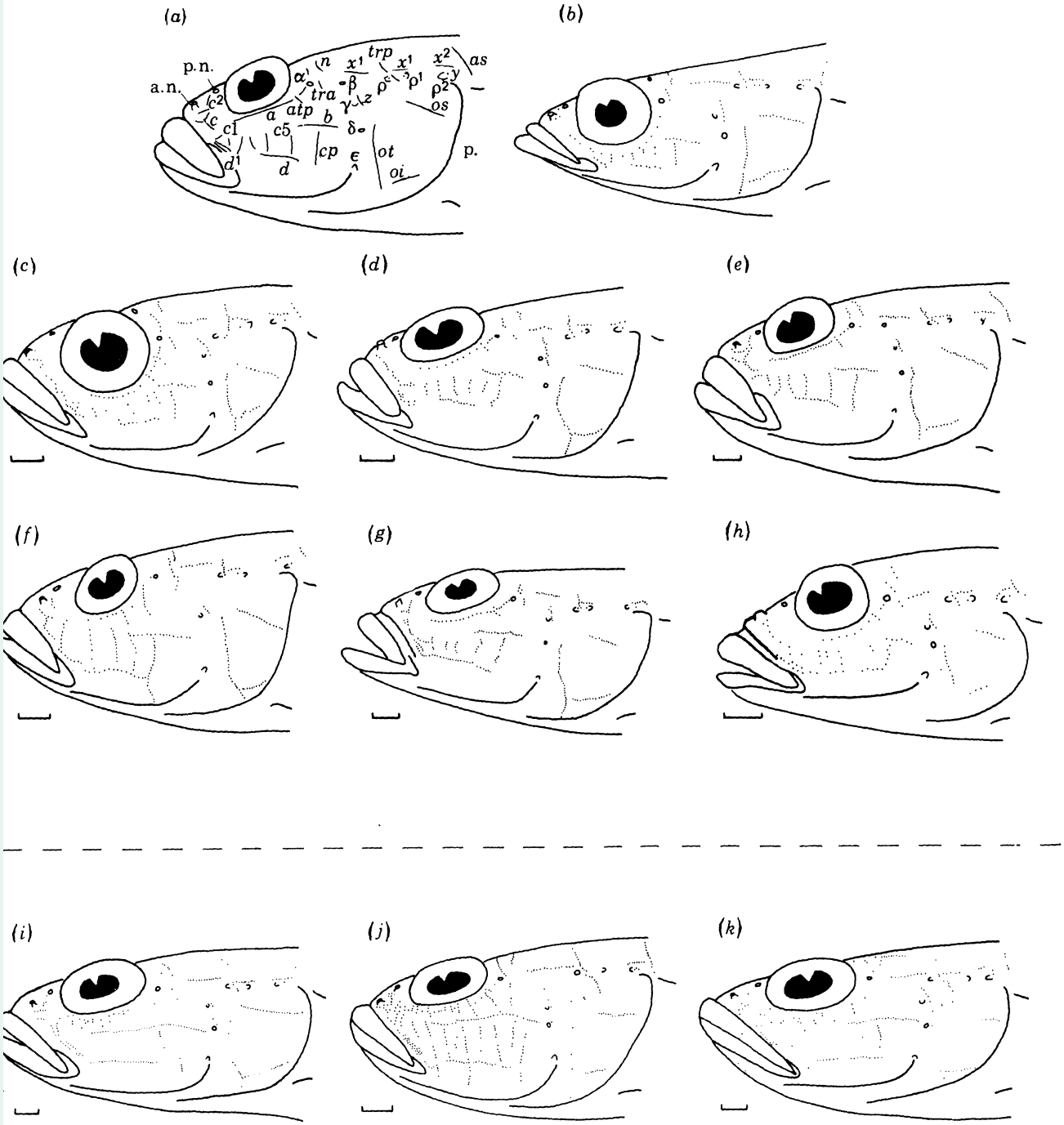

FIGURE 7. Lateral view of head sensory papillae and canal pores in *Pomatoschistus*, scale 2 mm. (*a*) Diagram of terminology; symbols and abbreviations as in figure 1. (*b*) *P. quagga*. (*c*) *P. knerii*. (*d*) *P. pictus*. (*e*) *P. marmoratus*. (*f*) *P. canestrinii*. (*g*) *P. microps*. (*h*) *P. tortonesei*. (*i*) *P. minutus*. (*j*) *P. lozanoi*. (*k*) *P. norvegicus*. (*a*), (*h*) After Miller (1968); (*b*) after Sanzo (1911); (*c*), (*e*), (*f*), (*j*) after P. J. Miller, unpublished; (*d*) after Miller (1972); (*g*), (*i*) after Miller (1963); (*k*) after Webb & Miller (1975).

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The ideal method for comparing homologous proteins is to determine their amino acid sequences, but a simpler means of comparison, gel electrophoresis, has been adopted in many systematic studies. The electrophoretic mobility of a protein is a function of its net electric charge, size and shape, and proteins that migrate at different rates usually differ by at least one amino acid and are the products of different gene loci, or of different alleles at the same locus (Shaw 1971; Avise 1974).

Electrophoretic data have been employed in systematic investigations of many organisms, including fishes, where they have been applied to a wide range of systematic problems (Utter *et al.* 1974). Electrophoretic studies of the Gobioidea are, however, limited. The main contribution has been made by El-Tawil (1974), who described the electrophoretic behaviour of haemoglobin, muscle 'myogens' (Hamoir 1955) and lactate dehydrogenase isozymes of twenty-three species and gave details of genetic intraspecific haemoglobin polymorphism in *Pomatoschistus microps*, *Gobius paganellus* (L. 1758) and *G. niger* (see Miller & El-Tawil 1974). Haemoglobin polymorphism in *G. niger* (as *G. jozo* L.) has also been discussed by Raunich *et al.* (1966, 1967), while Cucchi & Callegarini (1969) reported haemoglobin polymorphism in *Padogobius martensi* (Günther 1861) (as *G. fluviatilis* Nardo). The electrophoretic behaviour of serum and water-soluble muscle proteins of various Ponto-Caspian gobies has been studied by Sulman & Kulikova (1966), Rodino (1968) and Tesio & Mester (1970) and a study of the products of 26 gene loci in three species of the circumtropical genus *Bathygobius* was carried out by Gorman *et al.* (1976).

No electrophoretic data are published for the *P. minutus* complex, although Fonds (1973) made a preliminary investigation of haemoglobin in *minutus* and *lozanoi*, using the technique of electrofocusing. Here the electrophoretic behaviour of haemoglobin and of proteins from white muscle, including myogens and the enzymes creatine kinase (CK, EC 2.7.3.2), phosphoglucose isomerase (PGI, EC 5.3.1.9) and lactate dehydrogenase (LDH, EC 1.1.1.27), is described in putative *minutus–lozanoi* hybrids and in *minutus*, *lozanoi* and *norvegicus*.

3.2. *Material and methods*

Individuals were identified on the basis of suborbital papillae patterns or meristic features. Details of the specimens used are given in appendix 2.

White muscle protein and haemoglobin samples were obtained by the methods of Miller & El-Tawil (1974). Horizontal thin-layer starch gel electrophoresis with hydrolysed starch (130 g/l; BDH) and Tris–EDTA–borate buffer solution (Smithies 1959; Tsuyuki *et al.* 1966) was employed in a modified Shandon U77 tank, as described by Miller & El-Tawil (1974). Electrophoresis was performed at 25–35 V/cm for 2.5–5 h, during which time the tank was situated in a refrigerator and water at 4 °C circulated beneath the gel.

After electrophoresis, muscle myogens and haemoglobins were revealed by staining with naphthalene black 12B (10 g/l; BDH) for 1 min and destaining with four washes of a 5 : 1 : 5 mixture of absolute alcohol, glacial acetic acid and water. For lactate dehydrogenase, gels were stained by the deposition of a reduced tetrazolium salt in the regions of enzyme activity (Whitt 1970) with the staining mixture of Davidson *et al.* (1965). Creatine kinase and phosphoglucose isomerase were identified by the positive filter paper staining method of Scopes (1968). Dried filter papers and destained gels were photographed and stored for reference.

3.3. *Results*

3.3.1. *White muscle proteins*

(*a*) *Muscle myogens and creatine kinase.* All the muscle myogens studied separated on electrophoresis into four or five anodic zones, here referred to by an arbitrary lettering system based on that of Tsuyuki *et al.* (1968) and Miller & El-Tawil (1974). Three phenotypes were observed (figure 8*a*). Type 1 comprises (in order of increasing mobility) zones a, j, o and q. Zones a, o and q also occur in type 2, but j is replaced by a faster zone k, while zones a, j, k, o and q are all present in type 3. Zones j and k, which stained more intensely than the other components, were identified as creatine kinase.

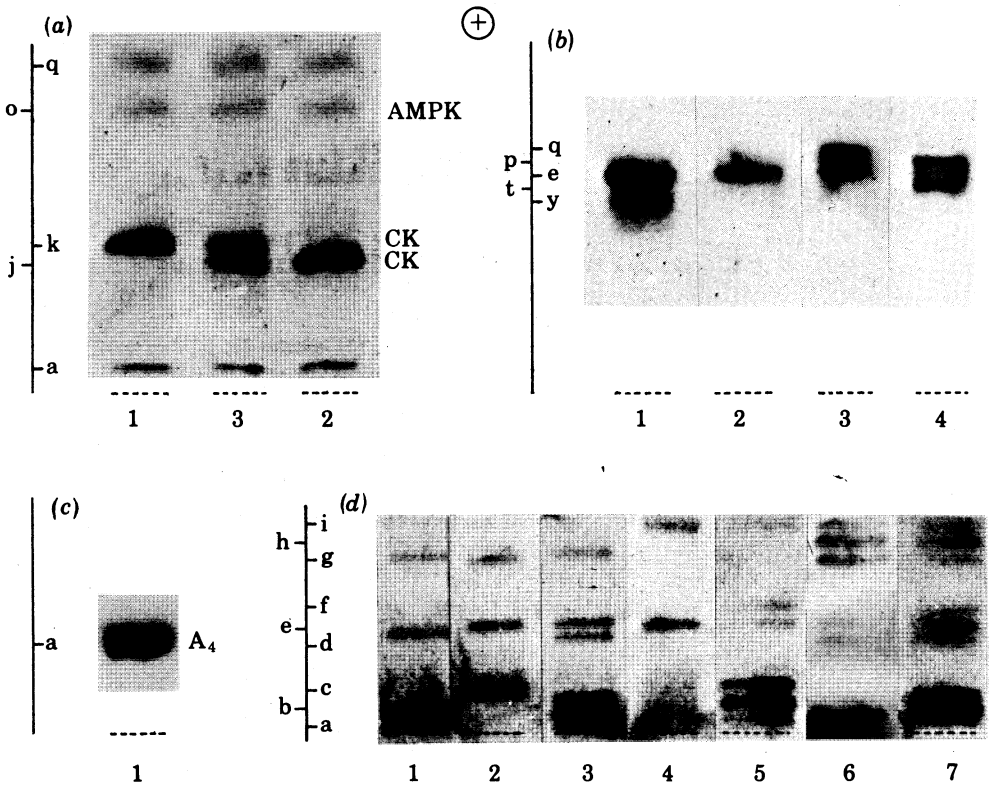

FIGURE 8. Electrophoretic phenotypes of (*a*) muscle myogens, (*b*) haemoglobin, (*c*) lactate dehydrogenase, (*d*) phosphoglucose isomerase.

Irrespective of sex, size or place of capture, all specimens of *minutus* and *norvegicus* possessed type 1 myogens while the *lozanoi* and *minutus–lozanoi* intermediates had type 2 and type 3 respectively (table 5; figure 9).

(*b*) *Phosphoglucose isomerase.* All PGI enzymes investigated were separable into a minimum of three, and a maximum of nine, anodic zones, referred to by an arbitrary lettering system in which the zones are lettered from a to i in order of increasing mobility. Seven phenotypes were observed (figure 8*d*). Types 1, 2 and 4 comprise three zones, while six zones occur in types 3, 5 and 6, and all nine zones are present in type 7.

All but one of the *minutus* examined possessed type 4 PGI, and the solitary exception, a 51.0 mm female from the Rame Mud fishing grounds off Plymouth, had type 5 (table 5; figure 10). *P. lozanoi* possessed either types 1, 2 or 3 (table 5; figure 10). The occurrence of these

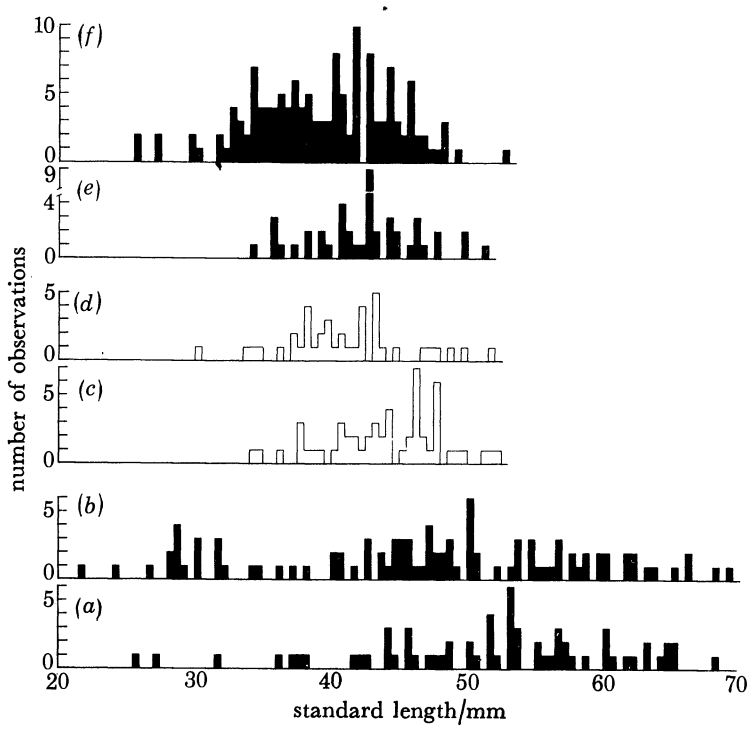

FIGURE 9. Occurrence of muscle myogen phenotypes in (*a*) *minutus* ♂♂, (*b*) *minutus* ♀♀, (*c*) *lozanoi* ♂♂ (*d*) *lozanoi* ♀♀, (*e*) *norvegicus* ♂♂ and (*f*) *norvegicus* ♀♀ *of* varying size. ■ Type 1, □ type 2.

TABLE 5. OCCURRENCE OF DIFFERENT MUSCLE MYOGENS, PGI AND HAEMOGLOBIN PHENOTYPES IN *MINUTUS*, *LOZANOI*, *NORVEGICUS*, AND PUTATIVE *MINUTUS–LOZANOI* HYBRIDS

phenotypes	*minutus*	*lozanoi*	*norvegicus*	*minutus–lozanoi* hybrids
myogens				
1	163	—	184	—
2	—	94	—	—
3	—	—	—	6
PGI				
1	—	10	6	—
2	—	16	33	—
3	—	30	14	—
4	77	—	—	—
5	1	—	—	—
6	—	—	—	1
7	—	—	—	3
haemoglobin				
1	102	—	—	
2	—	20	—	
3	—	17	—	
4	—	—	33	

phenotypes was not linked with sex, and there was no significant difference in the mean standard length of fish with type 2 (43.2 ± 1.14 mm) and type 3 (43.7 ± 0.86 mm), but the mean length of specimens with type 1 (39.7 ± 0.69 mm) was significantly different from the mean of fish with type 2 ($p = 0{\cdot}01$–$0{\cdot}02$) and type 3 ($p < 0.01$) (figure 10). Specimens of *norvegicus* also possessed either type 1, type 2 or type 3 PGI, although types 1 and 3 were less frequent and type 2 was

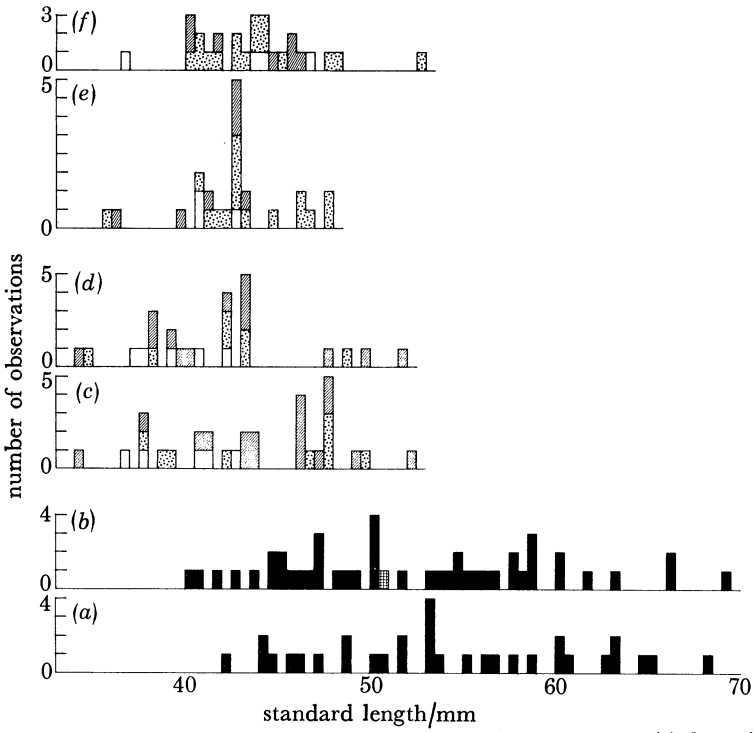

FIGURE 10. Occurrence of PGI phenotypes in (*a*) *minutus* ♂♂, (*b*) *minutus* ♀♀, (*c*) *lozanoi* ♂♂, (*d*) *lozanoi* ♀♀, (*e*) *norvegicus* ♂♂ and (*f*) *norvegicus* ♀♀, of varying size. □ Type 1, ▦ type 2, ▨ type 3, ■ type 4, ▦ type 5.

more frequent than in *lozanoi* (table 5). The occurrence of phenotypes was not sex-linked and there was no significant difference between the mean lengths of fish with type 1 (43.3 ± 0.94 mm), type 2 (43.5 ± 0.58 mm) and type 3 (42.5 ± 0.73 mm) (figure 10). All three phenotypes occurred in samples from Plymouth, but only types 2 and 3 among fish from Dunstaffnage Bay near Oban. Putative *minutus–lozanoi* hybrids possessed either type 6 or type 7 PGI (table 5).

(*c*) *Lactate dehydrogenase*. LDH from all specimens of the *minutus* complex separated on electrophoresis into a single anodic zone (a), which migrated faster than myogen zone a but slower than either of the myogen zones identified as creatine kinase (figure 8*c*).

3.3.2. *Haemoglobin*

Haemoglobins were separable into one or more anodic zones, referred to by an arbitrary lettering system based on, but supplementing, that of Raunich *et al.* (1967) and Miller & El-Tawil (1974). Four phenotypes were observed (figure 8*b*). Type 2 comprises a single zone, while two zones occur in types 1 and 3, and type 4 consists of a minor and two major zones.

All specimens of *minutus* possessed type 1 haemoglobin, but representatives of *lozanoi* had either type 2 or type 3 (table 5; figure 11). The occurrence of types in *lozanoi* was not sex-linked (figure 11) and although the mean standard lengths of fish with type 2 (42.4 ± 0.94 mm) and type 3 (44.9 ± 0.71 mm) were significantly different (p = 0.05–0.02) there was no obvious difference in the maturity of these fish. Both types of haemoglobin were present in samples from the Dutch North Sea and from Burnham-on-Crouch, but only type 3 occurred in a small sample from Oldbury-on-Severn. All specimens of *norvegicus* had type 4 haemoglobin (table 5; figure 11).

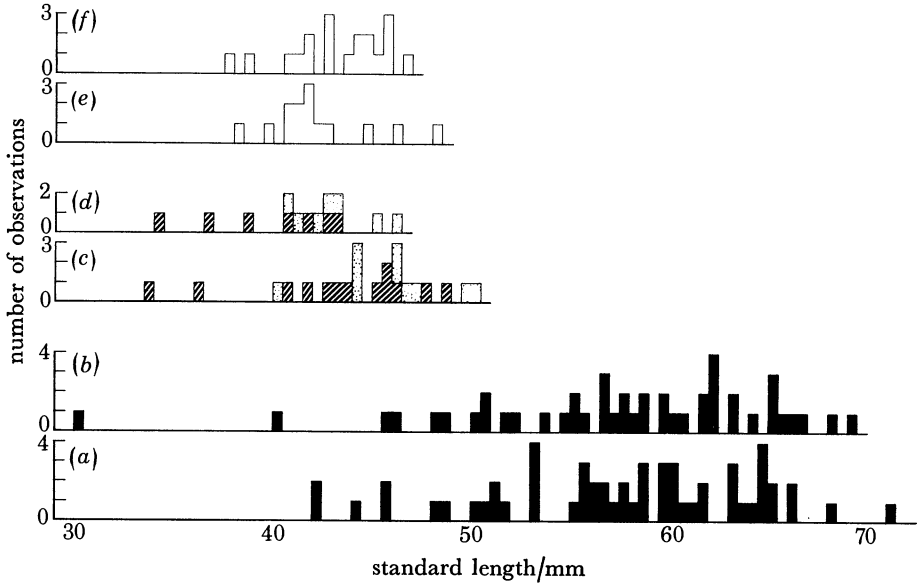

FIGURE 11. Occurrence of haemoglobin phenotypes in (*a*) *minutus* ♂♂, (*b*) *minutus* ♀♀, (*c*) *lozanoi* ♂♂, (*d*) *lozanoi* ♀♀, (*e*) *norvegicus* ♂♂ and (*f*) *norvegicus* ♀♀ of varying size. ■ Type 1, ▨ type 2, ▩ type 3, □ type 4.

3.4. *Discussion*

In the *minutus* complex, as in other fish, creatine kinase is an important component of sarcoplasmic protein. In most teleosts it appears to be a dimer controlled by two independent gene loci which may be differentially expressed in different tissues (Eppenberger *et al.* 1971; Scholl & Eppenberger 1972). Here it is not clear from its electrophoretic behaviour that CK from white muscle has a dimeric structure controlled by more than one locus. However, it does seem to be determined by at least one locus that is monomorphic for the same allele (A^1) in *minutus* and *norvegicus*, but that is fixed for a different allele (A^2) in *lozanoi* and is heterozygous for A^1 and A^2 in putative *minutus–lozanoi* hybrids. Other myogen components were not identified with specific proteins, but probably represent the products of at least three loci, which seem to be monomorphic for the same allele in all members of the *minutus* complex.

PGI and LDH are both enzymes involved in glucose metabolism. In most teleosts examined so far, PGI is determined by two loci, *PGIA* and *PGIB*, which encode A and B subunits that polymerize randomly to yield dimers (Avise & Kitto 1973; Dando 1974). In fish, *PGIB* activity predominates in white skeletal muscle while *PGIA* is more active in kidney, liver and brain. Isozymes composed of B subunits migrate more slowly towards the anode than heterodimers which, in turn, migrate at a slower rate than isoenzymes made up of A subunits (Avise & Kitto 1973; Dando 1974). The electrophoretic behaviour of PGI from white muscle of the *minutus* complex is also consistent with a dimeric structure controlled by two loci, one of which (*PGIB*) is more active than the other and at both of which two alleles occur (figures 8*d*, 12; table 6). The samples of *lozanoi* and *norvegicus* are monomorphic for $PGIA^1$, while the population of *minutus* is monomorphic for $PGIA^2$. The sample of *minutus* is also essentially monomorphic for $PGIB^1$, the B^2 allele occurring at a frequency of only 0.01, while *lozanoi* and *norvegicus* segregate for B^1 and B^2 (table 6). The B^2 allele, commoner than B^1 in both *norvegicus* and *lozanoi*, occurs at a higher frequency in the former and is also markedly more frequent in *norvegicus* from Oban than

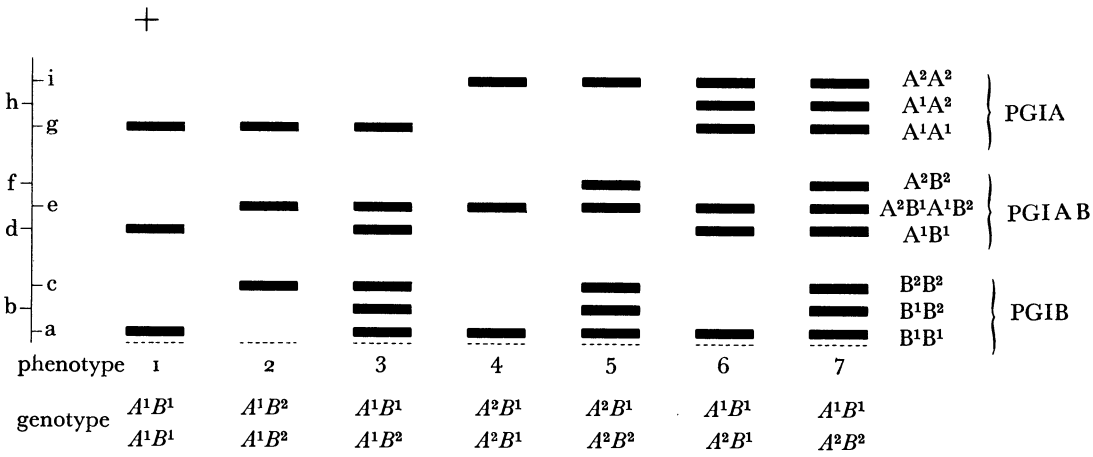

FIGURE 12. Genetic interpretation of PGI phenotypes.

TABLE 6. DISTRIBUTION OF *PGIB* GENOTYPES AMONG *MINUTUS*, *LOZANOI* AND *NORVEGICUS*

phenotype		1/4	3/5	2				
genotype		B^1B^1	B^1B^2	B^2B^2				
	‡				N	pB^2†	χ^2 (1)	p
norvegicus								
Plymouth	o.	6	13	19	38	0.67	1.9511	0.10–0.25
	e.	4.1	16.8	17.1				
Dunstaffnage	o.	—	1	14	15	0.97	0.0209	0.75–0.90
Bay	e.	0.02	0.97	14.02				
total	o.	6	14	33	53	0·75	4.3096	0.02–0.05
	e.	3.2	19.6	30.2				
lozanoi								
Oldbury	o.	10	29	14	53	0.54	0.5162	0.25–0.50
	e.	11.3	26.4	15.3				
total	o.	10	30	16	56	0.55	0.4033	0.50–0.75
	e.	11.2	27.7	17.2				
minutus								
total	o.	77	1	—	78	0.01	0.0001	>0.90
	e.	77.002	0.991	0.003				

† pB^2, Frequency of B^2 allele. ‡ Observed value, o.; expected value, e.

in *norvegicus* from Plymouth (table 6). Putative *minutus–lozanoi* hybrids are all heterozygous at *PGIA* and are either homozygous for B^1 or heterozygous at *PGIB*.

Lactate dehydrogenase is ubiquitous in vertebrate tissues. All vertebrates possess at least two loci for LDH, *A* and *B*, which encode biochemically distinct subunits that polymerize to yield tetramers (Markert 1963; Wilson & Kaplan 1964). In many fishes the assembly of LDH polypeptides is restricted, so that fewer than the total number of possible isozymes are formed, and, as in other groups, there is a different tissue-specific expression of the *A* and *B* loci (Kaplan 1964; Markert & Faulhaber 1965). Evidence also suggests that in addition to the *A* and *B* genes, tissues of several teleosts possess a third and perhaps also a fourth LDH locus (see, for example: Whitt 1968; Sensabaugh & Kaplan 1972), though these are rarely active in muscle tissue (Whitt *et al.* 1973). LDH from skeletal muscle in the *minutus* complex, like that of many flatfish and a number of gobioids (Lush *et al.* 1969; El-Tawil 1974), separates on electrophoresis into a single zone of

moderate anodic mobility. This presumably represents the A_4 isozyme, composed of subunits synthesized by a single locus (*LDHA*), which seems to be monomorphic for the same allele in all members of the *minutus* complex.

Like LDH, most vertebrate haemoglobins have a tetrameric structure and, in fishes, haemoglobins can contain two, three or even four different polypeptides, and as many as eight structural genes may be involved in their synthesis (see, for example: Tsuyuki & Ronald 1970; Wilkins 1970). In common with many fish and nearly all studied gobies (Riggs 1970; El-Tawil 1974), several members of the *minutus* complex possess more than one haemoglobin. Multiple components in haemolysates usually represent tetrameric combinations of different globin molecules and reflect the occurrence of multiple loci or allelic variants (see, for example, Wilkins 1968, 1970), although apparent multiple haemoglobins can also arise as technical artefacts, caused, for example, by polymerization or methaemoglobin formation, particularly

TABLE 7. DISTRIBUTION OF HAEMOGLOBIN GENOTYPES AMONG *LOZANOI* FROM THE DUTCH NORTH SEA AND BURNHAM-ON-CROUCH

(Symbols and abbreviations as in table 6.)

phenotype		2	3	—				
genotype		B^1B^1	B^1B^2	B^2B^2	N	pB^1	$\chi^2(1)$	p
Dutch North	o.	15	10	—	25	0.80	1.5625	0.10–0.25
Sea	e.	16	8	1				
Burnham	o.	5	3	—	8	0.81	0.4233	0.50–0.75
	e.	5.3	2.4	0.3				
total	o.	20	13	—	33	0.80	2.0293	0.10–0.25
	e.	21.3	10.4	1.3				

during sample storage (see, for example, Yamanka *et al.* 1965 *a*, *b*). Haemolysates were not stored in this study and it seems reasonable to assume that the major zones observed in phenotypes represent tetrameric combinations of at least two distinct polypeptide chains. If so, it follows that the haemoglobin of *minutus* and *norvegicus*, which invariably separates into two main components, is determined by three monomorphic loci (*HbA*, *HbB*, *HbC*), while the behaviour of *lozanoi*, which occurs as a single or double-banded form, is consistent with a model of control by two loci (*HbA*, *HbB*), at one of which (*HbB*) two codominant alleles (B^1, B^2) segregate. Haemoglobin polymorphism has been reported in several fishes (reviews by De Ligny (1969) and Manwell & Baker (1970)) and is often determined by codominant alleles (see, for example: Sick 1965; Wilkins 1971), but can also result from a differential ontogenetic expression of loci encoding different globin subunits (see, for example: Wilkins & Iles 1966; Perez & Maclean 1974). Although the mean length of *lozanoi* with the single-banded form was different from that of *lozanoi* with the double-banded form, there was no obvious relationship between haemoglobin phenotype and maturity, and the hypothesis that the polymorphism observed is determined by two allelic genes is supported by the distribution of supposed genotypes in samples from Burnham-on-Crouch and the Dutch North Sea, which is consistent with the Hardy–Weinberg distribution (table 7). The electrophoretic behaviour of haemoglobin from the *minutus* complex suggests that *minutus* and *lozanoi* have the same allele at *HbA* and that *minutus* is monomorphic at *HbB* for one of the alleles (B^1), which segregates at this locus in *lozanoi*. On the other hand, it seems that *norvegicus* and *lozanoi* are allelically distinct at either *HbA* or *HbB*, while *minutus* and *norvegicus* must have different alleles at a minimum of two of their three haemoglobin loci.

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In all, the proteins studied represent the products of some ten or eleven loci, and estimates of genetic distance (table 8), calculated, according to the method of Nei (1972), from the frequency of alleles at these loci, indicate that all pairs of taxa in the *minutus* complex are allelically distinct at 20–30 % of their loci. Recent multi-loci studies suggest that this sort of genetic divergence is more typical of congeneric than conspecific populations (see, for example, Ayala 1975). However, there is no strict rule relating the genetic similarity of two populations to their

TABLE 8. GENETIC IDENTITY (BELOW DIAGONAL) AND GENETIC DISTANCE (ABOVE DIAGONAL) BETWEEN *MINUTUS*, *LOZANOI* AND *NORVEGICUS*

	minutus	*lozanoi*	*norvegicus*
minutus	—	0.278	0.302
lozanoi	0.757	—	0.223
norvegicus	0.739	0.800	—

taxonomic status. Thus low levels of genetic differentiation seem to be characteristic of populations that are reproductively isolated as a result of changes in chromosome structure or number (see, for example, Nevo & Shaw 1972), a point worth noting since, as shown in the following section, karyological changes may have played a role in the evolution of the *minutus* complex. While the biochemical data clearly do not support the hypothesis that *lozanoi* is a simple F_1 hybrid between *minutus* and *norvegicus*, they confirm the hybrid status of specimens that are morphologically intermediate between *minutus* and *lozanoi*. Thus, as might be expected for F_1 hybrids (see, for example, Payne *et al.* 1972), *minutus–lozanoi* intermediates are heterozygous at loci (for example, *PGIA* and *CKA*) at which different alleles are fixed in the presumed parental populations. There is no biochemical indication of backcrossing, and so, although *minutus* and *lozanoi* can *interbreed*, they may still be *reproductively isolated* (cf.: Sibley 1961; Bigelow 1965). Possible isolating mechanisms are discussed below.

4. KARYOLOGICAL STUDIES

4.1. *Introduction*

Systematic investigations of various animal groups have long and profitably included chromosome studies. Progress depends on the availability of satisfactory techniques. Fishes often have many small chromosomes and methods for their preparation have only been developed recently (see reviews by Denton (1973) and Blaxhall (1975)). Karyological information is therefore available for only 2–3 % of the known species and most of this consists of numerical estimates (Denton 1973). Chromosome homologies have usually been inferred from size and centromere position as most fish chromosomes are devoid of other obvious markers, although secondary constrictions and satellites have been observed occasionally (Svarsdon 1945; Roberts 1964). Sex chromosomes have been recognized in some species and there is evidence of male and female heterogamety and multiple sex chromosome systems (Chen & Ebeling 1966, 1968; Uyeno & Miller 1971).

From the karyological viewpoint, gobies are among the better studied teleosts (Denton 1973), although data are published on less than 50 of the 1300 species. The only karyological information available for the *P. minutus* complex, indeed for the genus *Pomatoschistus*, is an estimate of the diploid number in *P. minutus* (Webb 1974).

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4.2. *Material and methods*

Specimens were identified on the basis of their suborbital papillae arrangements. Representatives of *minutus* (4♂♂ and 1♀), *lozanoi* (6♂♂ and 2♀♀) and *norvegicus* (4♂♂ and 1♀) were analysed. For details of their size and source see appendix 3.

4.2.1 *Mitotic preparations*

Chromosomes were observed in gill and spleen tissue squashes and in smears of gill tissue from colchicine-treated specimens (McPhail & Jones 1966; Webb 1974), except in *norvegicus*, individuals of which were usually captured in a moribund condition and did not survive colchicine injection. Here chromosomes were obtained from monolayer cultures of cells derived from fin or gill tissue and maintained in a small-scale *in vitro* system (Webb 1975). Digests were seeded in culture chambers at a density of 5×10^5–3×10^6 cells per millilitre in Leibovitz L15 medium (Gibco–Biocult) supplemented with antibiotics, sera and additional sodium chloride as described by Webb (1975). Cultures were maintained at 18–20 °C for 5–7 days, treated with colcemid at a concentration of 1 μg/ml for 4–6 h, detached from the culture surface by a short treatment with trypsin–pancreatin (0.25 mg/ml; Difco) prepared in Ca^{2+} and Mg^{2+}-free Hanks balanced salt solution, transferred to a hypotonic solution (0.075 M KCl, or sodium citrate (0.5 mg/ml)) for 10 min, and fixed in two 15 min changes of freshly prepared cold Carnoy's fixative. Cells were then resuspended in a small volume of fixative, drops of this suspension being deposited on slides, air dried, stained with Giemsa and mounted in Euparol.

The number and morphology of mitotic chromosomes in each individual were determined from a minimum of ten well spread metaphase figures. Chromosomes were classified as m (metacentric), sm (submetacentric), st (subtelocentric) and t (acrocentric or telocentric) on the basis of long arm : short arm ratio, as proposed by Levan *et al.* (1964). Following Arai & Sawada (1974), in calculations of the arm number (*nombre fondamental*, N. F.; Matthey 1949) metacentrics and submetacentrics were scored as two- and one-armed chromosomes respectively.

4.2.2. *Meiotic preparations*

Chromosomes were observed in squashes of testis tissue (Webb 1974). Meiotic figures were most readily available from developing or ripening testes (maturation stage II of Swedmark (1958)), which occurred from November to February. The number and morphology of meiotic chromosomes at metaphase I in each individual was established from a minimum of ten figures.

4.2.3. *Relative DNA content*

The relative amount of DNA in a male and female of *minutus*, *lozanoi* and *norvegicus* was estimated by measuring the Feulgen stain content of erythrocyte nuclei with an integrating microdensitometer (Atkin *et al.* 1965). For each individual, a mean value for nuclear Feulgen stain content was calculated from measurements of a 100 cells and the stain content of erythrocytes from different specimens compared with a *t*-test.

4.3. *Results*

4.3.1. *Chromosome studies*

(*a*) *P. minutus*. All specimens examined had a modal diploid number of 46 chromosomes. No obvious intra- or inter-individual variation in chromosome morphology was observed and there

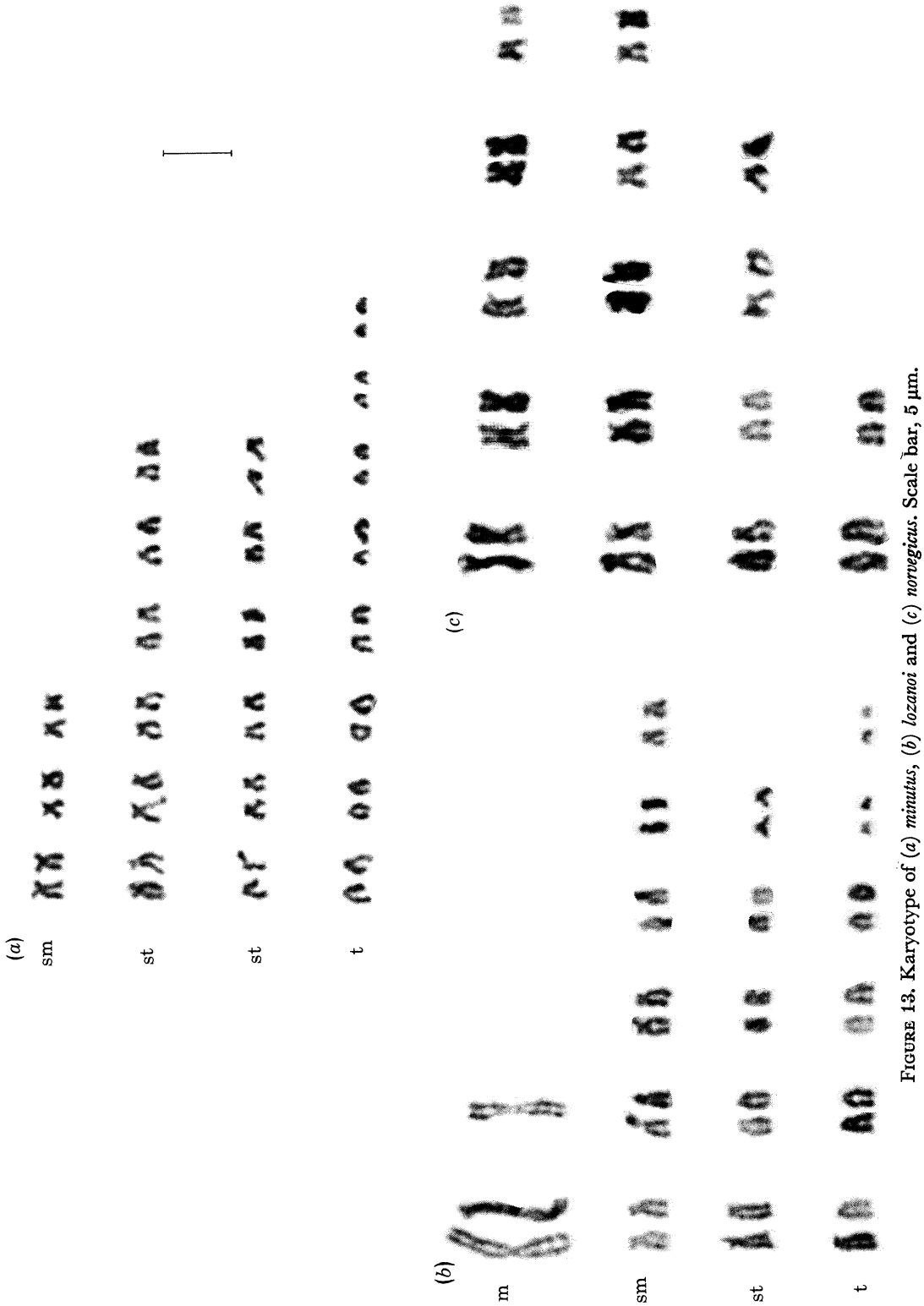

FIGURE 13. Karyotype of (*a*) *minutus*, (*b*) *lozanoi* and (*c*) *norvegicus*. Scale bar, 5 μm.

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was no heteromorphy between sexes. The karyotype (figure 13*a*) consisted of three pairs of submetacentric, twelve pairs of subtelocentric and eight pairs of telocentric chromosomes, giving an N.F. of 52. Meiotic figures contained 23 bivalents at metaphase I, the vast majority of which were short and rod-like (figure 14*a*). None of them appeared to be asymmetric and heteropycnotic bodies were not apparent in meiotic prophase nuclei.

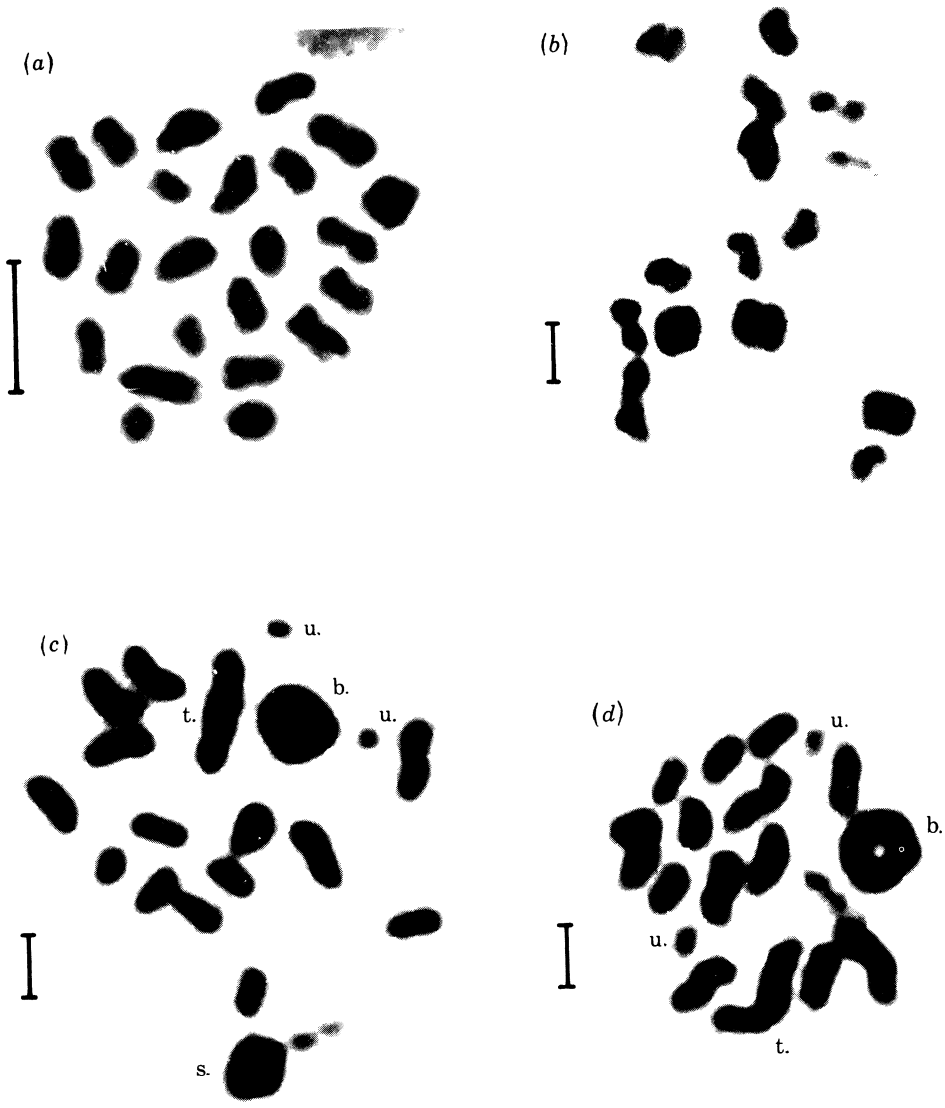

FIGURE 14. Metaphase I of male meiosis in (*a*) *minutus*, (*b*) *norvegicus*, (*c*), (*d*) *lozanoi*; scale bars 2 μm; b., large ring bivalent; t., trivalent association; u., univalent; s., sperm head.

(*b*) *P. lozanoi*. All specimens analysed had a modal diploid number of 37 chromosomes. Intra- or inter-individual variation in chromosome morphology was not recorded and there was no heteromorphy between sexes. The karyotype (figure 13*b*) consisted of one large pair of metacentrics, six pairs of submetacentrics (one of which usually had satellites), five pairs of subtelocentrics, six pairs of telocentrics and one large unpaired metacentric chromosome, giving an N.F. of 52. Metaphase I of meiosis regularly contained 16 bivalents (15 rods and one large ring bivalent), two univalents and one trivalent (figure 14*c*, *d*). None of the bivalents was noticeably asymmetric and heteropycnotic bodies were absent at pachytene of meiosis.

(*c*) *P. norvegicus*. All specimens investigated had a modal diploid number of 32 chromosomes. Intra- or inter-individual variation in chromosome morphology was not observed and there was no heteromorphy between the sexes. The karyotype (figure 13*c*) consisted of five pairs of metacentric, five pairs of submetacentric, four pairs of subtelocentric and two pairs of telocentric chromosomes, giving an N.F. of 52. Meiotic figures contained 16 bivalents at metaphase I, of which four or five were ring type and the remainder rod type associations. None of the bivalents was noticeably asymmetric and heteropycnotic bodies were not observed in pachytene nuclei.

4.3.2. *Relative* DNA *content*

There was no significant difference between the Feulgen stain content of erythrocyte nuclei from male and female *minutus*, *lozanoi* and *norvegicus* (figure 15), which indicates that they have a similar amount of DNA.

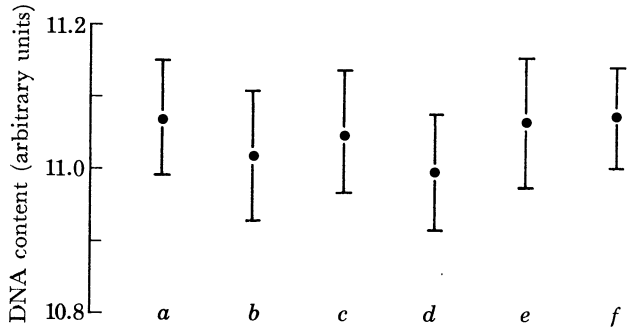

FIGURE 15. Relative mean DNA content (± 2 s.e.) of erythrocytic nuclei of (*a*) ♂ *minutus*, (*b*) ♀ *minutus*, (*c*) ♂ *lozanoi*, (*d*) ♀ *lozanoi*, (*e*) ♂ *norvegicus*, (*f*) ♀ *norvegicus*.

4.4. *Discussion*

The occurrence of an unpaired metacentric chromosome in the karyotype of *lozanoi* and the observation of a trivalent association at first metaphase of meiosis in males indicates that the individuals examined were heterozygous for a Robertsonian transformation, a type of chromosomal rearrangement by which two non-homologous rod-shaped elements are derived by fission from, or give rise by fusion to, a metacentric chromosome (see, for example, White 1973). Proposed mechanisms of Robertsonian change depend on the type of rod-shaped element involved in the rearrangement (White 1957; John & Hewitt 1966, 1968). The arms of the unpaired metacentric chromosome in the *lozanoi* karyotype appear, on the basis of size, to be homologous with either the two largest telocentrics or the two largest subtelocentric elements. However, in the absence of more detailed evidence of homology it is impossible to determine which of these rod-shaped chromosomes have been involved in the transformation and therefore by which mechanism the Robertsonian event has occurred.

Whatever the mechanism, the resulting rearrangement has obviously not reached fixation, so that, as well as the observed heterozygotes, *P. lozanoi* probably embraces individuals homozygous for the structural change and others in which rearranged elements are absent (basic homozygotes). There is evidence that Robertsonian polymorphism is maintained by heterozygote advantage (Ford & Hamerton 1970) and it is perhaps not surprising that structural and basic homozygotes were not encountered in the small sample of *lozanoi*.

Unpaired metacentric chromosomes have also been observed in the gobiines *Gobiodon citrinus*,

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Gobius paganellus and *Padogobius martensi* by Arai & Sawada (1974) and Cataudella *et al.* (1973), who interpreted them as sex chromosomes. The unpaired metacentric of *lozanoi* cannot be a sex element since it is present in both sexes. In fact, there was no evidence for the occurrence of heteromorphic chromosomes associated with sex in *minutus*, *lozanoi* or *norvegicus*. In the past, sex chromosomes in fishes were regarded as exceptional but there have been sufficient recent reports to suggest that in some groups overt karyological heterogamety is a widespread phenomenon. However, in few reported cases has analysis of ovarian metaphase, a necessary procedure for the definitive identification of a heteromorphic pair as sex elements, been possible (Denton 1973). The occurrence of sex chromosomes has often been inferred solely on the inadequate basis of mitotic evidence. This is so for the gobiines and, until meiotic analyses have been performed, the status of their heteromorphic pairs as sex elements must remain provisional.

The absence of recognizable sex elements appears to be one of the few karyological features shared by members of the *minutus* complex, within which differences in the diploid numbers exceed those previously observed between species of any gobioid genus, with the possible exception of *Odontobutis* if Nogusa's estimate (1955, 1960) is correct. It may be noted, however, that the range of chromosome number in the *minutus* complex is less spectacular than that recorded between or even within species of certain teleosts (see, for example, Booke 1968).

Although the chromosome complements of *minutus*, *lozanoi* and *norvegicus* are different, all have a similar amount of DNA and the same number of major chromosome arms. This suggests (Matthey 1973) that karyotypic differences in the *minutus* complex are, at least in part, the result of Robertsonian chromosomal rearrangement. Robertsonian transformation, particularly fusion, has often been invoked to explain differences in the karyotypes of related taxa and it seems likely that fusion has been involved in the karyotypic evolution of the *minutus* complex since *lozanoi* and *norvegicus* have the lowest diploid numbers so far recorded in the whole of the Gobioidea.

It has been argued (Matthey 1973; White 1973) that, unlike some other types of chromosomal rearrangement, Robertsonian transformation cannot promote reproductive isolation because the trivalent associations formed in Robertsonian heterozygotes are preadapted for regular segregation and that such individuals suffer no loss of viability or fertility on meiotic grounds. However, trivalents do not always disjoin in a regular manner and the fecundity of some Robertsonian heterozygotes is undoubtedly reduced, especially where the degree of heterozygosity is large (Capanna *et al.* 1976). As hybrids within the *minutus* complex will be heterozygous for several Robertsonian changes, the fertility of such individuals may be impaired and in consequence gene exchange between the taxa restricted.

5. Geographical and ecological studies

In this section earlier work is summarized and linked with field observations made in the Plymouth area. Data are presented as follows: (*a*) general statement of geographical distribution in terms of faunistic regions defined by Ekman (1953); (*b*) European distribution and (*c*) ecological distribution, including breeding habitats.

P. minutus

Black Sea – Mediterranean – Lusitanian – Boreal – Baltic Sea. In the Atlantic, *minutus* extends from near Tromso, Norway, at *ca.* 69° N (Johnsen 1936) and the Faeroes (Tåning 1940) to Brittany and northwestern Spain (Duncker 1928). It also occurs in the Baltic, to the southern

Gulf of Bothnia and the Gulf of Finland (Lawacz 1965). Although thought by some (see, for example, Fage 1918) to be absent from the Mediterranean, *minutus* has been clearly identified on the southeast coast of Spain (De Buen 1923) and in the northern Adriatic (Cavinato 1952) and has been reported from the Black Sea by Slastenenko (1939). It is generally distributed around the British Isles.

P. minutus characteristically inhabits sandy grounds from low water to about 30 m (Miller 1961 *a*) but can be found in deeper water at certain times of the year (Jones & Miller 1966). In the Plymouth area, for example, it was taken at depths of 45–50 m from the offshore Rame Mud fishing grounds during autumn and winter months of 1972–1974. It is rarely found in shore pools (Boulenger 1911; Miller 1963), but regularly occurs in the channels of larger estuaries and rias, where adults are usually restricted to lower parts (Hartley 1940; Healey 1971), but juveniles, which are less stenohaline, may penetrate into higher reaches (Miller 1963; Lee 1974). In the Plymouth area, adults were common in the lower part of the Tamar estuary (Neal Point and St John's Lake) during late summer and autumn 1972–1974, while a trawling survey of the Tamar made from April 1973 to May 1974 by Dr P. Dando of the Marine Biological Association indicated that young penetrate as far as 25 km upstream, where bottom salinity is less than 5‰ (by mass).

Individual *minutus* spawn several times during their protracted season, which generally extends from spring to early summer, although its precise onset and duration vary according to locality (see review by Russell (1976)). Healey (1971) demonstrated that eggs survived best in laboratory experiments at salinities of 10–25‰ (by mass), the range prevailing in estuaries. However, nests, which are usually constructed from a lamellibranch shell (Petersen 1892; Lebour 1920; Reese 1964), have been found in coastal areas under conditions of high salinity at depths of 0.3–25 m (Petersen 1892; Kinzer 1960; Fonds 1973). During this study, ripe fish were obtained in spring and summer from coastal areas (Cawsand and Whitsand Bay) at depths of 10–30 m.

P. lozanoi

Lusitanian – Boreal. The most northerly record is from Kames Bay, near Millport, Scotland, at *ca.* 56° N, and records extend south to the French Channel coast and northwest Spain (Le Danois 1913; De Buen 1923; Cantacusene 1956). There are no Mediterranean, Baltic or Belt Sea records and *lozanoi* has an erratic distribution around the British Isles.

Fonds (1973) demonstrated experimentally that *lozanoi* is more stenohaline than *minutus* and suggested that it is less adapted to estuarine conditions and also is more neritic in habit. Fish attributable to *lozanoi* have been reported from a tidal reservoir in the Severn Estuary (Williams 1977), but *lozanoi* seems usually to occur at the mouth of estuaries or in inshore coastal waters, where it can be found in mixed populations with *minutus* (Le Danois 1913; De Buen 1923; Fonds 1973).

Fonds (1973) suggested that, at least in the Dutch North Sea, *lozanoi* starts spawning later than does *minutus*. However, his data show that the breeding season, which extended from May to August, overlapped that of *minutus*. He found that Dutch *lozanoi* and *minutus* had the same spawning ground, situated at depths of 10–25 m in the coastal area off Texel, but that there was a tendency for *lozanoi* to select for nest building shells of lamellibranch species different from those of shells used by *minutus*, although some of these shells (e.g. that of *Cardium echinatum*) may be used by *minutus* elsewhere (see Lebour 1920).

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P. norvegicus

Mediterranean – Lusitanian – Boreal, from the Lofotens, Norway, at 68° N (Collett 1903) to the Aegean (Fage 1918). It occurs in the Skagerrak but not in the Belt Seas or the Baltic (Duncker 1928). Full records and details of the entire European distribution are given by Webb & Miller (1975).

Lebour (1919) remarked that *norvegicus* appears to take the place of *minutus* in deeper waters off Plymouth. It has usually been obtained from relatively deep water but can be found at lesser depths and has been recorded from 6.3–325 m on mud, muddy sand and coarse shell gravel (see Webb & Miller 1975).

Clark (1920) recorded postlarvae off Plymouth in July, August and September. Young stages were found to be common at 42–66 m, also off Plymouth, by Lebour (1919) and, although eggs have not been described, Miller (1963) suggested that breeding occurs offshore. In this study, ripe specimens were obtained from the Rame Mud fishing grounds off Plymouth at depths of 45–55 m during spring and early summer, but occurred simultaneously at 18–20 m in Whitsand Bay, Plymouth, together with ripe specimens of *minutus*.

Hybrids

Putative *lozanoi–norvegicus* hybrids have been recorded from the Clyde Sea area and the Dutch North Sea, and *lozanoi–minutus* hybrids occurred at Oxwich Bay near Swansea, Oldbury-on-Severn, and the Dutch North Sea.

The geographical ranges of *minutus*, *lozanoi* and *norvegicus* overlap to a considerable extent. While the known distribution of *lozanoi* is centred in the more northern part of the overall ranges of *minutus* and *norvegicus*, future investigation will probably reveal that it also extends into southern regions including the Mediterranean. Within the area of overlap the three taxa appear to occupy somewhat different ecological niches. However, their breeding distribution is such that mature individuals may encounter each other and they must therefore be regarded as being truly sympatric.

6. Discussion

Clarification of the systematic relationships of the *P. minutus* complex largely depends on a determination of the status of *lozanoi*. This investigation has indicated that, while it is morphologically intermediate between *minutus* and *norvegicus*, it has a degree of genetic identity and is clearly not a simple hybrid between them. In fact it seems reasonable, on phenetic grounds, to regard *lozanoi*, *minutus* and *norvegicus* as separate species since they display morphological, biochemical and karyological differences comparable to those defining species in *Pomatoschistus* and other gobiid genera.

Another criterion of specific status is reproductive isolation. Isolating mechanisms have usually been classified into pre-mating mechanisms which prevent interspecific crosses and post-mating mechanisms which reduce the success of interspecific crosses. Pre-mating mechanisms, which include temporal, habitat, mechanical and ethological isolation, are obviously not completely efficient in isolating all the *minutus* complex taxa since *minutus* and *lozanoi* clearly cross in the wild and there is evidence that *lozanoi* and *norvegicus* also hybridize. However, the rarity of F_1 hybrids (less than 1 % of the total population sampled and from 2.5–3.5 % of local populations investigated) suggests that mechanisms operate to restrict interbreeding. Crossing

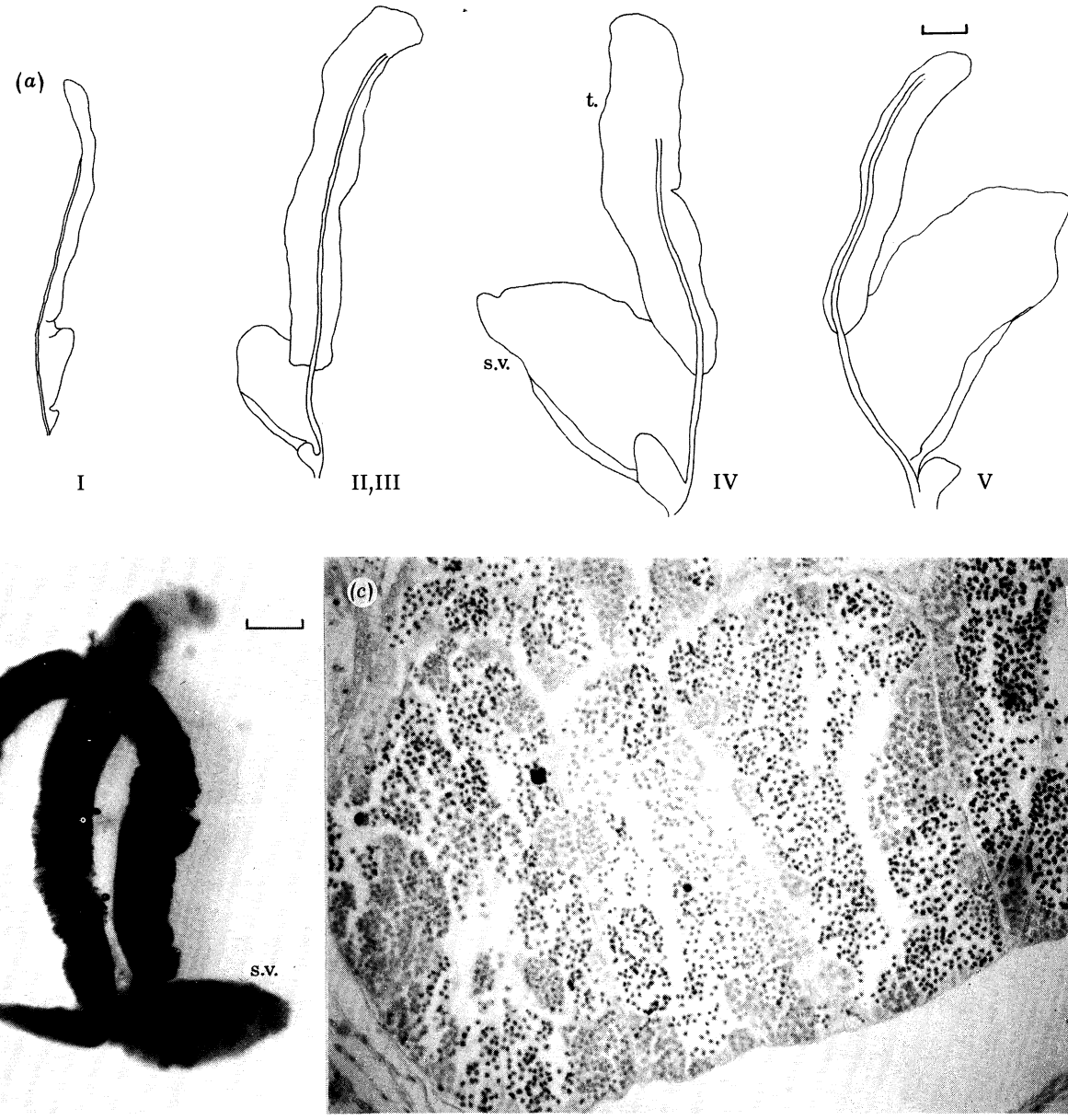

FIGURE 16. (*a*) Maturity stages in male genitalia of *minutus* (after P. J. Miller, unpublished). I immature; II, III ripening and maturing; IV ripe; V spent; scale bar 1 mm. (*b*) Genitalia of male *minutus–lozanoi* hybrid, s.l. 46.7 mm, from Dutch North Sea, February 1974; scale bar 1 mm. (*c*) Section of testis from above hybrid; scale bar 20 μm. Abbreviations: s.v., seminal vesicle; t., testis.

demands the coexistence of forms in breeding condition and Fonds (1973) and Williams (1977) have claimed that different breeding seasons prevent hybridization between *minutus* and *lozanoi*. However, information available on the onset, duration and place of spawning of *minutus*, *lozanoi* and *norvegicus* (see §5) suggests that neither temporal nor habitat isolation effectively isolates any of these taxa.

If two forms are sympatric and in breeding condition, successful crossing still depends on their mutual ability to participate in reproductive activity, and ethological isolation is often the most important mechanism preventing interbreeding (Mayr 1963). Gobiid fishes have a

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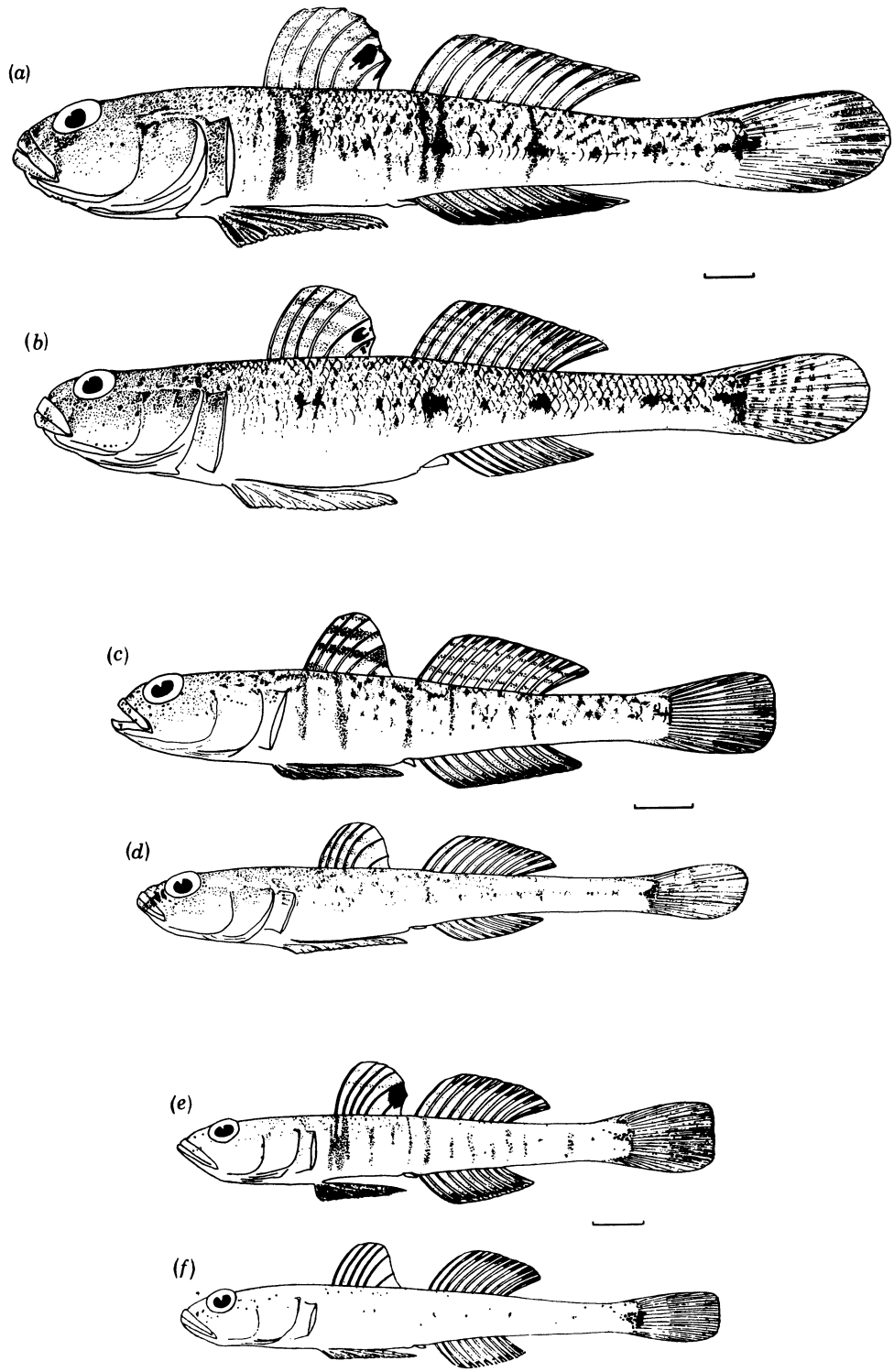

FIGURE 17. (*a*) *P. minutus*, male, from St Andrews, Scotland, March 1974; (*b*) *P. minutus*, female, from St Andrews, Scotland, March 1974; (*c*) *P. lozanoi*, male, from Dutch North Sea, March 1974; (*d*) *P. lozanoi*, female, from Oxwich Bay, Swansea, June 1974; (*e*) *P. norvegicus*, male, from Plymouth, March 1974; (*f*) *P. norvegicus*, female, from Plymouth, March 1974. Scale bars 5 mm. (*a–d*) After P. J. Miller, unpublished; (*e*), (*f*) after Webb & Miller (1975).

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complicated courtship behaviour (see, for example, Kinzer 1960) and the coloration of breeding males, which is usually species-specific, is probably important in this respect. It is therefore of some significance that the nuptial coloration of *minutus*, *lozanoi* and *norvegicus* males is different (see §7) and these differences may be the basis of specific recognition.

Post-mating mechanisms include gametic and zygotic mortality, hybrid inviability and sterility. Fonds (1970, 1971, 1973) demonstrated that eggs from *minutus–lozanoi* hybrids develop normally and show the same sort of mortality as eggs from parental crosses reared under similar conditions. This result, coupled with observation of natural adult *minutus–lozanoi* and putative *lozanoi–norvegicus* hybrids, suggests that embryonic mortality and hybrid inviability are not effective in isolating these pairs of taxa. The external appearance of gonads from *minutus–lozanoi* and putative *lozanoi–norvegicus* hybrids is similar to that of gonads from parental forms of equivalent maturity, and sections of gonads from hybrids reveal gametes (figure 16). However, because of differences in the chromosome complements of *minutus*, *lozanoi* and *norvegicus*, meiosis in hybrids between them is probably irregular (see §4) and reduced hybrid fertility may be an important isolating mechanism in this complex. Certainly there is no evidence of backcrossing of hybrids or suspected hybrids and it seems that *lozanoi*, *minutus* and *norvegicus* are all effectively isolated and deserve specific status on reproductive criteria.

7. Diagnosis of species in the *P. minutus* complex

Pomatoschistus species with villose anterior pelvic membrane; branchiostegal membrane not attached to more than half of lateral margin of isthmus; caudal peduncle slender and caudal fin short; sensory papillae row *a* with several to many transverse rows; at least seven transverse *c* rows; *n* to near dorsal midline, median to *g*; *trp* to *m*; *as* to *h*; scales to above opercle; nuptial warts on inner surface of pectoral fin.

Pomatoschistus minutus (Pallas 1770)

Background coloration sandy or grey with fine reticulation and ferruginous specks, males with four transverse bars and breast usually unpigmented (figures 17, 18); D1 spot present and not reaching edge of membrane in immature and ripe specimens of both sexes; sensory papillae row *b* usually long, ending under front part of orbit; 9–12 (range 8–12) transverse *c* rows, of which usually only *cp* extends below row *d* although 1–2 more anterior rows may penetrate a short distance; *d′* and anterior *i* are double; *m* and *g* separate; maximum size 110 mm; branchiostegal membrane attached to about half of lateral isthmus; breast usually scaled; LL 55–75; D2 I/9–12; A I/9–12; P 18–21; vertebrae 33 (range 32–34); meristic index (see §2) 49–84; monomorphic for allele A^1 at creatine kinase locus *A*; monomorphic for allele A^2 at phosphoglucose isomerase locus *A*; $2n = 46$; habitat inshore and estuarine, typically on sand; Black Sea – Mediterranean – Lusitanian – Boreal – Baltic Sea. Synonymies in Miller (1973*b*) and Tortonese & Hureau (1979).

Pomatoschistus lozanoi (De Buen 1923)

Background coloration brown, marbled with fine reticulation and irregularly dispersed orange spots, males with 7–9 lateral stripes and usually unpigmented breast (figures 17, 18); D1 spot absent in females and present only in ripening males, where it reaches the edge of the membrane; sensory papillae row *b* long, ending under the front part of the orbit; 9–10 (range

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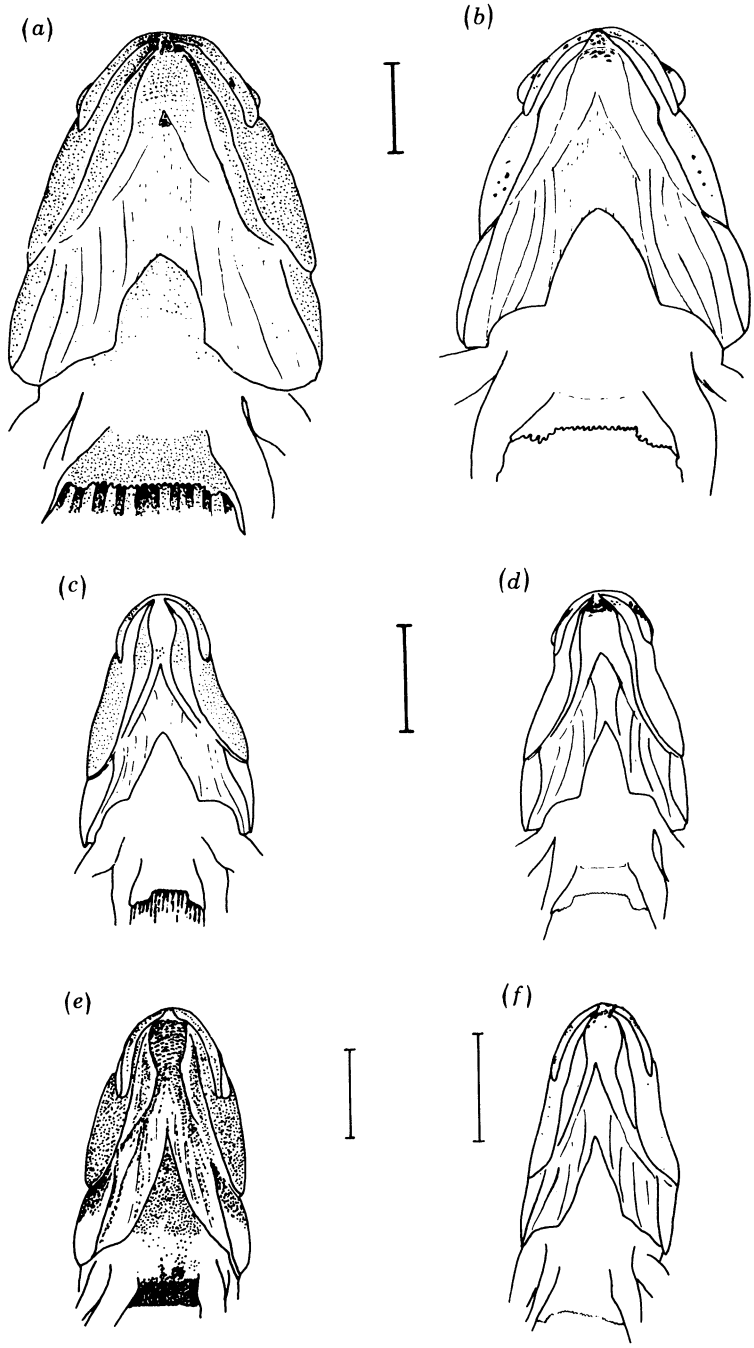

FIGURE 18. Ventral pigmentation of head and breast region in (*a*) *P. minutus*, male, from St Andrews, Scotland, March 1974; (*b*) *P. minutus*, female, from St Andrews, Scotland, March 1974; (*c*) *P. lozanoi*, male, from Dutch North Sea, March 1974; (*d*) *P. lozanoi*, female, from Oxwich Bay, Swansea, June 1974; (*e*) *P. norvegicus*, male, from Plymouth, March 1974; (*f*) *P. norvegicus*, female, from Plymouth, March 1974. Scale bars 5 mm. After P. J. Miller, unpublished.

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7–11) transverse *c* rows, usually with second, fourth and last all extending below row *d*; *d'* and anterior *i* doubled; *m* and *g* separate, maximum size 80 mm; branchiostegal membrane attached to one half to one quarter of lateral isthmus; breast scaled; LL 57–65; D2 I/9–12; A I/9–12; P 18–21; vertebrae 32 (range 30–33); meristic index 33–67; monomorphic for allele A^2 at creatine kinase locus *A*; monomorphic for allele A^1 at phosphoglucose isomerase locus *A*; $2n = 36–38$; habitat inshore, and estuarine rarely; northern Lusitanean and Boreal. Synonymies in Tortonese & Hureau (1979).

Pomatoschistus norvegicus (Collett 1903)

Background coloration pale fawn or translucent with faint reticulation and scattered orange dots, males with 10–12, mostly thin, vertical dark striae and pigmented breast (figures 17, 18). D1 spot present in maturing fish of both sexes; sensory papillae row *b* short, ending anteriorly under rear half of orbit; 7–8 (range 7–9) transverse *c* rows, usually with second, fourth and last all extending below level of *d*, *d'* and anterior part of *i* usually single, *m* and *g* fused; maximum size 63 mm; branchiostegal membrane attached to not more than one quarter of lateral isthmus; breast usually naked; LL 55–60; D2 I/8–10; A I/8–11; P 16–18; vertebrae 32 (range 30–33); meristic index 5–38; monomorphic for allele A^1 at creatine kinase locus *A*; monomorphic for allele A^1 at phosphoglucose isomerase locus *A*; $2n = 32$; habitat offshore; Mediterranean – Lusitanian – Boreal. Synonymies in Webb & Miller (1975).

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Appendix 1. Sex, size range and source of specimens used for morphological studies

	♀♀	♂♂	standard length mm	supplied by
Dunstaffnage Bay, Oban	77	59	29.0–70.0	R.N. Gibson
Millport	21	6	21.0–62.0	P.J. Miller
Port Erin, Isle of Man	21	17	48.5–59.0	P.J. Miller
Oxwich Bay, Swansea	38	—	35.8–62.9	J. Rylands
Oldbury-on-Severn	22	22	36.8–57.8	I.C. Potter
Plymouth	143	126	21.9–71.5	C.J. Webb
Burnham-on-Crouch	18	42	41.8–68.5	A. Howard
Mweenish Bay, Co. Galway	1	1	51.7–53.5	J. Rylands
Dutch North Sea	149	94	26.0–59.8	M. Fonds

Appendix 2. Sex, size range and source of specimens used for biochemical studies

	♀♀	♂♂	standard length mm
myogens/CK			
minutus			
Oldbury-on-Severn†	14	9	40.6–60.8
Plymouth	66	40	21.9–69.3
Burnham-on-Crouch‡	17	16	40.4–68.5
Dutch North Sea§	1	—	32.0
lozanoi			
Oldbury-on-Severn†	29	34	33.9–52.5
Burnham-on-Crouch‡	1	13	41.8–48.7
Dutch North Sea§	10	7	30.5–52.0
norvegicus			
Dunstaffnage Bay‖	5	18	42.0–52.7
Plymouth	134	27	25.9–49.3
min.-loz. hybrids			
Oldbury-on-Severn†	1	1	46.0–46.4
Dutch North Sea§	3	1	40.0–47.0
PGI			
minutus			
Oldbury-on-Severn†	13	9	40.6–60.8
Plymouth	20	11	45.1–69.3
Burnham-on-Crouch‡	13	12	40.4–66.1
lozanoi			
Oldbury-on-Severn†	24	29	34.1–52.5
Burnham-on-Crouch‡	1	—	47.6
Dutch North Sea§	—	2	39.5–48.0
norvegicus			
Dunstaffnage Bay‖	3	12	42.0–52.7
Plymouth	24	14	35.9–48.5
min.-loz. hybrids			
Oldbury-on-Severn†	1	1	46.0–46.4
Dutch North Sea§	1	1	46.7–47.0

For footnote, see facing page.

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APPENDIX 2 (*cont.*)

LDH			
minutus			
Plymouth	12	11	52.2–66.1
Burnham-on-Crouch‡	3	2	54.3–63.5
Dutch North Sea§	1	—	32.0
lozanoi			
Oldbury-on-Severn†	6	4	33.9–52.7
Burnham-on-Crouch‡	—	2	44.1–46.4
Dutch North Sea§	6	5	35.0–52.0
norvegicus			
Plymouth	56	10	29.6–47.0
min.-loz. hybrids			
Dutch North Sea§	2	1	45.5–46.7
haemoglobin			
minutus			
Oldbury-on-Severn†	1	—	48.7
Plymouth	29	33	30.2–71.5
Burnham-on-Crouch‡	16	23	42.2–68.5
lozanoi			
Oldbury-on-Severn†	1	3	42.1–50.0
Burnham-on-Crouch‡	—	8	42.8–49.0
Dutch North Sea§	13	12	34.0–50.5
norvegicus			
Plymouth	19	14	38.0–48.5

† Supplied by I. C. Potter; ‡ supplied by A. Howard; § supplied by M. Fonds; ‖ supplied by R. N. Gibson.

APPENDIX 3. SEX, SIZE AND SOURCE OF SPECIMENS USED FOR KARYOLOGICAL STUDIES

	sex	standard length mm	source
minutus	♀	54.3	River Tamar, Plymouth, December 1972
	♂	51.1	River Tamar, Plymouth, December 1972
	♂	57.3	Cawsand Bay, Plymouth, December 1972
	♂	58.9	Cawsand Bay, Plymouth, December 1972
	♂	60.0	River Tamar, Plymouth, November 1974
lozanoi†	♀	39.9	Dutch North Sea, October 1973
	♀	40.0	Dutch North Sea, February 1974
	♂	39.8	Dutch North Sea, October 1973
	♂	38.5	Dutch North Sea, February 1974
	♂	41.0	Dutch North Sea, February 1974
	♂	42.0	Dutch North Sea, February 1974
	♂	42.5	Dutch North Sea, February 1974
	♂	47.5	Dutch North Sea, February 1974
norvegicus	♀	48.5	Eddystone, Plymouth, February 1975
	♂	43.4	Rame Mud, Plymouth, March 1974
	♂	48.0	Rame Mud, Plymouth, January 1974
	♂	48.5	Rame Mud, Plymouth, January 1974
	♂	43.5	Rame Mud, Plymouth, February 1975

† Supplied by M. Fonds.

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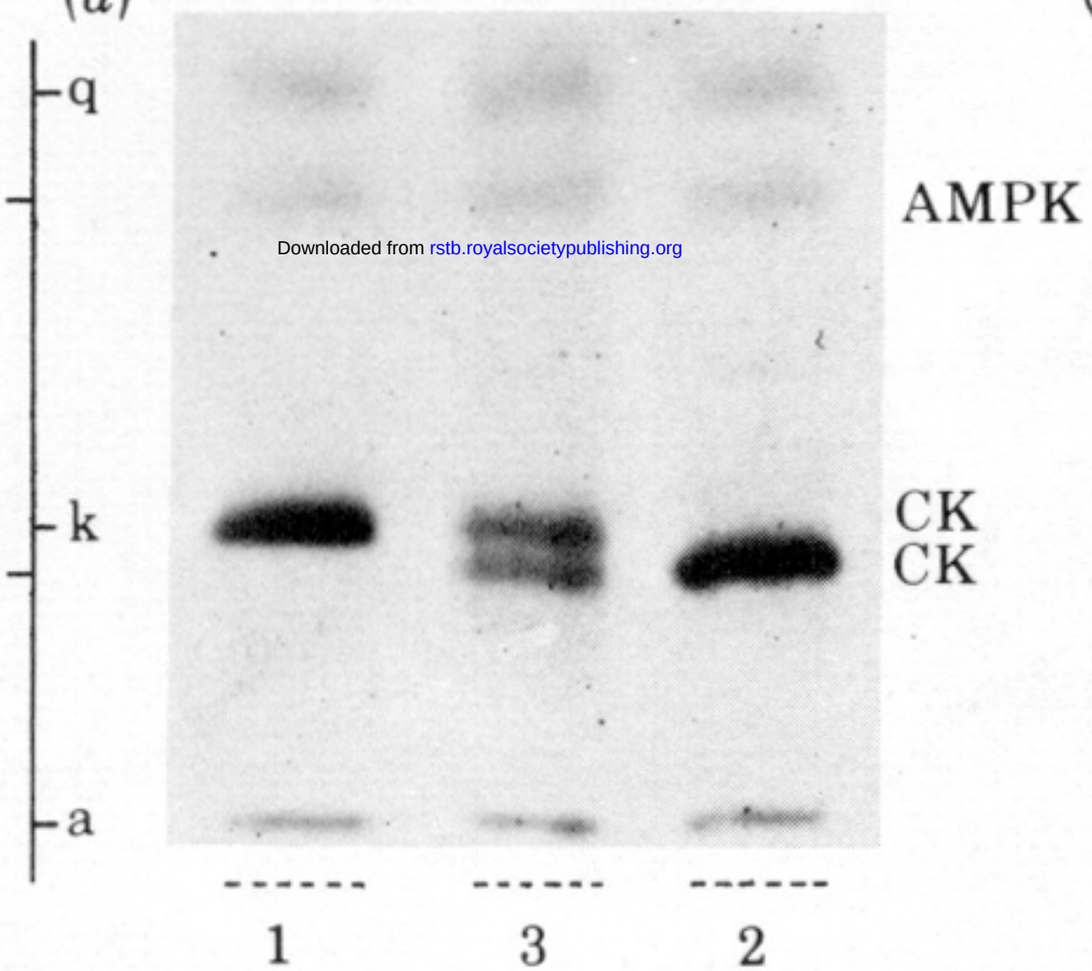

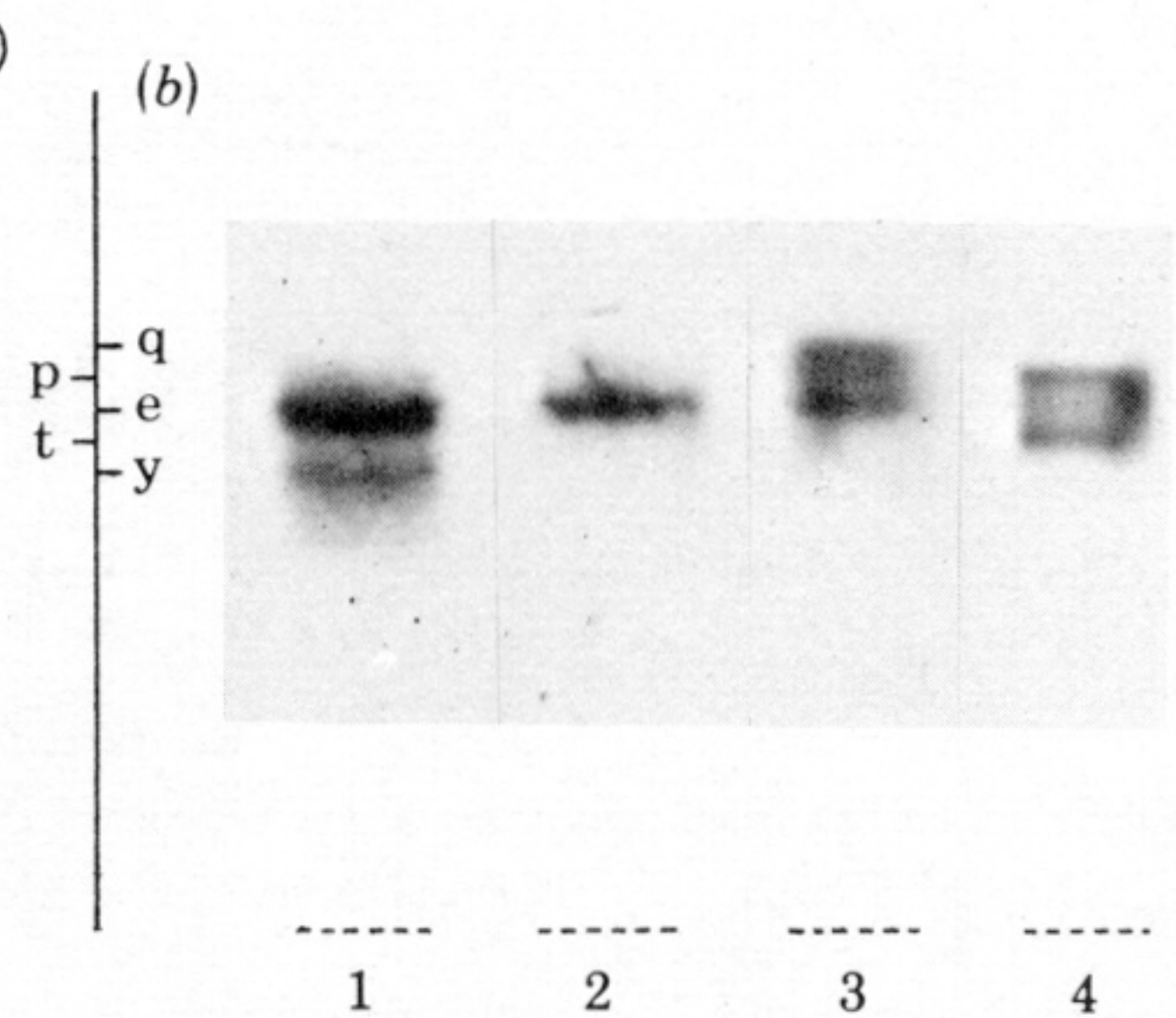

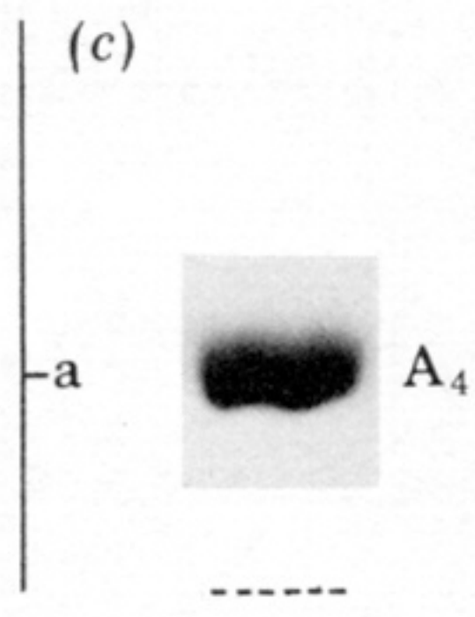

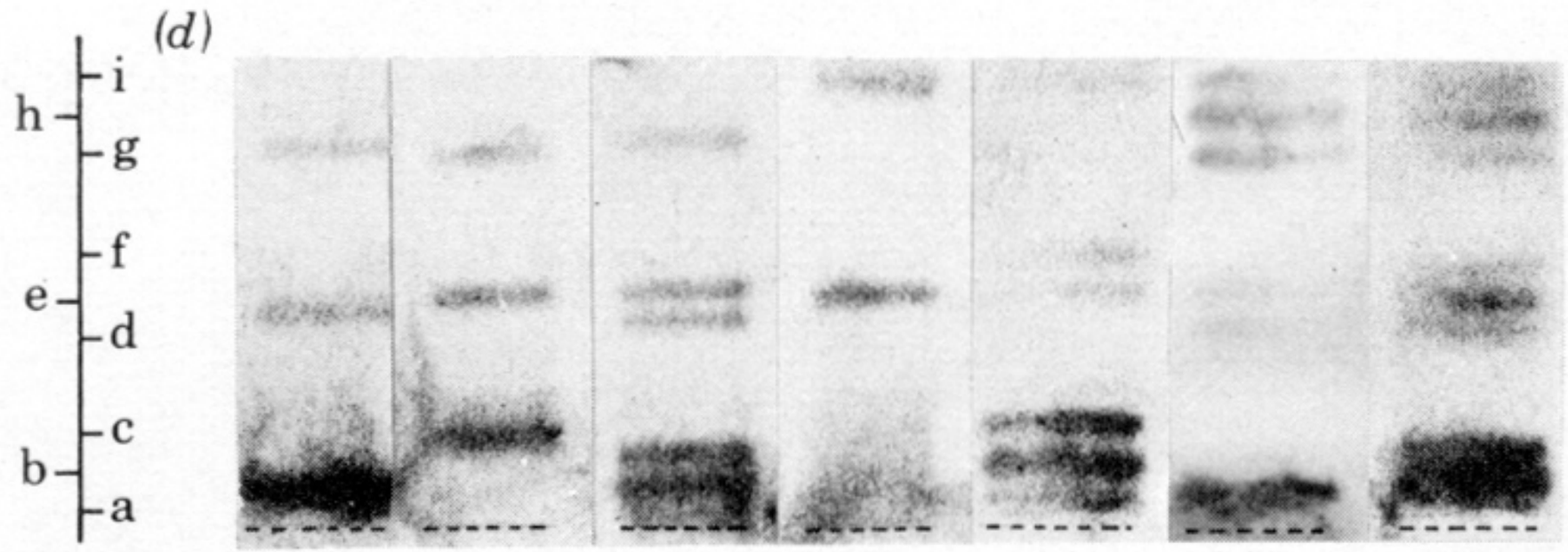

FIGURE 8. Electrophoretic phenotypes of (*a*) muscle myogens, (*b*) haemoglobin, (*c*) lactate dehydrogenase, (*d*) phosphoglucose isomerase.

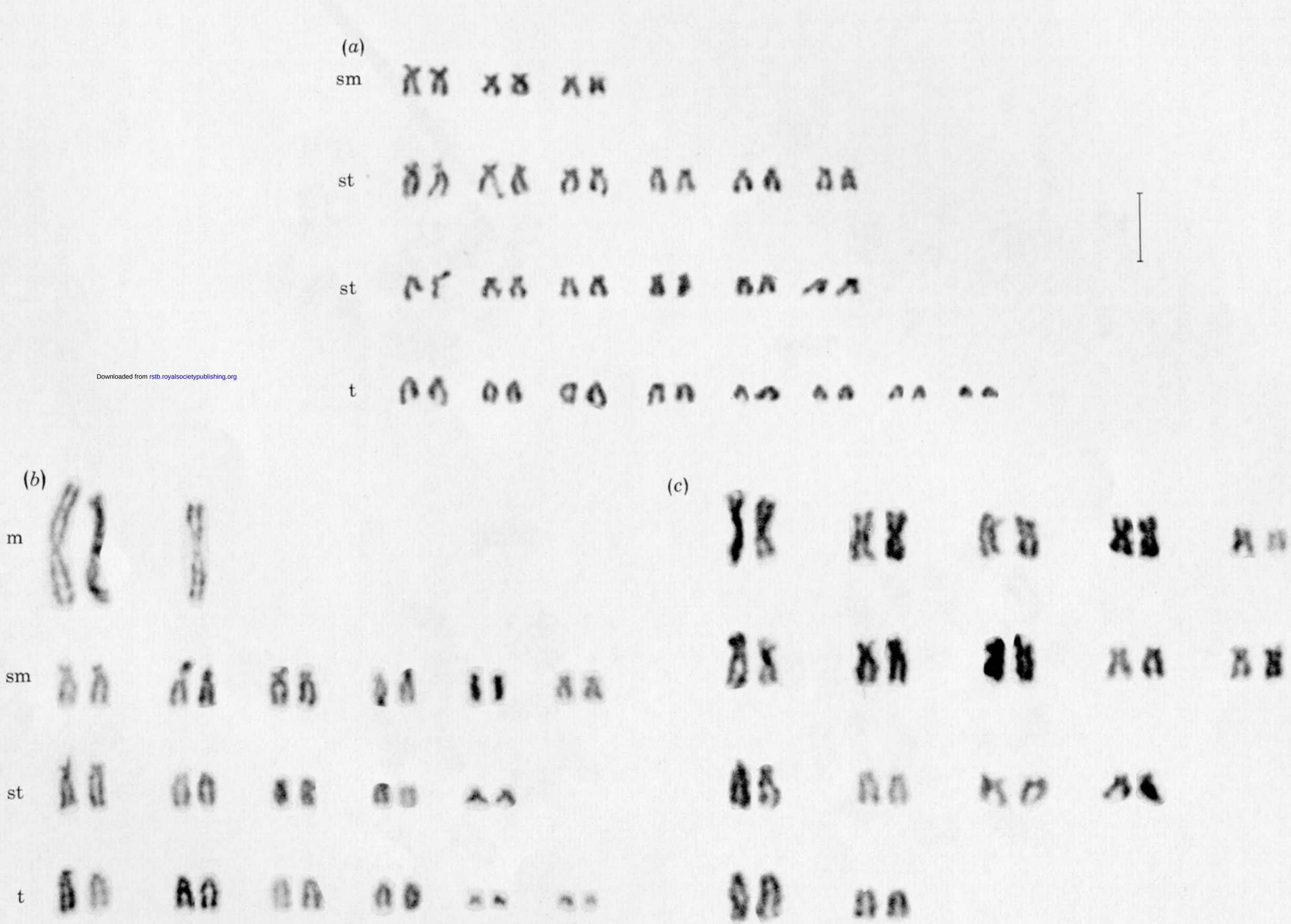

FIGURE 13. Karyotype of (*a*) *minutus*, (*b*) *lozanoi* and (*c*) *norvegicus*. Scale bar, 5 µm.

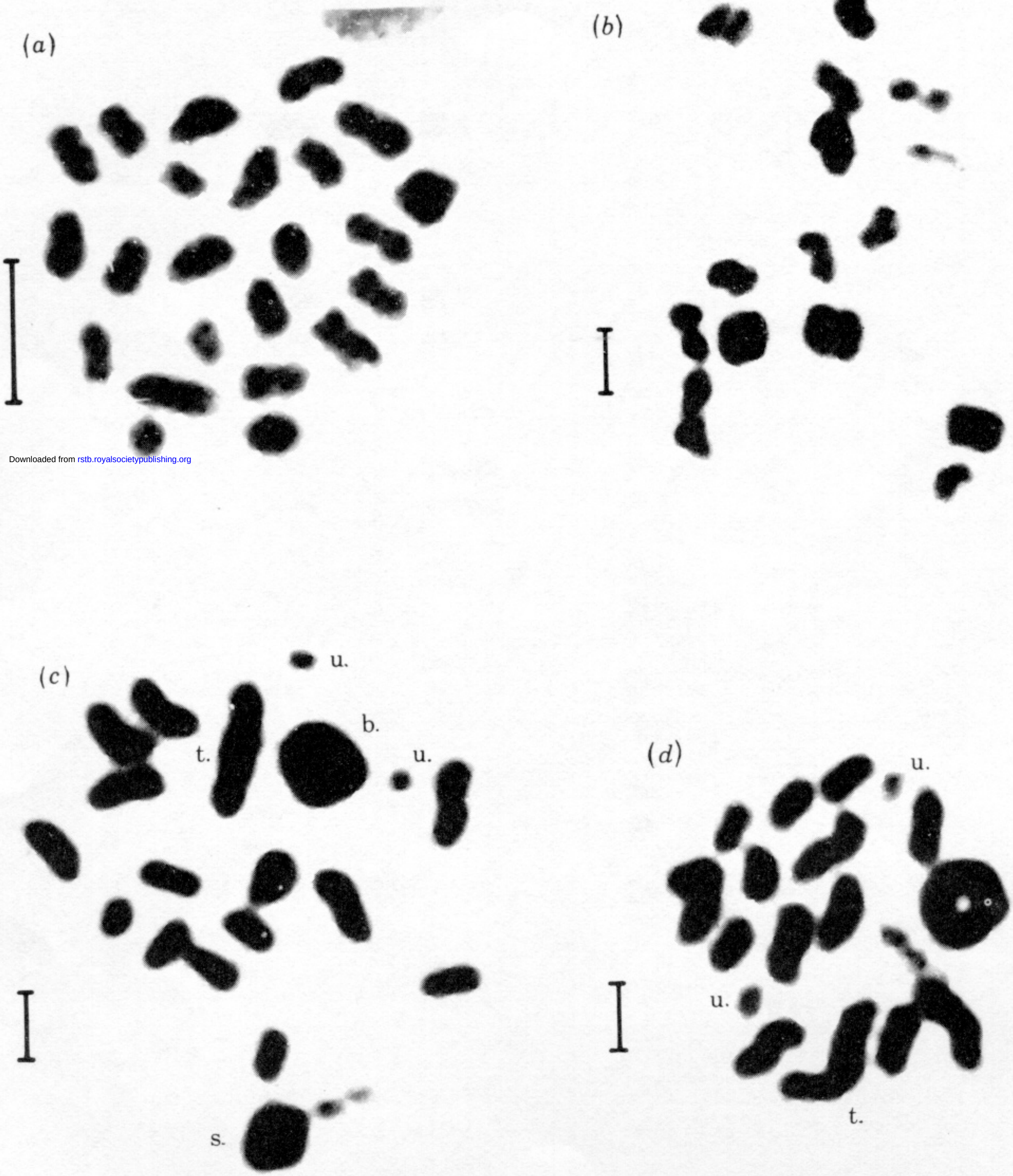

FIGURE 14. Metaphase I of male meiosis in (*a*) *minutus*, (*b*) *norvegicus*, (*c*), (*d*) *lozanoi*; scale bars 2 μm; b., large ring bivalent; t., trivalent association; u., univalent; s., sperm head.

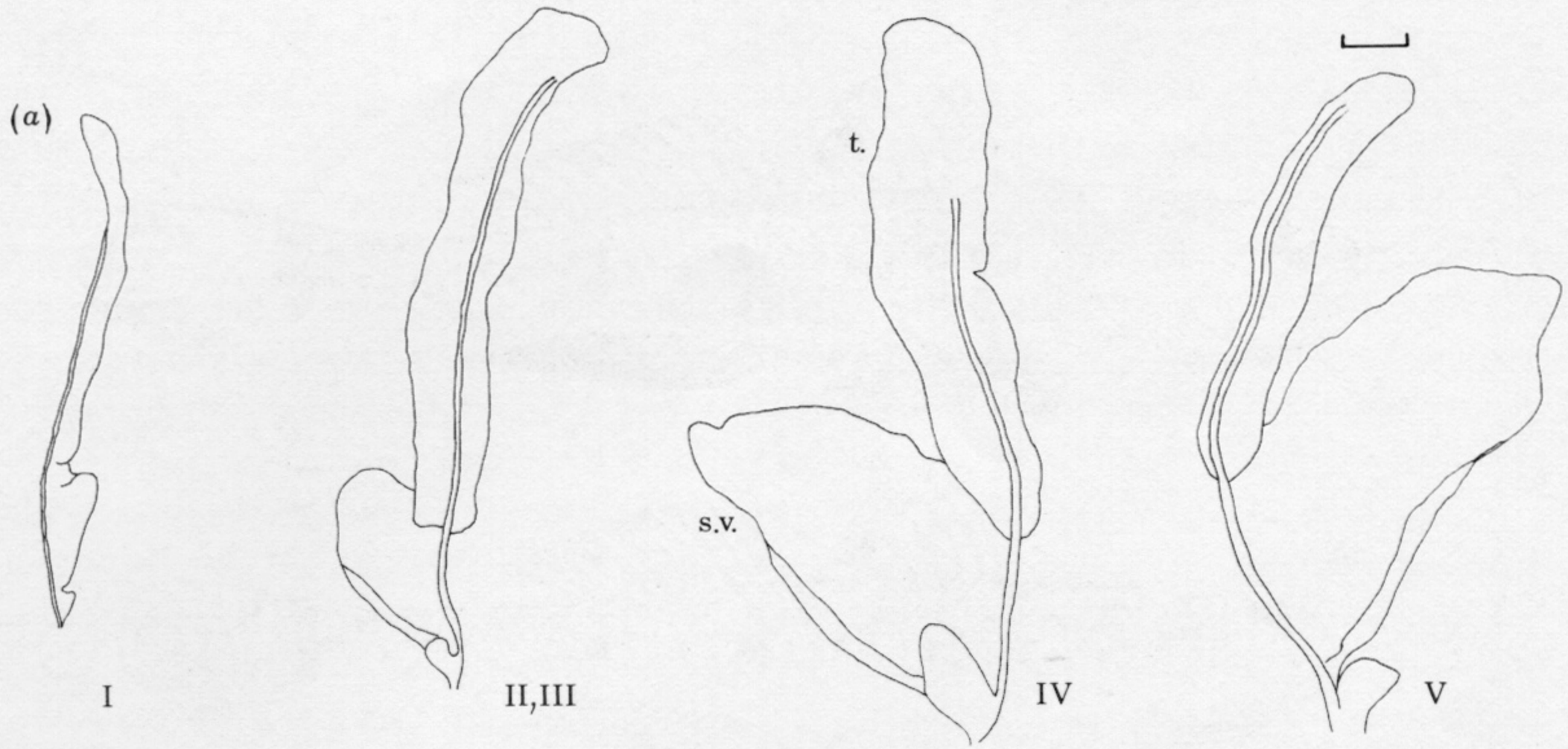

FIGURE 16. (*a*) Maturity stages in male genitalia of *minutus* (after P. J. Miller, unpublished). I immature; II, III ripening and maturing; IV ripe; V spent; scale bar 1 mm. (*b*) Genitalia of male *minutus–lozanoi* hybrid, s.l. 46.7 mm, from Dutch North Sea, February 1974; scale bar 1 mm. (*c*) Section of testis from above hybrid; scale bar 20 μm. Abbreviations: s.v., seminal vesicle; t., testis.

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