Taxonomy of some species of *Anguina* Scopoli, 1777 (*sensu* Brzeski, 1981) forming galls on Gramineae : value of diagnostic characters and present status of nominal species

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SUMMARY

Morphological and biometrical studies of species of *Anguina (sensu stricto)* causing galls on Gramineae, as represented in two nematode collections at Harpenden, England, confirmed that species are often difficult to distinguish apart from their hosts. Re-examination of characters used to separate species, supported in part by statistical analyses, showed that not all these characters had value at specific level. For example, relaxed body form (habitus) of females, structure of genital tract, and number of flexures of gonads are not useful below generic level. Shape of tail tip (both sexes) and direction of heat-relaxed curvature of males are among the most useful features; whereas, body length (L), details of œsophagus, V-value, form of spicules and gubernaculum and, possibly, presence of a knob of vestigial tissue on post-uterine sac may help to separate some species. Published evidence suggests that populations in the studied group are usually specific to one or two host genera, and may be a complex of host races within a few closely related species. *A. tritici, A. agrostis, A. graminis* and *A. funesta* are accepted as valid. The definitive description used by Steinbuch (1799) in naming *A. tritici* was that of Roffredi (1775), therefore the type locality is in Piedmont, North Italy and not Germany. A population from *Dactylis glomerata* is deemed conspecific with *A. graminis;* populations from *Puccinellia maritima* and *Holcus mollis* are considered undescribed species. Species not included in the study are reviewed from the literature. *A. phalaridis* (Steinbuch, 1799) Chizhov, 1980 is designated *species inquirenda;* most others listed by recent reviewers are accepted *pro tempore.* The poor condition of many specimens in slide collections is, at present, severely limiting and there is much scope for new work on this biologically important genus.

RÉSUMÉ

*Taxonomie de quelques espèces d'*Anguina *Scopoli, 1777 (*sensu *Brzeski, 1981) provoquant des galles sur les graminées :
valeur des caractères diagnostiques et statut actuel des espèces nominales*

L'étude morphologique et biométrique d'espèces du genre *Anguina (sensu stricto)* provoquant des galles sur les graminées — provenant de deux collections de nématodes, à Harpenden, Angleterre — confirme que ces espèces sont difficiles à distinguer les unes des autres, sinon par leur hôte. Un réexamen des caractères utilisés pour séparer les espèces, complété par des analyses statistiques, montre que ces caractères ne sont pas tous valables au niveau spécifique. Par exemple, la forme du corps des femelles relaxées (habitus), la structure du tractus génital et le nombre de replis de la gonade ne sont pas utilisables en-dessous du niveau générique. La forme de l'extrémité caudale, chez les deux sexes, et l'aspect de la courbure du corps, chez les mâles relaxés par la chaleur, figurent parmi les caractéristiques les plus utilisables; cependant, la longueur du corps (L), la structure fine de l'œsophage, la valeur de V, la forme des spicules et du gubernaculum et, peut-être, la présence de tissu vestigial à l'extrémité du sac post-utérin, peuvent aider à séparer certaines espèces. Les données publiées suggèrent à l'évidence que les populations relevant du groupe étudié sont généralement spécifiques d'un ou deux genres-hôtes et qu'elles pourraient constituer un complexe de races d'hôte comprenant un nombre limité d'espèces étroitement apparentées. *A. tritici, A. agrostis, A. graminis* et *A. funesta* sont considérés comme valides. La description utilisée par Steinbuch (1799) lorsqu'il a nommé *A. tritici* était celle de Roffredi (1775); il en résulte que le lieu type de cette espèce est le Piémont (Nord de l'Italie) et non l'Allemagne. Une population provenant de *Dactylis glomerata* est considérée comme conspécifique à *A. graminis;* des populations provenant de *Puccinellia maritima* et *Holcus mollis* sont considérées comme des espèces encore non décrites. *A. phalaridis* (Steinbuch, 1779) Chizhov, 1980 est rangé parmi les *species inquirendae.* La plupart des autres espèces citées dans les révisions récentes sont provisoirement acceptées. Le mauvais état de nombreux spécimens en collection est une limite importante à ce type d'étude et de nouveaux travaux sur ce genre de grande importance au point de vue biologique seraient nécessaires.

The first plant-parasitic nematodes to be observed (Needham, 1743; Roffredi, 1775 *a, b*, 1776; Steinbuch, 1799) were species of *Anguina*. Most of the original descriptions are inadequate although more detailed redescriptions exist for some. Type specimens are non-existent or unknown for the earlier named species, notably *A. tritici* (Steinbuch, 1799) Chitwood, 1935 (type species), *A. agrostis* (Steinbuch, 1799) Filip'ev, 1936, *A. phalaridis* (Steinbuch, 1799) Chizhov, 1980 and

A. graminis (Hardy, 1850) Filip'ev, 1936. This paper reviews these and species and populations morphologically close to them, which form a group causing characteristic galls on flowers, or occasionally vegetative parts, of Gramineae. Our concept of the genus follows Brzeski (1981) as modified by Fortuner and Maggenti (1987).

Preliminary studies confirmed that the members of this group were very similar in general morphology. Brzeski (1981) went so far as to state, " Adults of

Table 1

Populations studied

(RES = Rothamsted Experimental Station, Harpenden; MAFF = Ministry of Agriculture, Fisheries and Food, Harpenden Laboratory)

Host	Locality	Date	Collection	Nominal species (on slide label)
1. *Agrostis capillaris* (= *A. tenuis*)	RES museum plots	1959	RES	*A. agrostis*
2. *Agrostis* sp.	Hayling Island, Hampshire, England	1930	RES	*A. agrostis*
3. *A. stolonifera* (herbarium spec.)	Kent, England	1968	MAFF	*A. agrostis*
4. *Agrostis* sp.	New Zealand	1957	RES	*A. agrostis*
5. *A. stolonifera* v. *major*	Kings Co., Nova Scotia, Canada	1943	RES	*A. agrostis*
6. *A. borealis*	Gaspé Co., Québec, Canada	1947 (♀♀) 1952 (♂♂)	RES	*A. agrostis*
7. *Arctagrostis latifolia*	Southampton Is., N.W. Territory, Canada	1948	RES	*A. agrostis*
8. *Dupontia fisheri* v. *psilosantha*	Chesterfield Inlet, N.W. Terr., Canada	1950	RES	*A. agrostis*
9. *Lolium rigidum*	Manoora, S. Australia	1980	RES	*A. funesta*
10. *Lolium rigidum*	Katanning, W. Australia	1980	RES	*A. funesta*
11. *Festuca rubra*	Sports Turf Res. Inst., Bingley, Yorks, England	1959	RES	*A. graminis*
12. *Festuca rubra*	Slapton Ley, Devon, England	1963	MAFF	*A. graminis*
13 a.* *Dactylis glomerata* (shoot galls)	Norfolk, England	1964-66	MAFF	—
13 b.* *Dactylis glomerata* (flower galls)	Norfolk, England	1964-66	MAFF	—
14. *Puccinellia maritima*	North Wootton, Norfolk, England	1946-49	RES	*A. agrostis*
15. *Holcus mollis*	Silwood Park, Sunningdale, Surrey, England	1982	MAFF	—
16. *Triticum aestivum*	RES (plot culture)	1985	RES	*A. tritici*

* 13 a and 13 b are separate generations on inoculated host plant (see Southey, 1969), on vegetative shoots (first year) and flowers (second year).
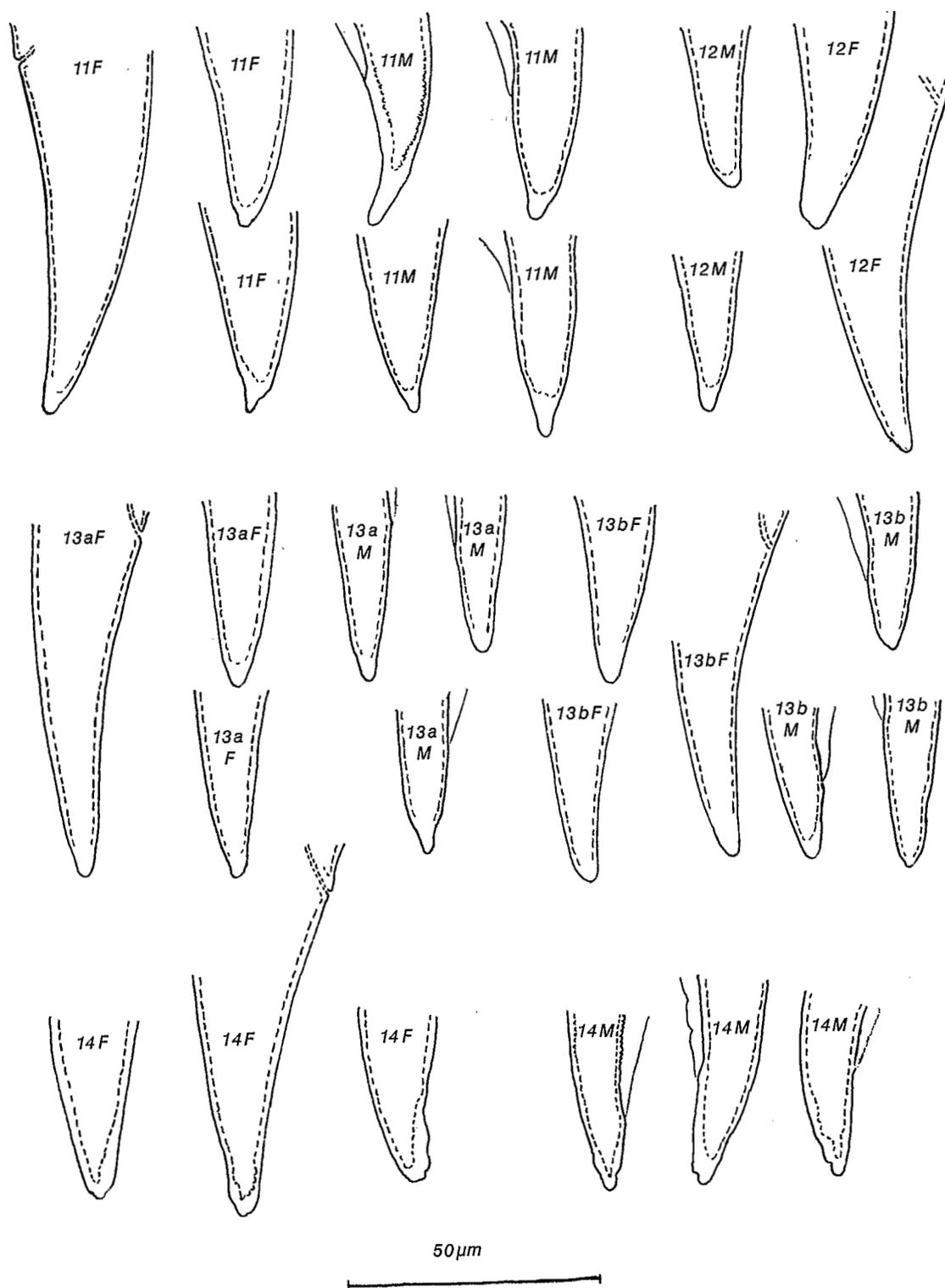
Fig. 1. Tail tips of examples from *Anguina* populations. Blunt rounded tips (Population numbers as in Table 1. F = female; M = male. Host genera : 1, 2, 4, 5, 6 : *Agrostis;* 7 : *Arctagrostis;* 8 : *Dupontia;* 9, 10 : *Lolium;* 11, 12 : *Festuca;* 13 : *Dactylis;* 14 : *Puccinellia;* 15 : *Holcus;* 16 : *Triticum).*
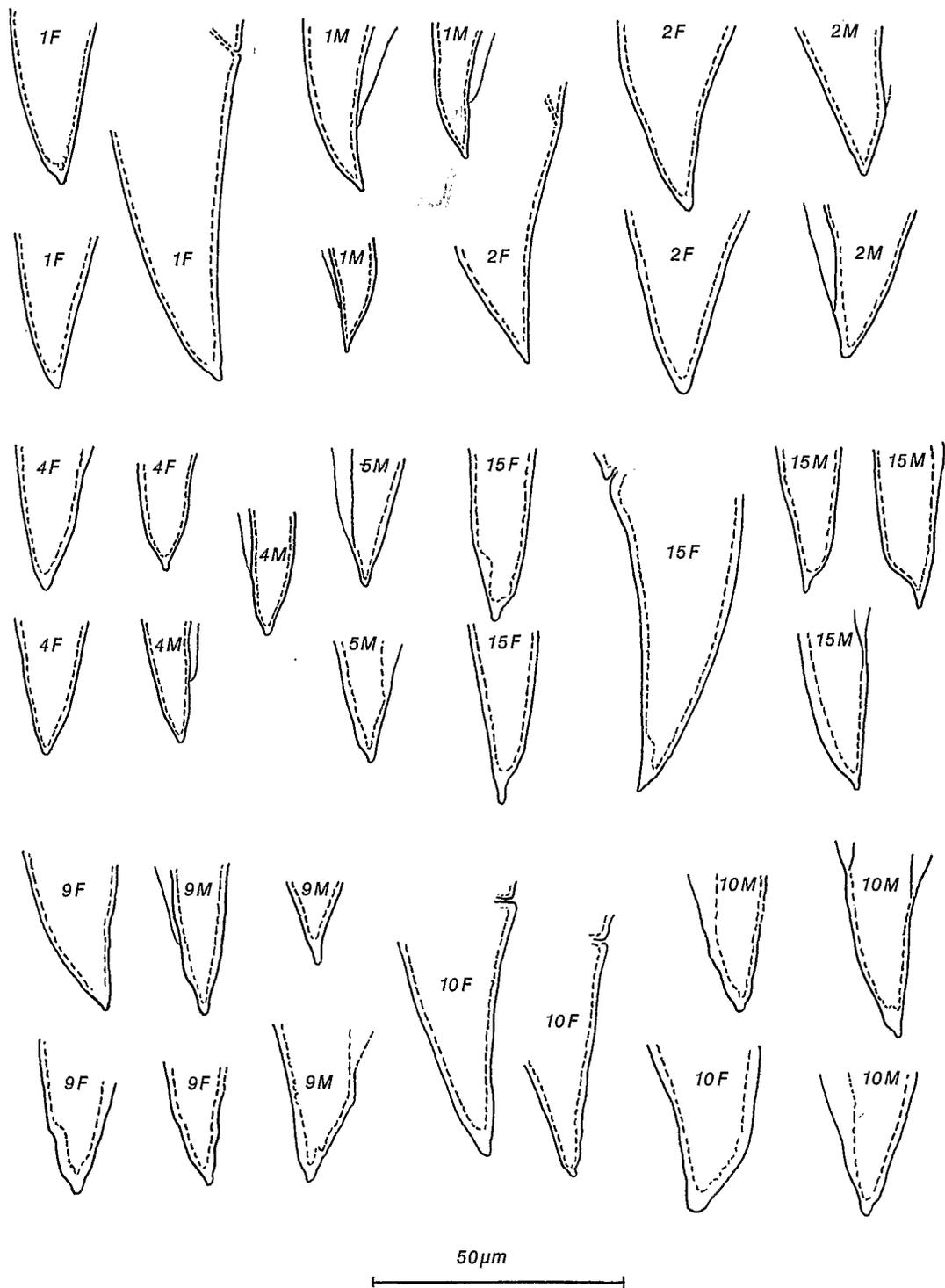
Fig. 2. Tail tips of examples from *Anguina* populations. Mostly acute pointed tips. (Letters and figures : see Fig. 1).
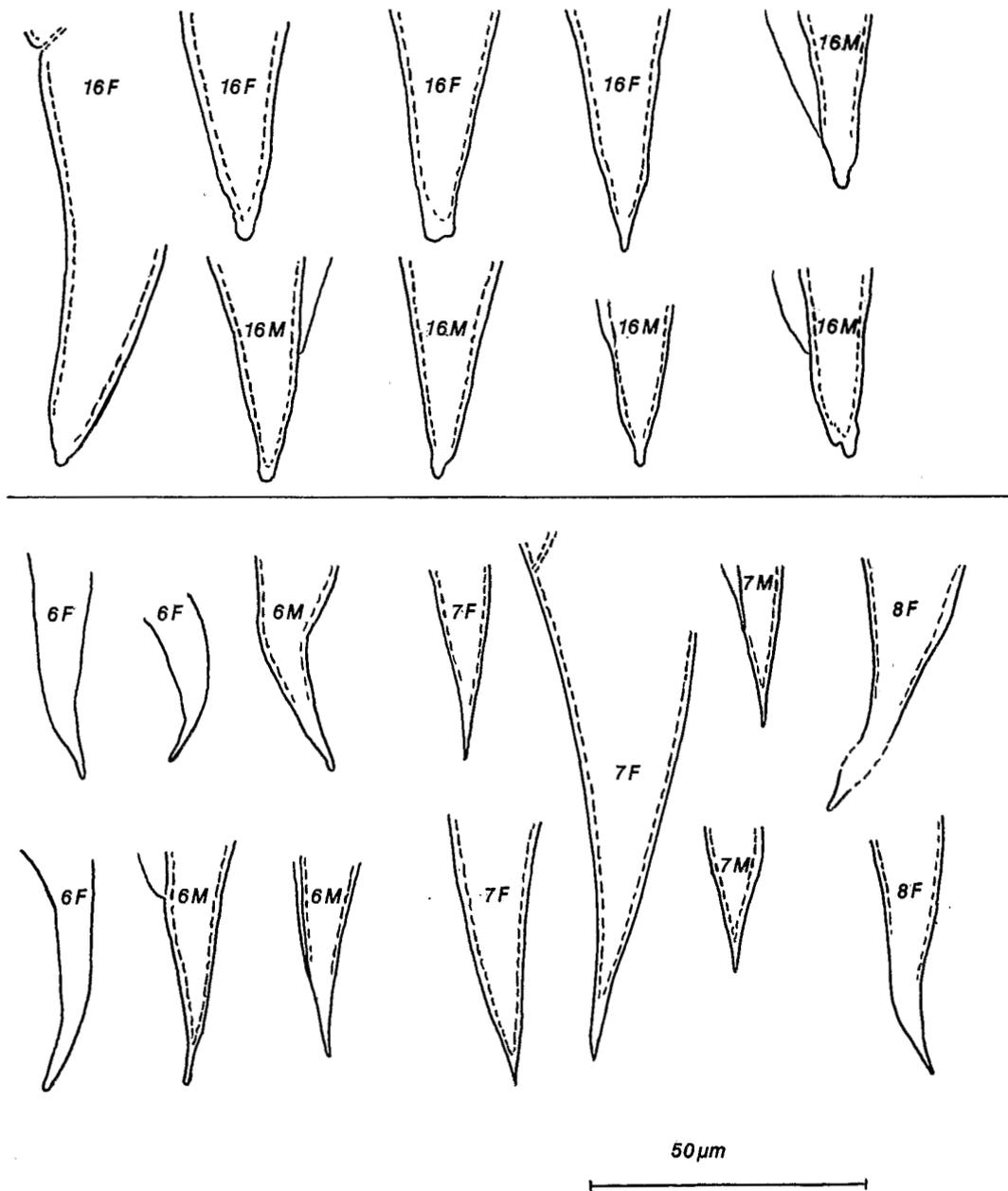
Fig. 3. Tail tips of examples from *Anguina* populations. Above : variable tips; below : attenuated tips. (Letters and figures : see Fig. 1).

different species of *Anguina* are morphologically indistinguishable although they develop on different hosts in widely separated geographical areas ". The objectives of the present review are to assess the value of characters which have been claimed to be useful in distinguishing species, to examine critically the morphological distinctness of species in the group on present evidence, and to indicate areas where further work is needed.

Materials and methods

All specimens examined were permanently mounted in glycerol on slides in the nematode collections of Rothamsted Experimental Station (RES) and Ministry of Agriculture, Fisheries and Food, Harpenden Laboratory, Harpenden, England. Table 1 shows that the specimens were of widely varying age and their condi-

tion was equally variable. " Populations " as listed in Table 1 consist of from 1 to 20 specimens collected from (or cultured from) the same host species and locality.

CHARACTERS ASSESSED

Measurements*, ratios and morphological features were recorded for adults from the above populations where condition of the specimens permitted (Figs 1-3). Some of the data were used for statistical analysis as detailed below. The characters studied included standard de Man ratios and measurements considered of possible value in *Anguina* (L, a, c, V, tail length [TL], stylet length), and the following features claimed by authors to be useful for differentiating species :

Both sexes

Distance (μm) from anterior of nematode to excretory pore (hex) : L ÷ hex.
    Stynes and Bird (1981) used position of excretory pore in relation to pharyngeal (= œsophageal) structures in characterising *A. agrostis* from *Lolium* (= *A. funesta*); Goodey (1927) and Southey (1974) used this character in defining *A. graminis*. However, it is probably unreliable since pharynx is not connected with body wall posteriorly.

Shape of extreme tip of tail (Figs 1-3).
    Southey (1969, 1973, 1974) considered that this character distinguished *A. agrostis* on *Agrostis* spp. from *A. graminis* and populations on several other grass genera.

Number of annules per 10 μm length in tail region.

Females only

Length (μm) of post-uterine sac (PUS)

Distance (μm) from vulva to anus (Van)

Distance (μm) from vulva to tail tip (Vt)

PUS ÷ Van

PUS ÷ Vt

Post-uterine sac with or without a knob of vestigial oviduct tissue at distal end (PUS-knob ±) (Southey, 1974).

    Stynes and Bird (1981 *b*) used size of PUS in defining *A. agrostis* (= *A. funesta*); Choi and Loof (1974) used ratio of PUS to Van in their key.

Males only

Length of spicules (μm) measured as a straight line joining anterior and posterior extremities (chord). A stable character because spicules are cuticular structures. Stynes and Bird (1981 *b*) claimed that it dis-

tinguished *A. agrostis* (= *A. funesta*) from some other species.

Distance (μm) from end of bursa to tail-tip. Used by Stynes and Bird (1981) in characterising *A. agrostis* from *Lolium* (= *A. funesta*).

Heat-relaxed curvature of body ventral, i.e. towards ventral surface, or dorsal (Fawcett, 1938; Southey, 1972, 1973, 1974).

Other morphological features have been observed when possible, e.g. presence and number of flexures in gonads (various authors); position of first flexure of ovary in relation to œsophageal glands, nerve-ring, etc (Stynes & Bird, 1981 *b*); presence and number of constrictions in pharynx (= œsophagus) (various authors); form of procorpus (Choi & Loof, 1974); shape and degree of overlap of pharyngeal glands (Fawcett, 1938; Southey, 1972, 1973, 1974); shape of spicules and gubernaculum (various authors); number of incisures on lateral field (Southey, 1972, 1973, 1974).

STATISTICAL ANALYSIS

Statistical analyses were made using the Genstat computer package, version 4.06 (Alvey, Galwey & Lane, 1982). The data were subjected to non-orthogonal analysis of variance to check for population and sex differences for each variate (character) and for population/sex interactions. Canonical variate analysis (CVA) was used on males and females separately, to assess to what degree the data supported assumed species or population groupings. However CVA requires complete datasets for all individuals. Owing to the poor condition of many of the available specimens some entire " populations " had to be omitted; for the remainder the number of variates was reduced to increase the number of specimens which qualified for analysis. This compromise was reached by selecting specimens which were complete for characters believed to be " useful " on the basis of published work or experience (females : L, c, V, PUS-knob [±], tail-tip shape; males : L, spicule length, tail-tip shape, body curvature), and then adding to the datasets other characters which were also recorded for all the specimens so selected (females : " a ", tail-length [TL], PUS, Vt, PUS/Vt; males : c, TL). On this basis 37 females with ten variates and 38 males with six variates were used separately for CVA and single-linkage cluster analysis. For males, populations 1, 5, 11, 13 b, 14, 15 and 16 were represented, and for females, populations 1, 6, 7, 8, 11, 12, 13 a, 13 b, 14, 15 and 16 (Table 1).

Results

STATISTICS

Analysis of variance

The analysis of variance showed significant differences (P = 0.01 or less) among populations for all

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* Morphometric data may be obtained by request from the senior author.

characters except PUS/Vt and PUS/Van. As expected in a group with marked sexual dimorphism, most characters showed significant differences between sexes and significant sex/population interaction (P = 0.05 for stylet and hex, P = 0.01 or less for the rest), confirming that the sexes must be treated separately for any multivariate analysis. An exception was tail-tip shape (Figs 1-3) which showed no significant sex difference or sex/population interaction; it is thus a useful feature for characterising species independently of sex (see also p. 138). Tail length (TL) also showed no significant difference between sexes.

Canonical variate analysis with single-linkage cluster analysis

Canonical variate analysis (CVA) uses numerical character data to calculate coordinates for individuals or population means in relation to a series of computed axes (canonical variates) such that the distances separating the points are inversely proportional to similarity; the first axis accounts for the maximum proportion of variance in the data, while the second, third, etc progressively maximise the remainder. With the *Anguina* males, 91.2 % of the variance was accounted for by the first two axes and 96.9 % by the first three; with the females the figures were, respectively, 76.8 % and 91.1 %. Thus, for both sexes, the results should be adequately represented by plotting on the first three axes, but less satisfactorily for the females using the first two only.

Figs 4-6 show, respectively, plot of population means for males on the first two axes, dendrogram for males, and plot of population means for females on axes 1 and 3. Radii for the 99 % confidence circles around the population-mean points were calculated as $\frac{t_{DF\ \infty}}{\sqrt{n}}$ where n = number of individuals (Mardia, Kent & Bibby,
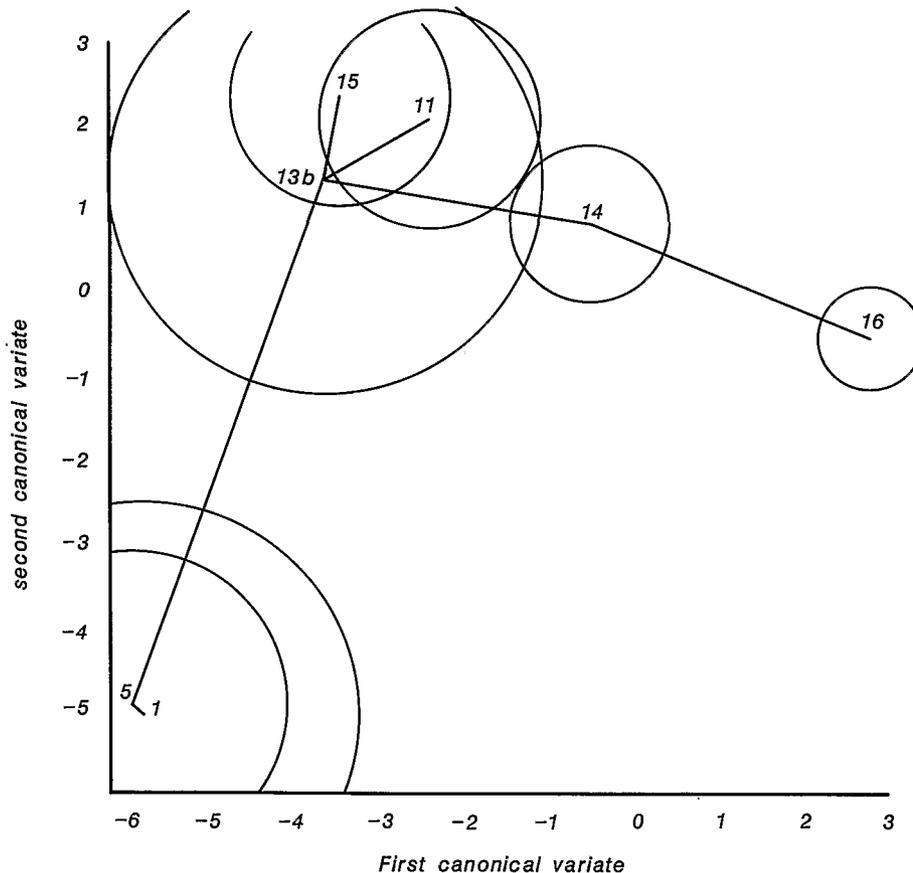

Fig. 4. Canonical variate analysis of numerical character data for *Anguina* males : position of population means with 99 % confidence circles, relative to the first two axes; points joined by minimum spanning tree. Population numbers (Table 1) and host genera : 1, 5 : *Agrostis;* 11 : *Festuca;* 13 : *Dactylis;* 14 : *Puccinellia;* 15 : *Holcus;* 16 : *Triticum.*

SIMILARITY LEVEL

Host genus   90   80   70   60   50   40   30

Agrostis   1

Agrostis   5

Dactylis   13b

Holcus   15

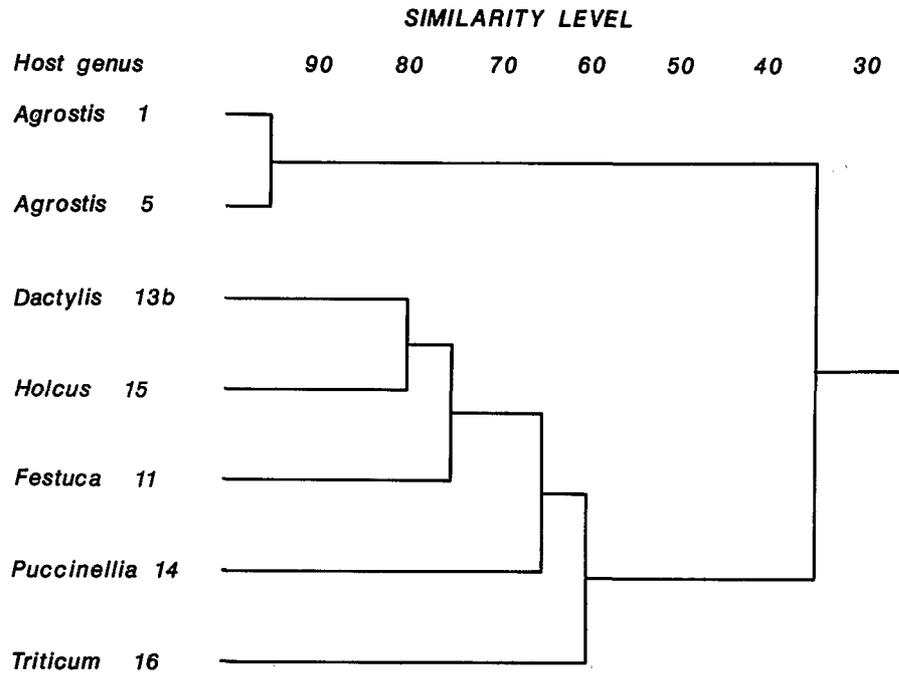Festuca   11

Puccinellia 14

Triticum   16

Fig. 5. Single linkage cluster analysis of numerical character data for *Anguina* males :
dendrogram to illustrate grouping of populations at different similarity levels. Population
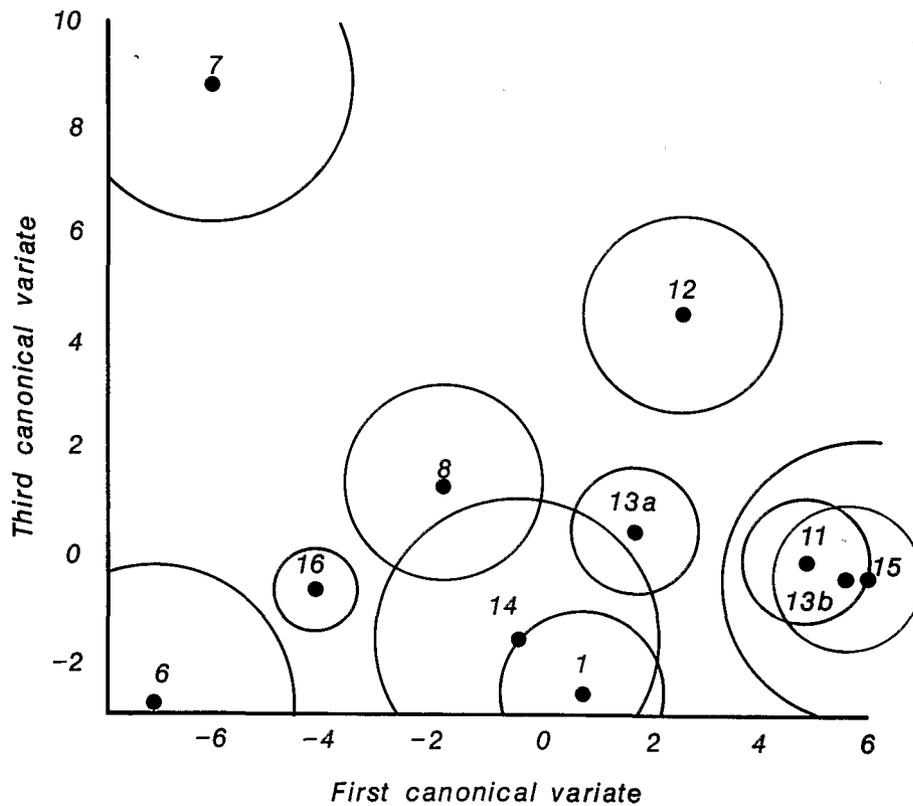numbers as in Table 1.

Fig. 6. Canonical variate analysis of numerical character data for *Anguina* females :
population means with 99 % confidence circles, relative to axes 1 and 3. (Population numbers
as in Table 1). Host genera : 1, 6 : *Agrostis;* 7 : *Arctagrostis;* 8 : *Dupontia;* 11, 12 : *Festuca;*
13 a, 13 b : *Dactylis;* 14 : *Puccinellia;* 15 : *Holcus;* 16 : *Triticum.*

1979). Distances between points on the graphs are approximations of the computed Mahalanobis's distances in multidimensional space. Reference to these showed that, for males (Fig. 4), each population mean was separated from every other by more than the sum of their 99 % confidence radii except populations 1 and 5 (both on *Agrostis* spp.), 13 b and 15 (from *Dactylis* flowers and *Holcus* respectively) and 13 b and 11 (*A. graminis* on *Festuca*), and for females (Fig. 6) all populations were so separated except 1 and 14 (from *Agrostis* and *Puccinellia* respectively), and 13 b and 11 (from *Dactylis* flowers and *Festuca* respectively) — the last two were, however, distinct at the 95 % level.

In the cluster analysis for males (Fig. 5), two populations from *Agrostis* (1 and 5) are separated from the rest at the 35 % level of similarity but remain grouped together up to the 95 % level. *A. tritici* (population 16) separates at the 60 % level and the *Puccinellia* population (14) at the 65 % level leaving populations from *Festuca* (*A. graminis*), *Holcus* and *Dactylis* together up to the 75 % level. The cluster analysis for females made less sense, but it included specimens of two of the Canadian arctic populations with attenuated tails (Fig. 3); that from *Agrostis borealis* (6) separated from the rest at the 35 % level and that from *Arctagrostis* (7) at the 50 % level, but these two were not grouped together as might have been expected. Population 11 (*A. graminis* from *Festuca*) was grouped with the population from *Dactylis* flowers (13 b) up to the 90 % level, consistent with the morphological similarity of the *Festuca* and *Dactylis* populations, but these two were separated from a second *Festuca* population (12) at the 70 % level and from the population from *Dactylis* shoots (13 a) at the 80 % level. *A. tritici* (population 16) was not isolated below the 75 % level.

Some measure of the relative importance of the characters used in CVA in causing separation of populations is obtained by multiplying the loadings (latent vectors) by standard deviations of the population means. Tables 2 and 3 give the " importance values " for males

Table 2

" Importance values " of variates in CVA
(SD population mean × loading) : *Anguina* males

Variate	Axis		
	1	*2*	*3*
L	3.1	5.4	5.9
TL	2.0	2.5	2.8
c	4.2	3.3	5.3
Spicules	0.65	0.39	1.30
Body curvature	0.21	2.7	0.39
Tail-tip	0.14	0.36	0.31

Table 3

" Importance values " of variates in CVA
(SD population mean × loading) : *Anguina* females

Variate	Axis		
	1	*2*	*3*
L	8.46	11.34	3.12
a	0.74	0.43	0.12
TL	8.76	9.92	7.05
c	9.67	11.66	4.64
V	1.28	0.03	1.51
PUS	1.90	1.68	2.07
Vt	1.09	1.86	0.82
PUS/Vt	1.49	1.27	1.40
Tail-tip	0.15	0.18	1.23
PUS knob (±)	0.45	0.68	0.31

and females respectively. Note however, that the values for the last two variates in each table are probably unreliable since these were morphological character states that were given a numerical rating rather than continuous variables. With both sexes the greatest effect is due to L, tail-length and c.

The results of the analyses clearly reflect differences in dimensions of the nematodes which are not necessarily specific but are probably related to nutritional differences between hosts or, in the case of the two populations from *Dactylis* (13a and 13b), between different growth stages of the same host. Populations 13a and 13b in fact were different generations (on vegetative shoots and flowers respectively) from the same culture; adults of both sexes of 13a are consistently larger than those of 13b, with related differences in some other measurements.

OBSERVATIONS OF MORPHOLOGICAL FEATURES

Heat-relaxed shape (habitus).

The more or less circular spiral shape of adult females with the ends overlapping is characteristic of the genus. The degree of overlap increases with the size and state of maturity of females. When live, they are semisedentary and tend to remain in this attitude when released from galls, though often more coiled than when heat-relaxed. Goodey (1927) illustrated a female of *A. graminis* of open-C shape and Goodey (1933) stated that the female of this species was less coiled than that of *A. tritici* or *A. agrostis;* however, no consistent differences in female habitus were observed among the studied populations, including those of *A. graminis* from *Festuca* species (see also Southey, 1974).

Males of *Anguina* are much less curved than females when heat-relaxed, and may be almost straight. Fawcett

(1938) was the first to note that those of *A. agrostis* were curved with the ventral surface inwards (as with most nematodes) whereas males of some other *Anguina* species curved the opposite way. This difference was confirmed in the present work. Males of population 1 (Table 1), regarded as the best available reference population of *Anguina agrostis* from the type host, consistently showed *ventral* curvature, a character which *A. agrostis (sens. strict.)* shares with *A. australis* among other described species. In spite of some variation, dorsal curvature predominated in apparently relaxed specimens of populations from all host genera other than *Agrostis*. In some specimens the body may twist along its length, but at the tail-end the bursa and spicules typically pointed outwards. Specimens from *Holcus mollis* (population 15) were not in good condition but four males were classed as having dorsal curvature. However, the senior author has since examined specimens of an Australian *Anguina* population from *Holcus lanatus* in the collection of the Waite Agricultural Research Institute, Adelaide (courtesy of Dr J. M. Fisher). These closely resembled the English material, but the males were well relaxed and mostly showed a characteristic habitus with head and tail slightly bent in a ventral direction while the body as a whole was bent dorsally at about the mid-point, i.e. tending to a sigma form. Typical dorsal curvature of males is illustrated for *A. graminis* by Goodey (1927), for *A. tritici* by Goodey (1932) and for *A. funesta* by Price, Fisher and Kerr (1979); Goodey (1930) figures typical ventral curvature in *A. agrostis*.

Flexures of gonads

Fully developed males and females of all studied populations had flexed gonads. All males showed a single flexure of the testis near the anterior end of the intestine. Adult females have one or more flexures of the ovary depending on degree of development; the ovary is rarely unflexed, two flexures are the most common. There were no consistent differences between studied populations; more than two flexures generally occurred in those with larger females. Choi and Loof (1974), in their key, distinguished *A. graminis* from other species in having one flexure of the ovary, but the studied material from *Festuca rubra* identified as this species (populations 11 and 12) more often had two flexures. Solov'eva and Gruzdeva (1974) also found two flexures of the ovary frequent in *A. graminis* from *F. rubra* in the Soviet Union.

Form of pharynx

Excluding *Anguina tritici* (population 16), the pharynx conformed to a common pattern among all populations in which its details could be seen (1, 11, 13 a, b, 14, 15, 16) : procorpus showing variable but usually slight swelling and separated from oval median bulb by a more or less marked constriction; isthmus often more or less

swollen posteriorly and usually separated from pharyngeal glands by a constriction; glands forming a more or less pyriform mass in females, often irregular or distorted by pressure of other organs in mature specimens, usually more regular and oblong in males. Price, Fisher and Kerr (1979) and Stynes and Bird (1981 *b*) illustrated and described similar pharyngeal structure in populations from *Lolium rigidum* (our populations 9 and 10). *A. tritici* (16) is distinguished from the rest by a stronger swelling of the procorpus (usually as wide, at least in part, as median bulb, which is spherical (rather than oval), as illustrated by Goodey (1932, 1933) and Siddiqi (1986).

Number of incisures on lateral field

Details of the lateral field in *Anguina* species are difficult to observe and usually not visible in adult females. According to Southey (1972, 1973, 1974), *A. tritici* has four or more, in adults visible only on young specimens, *A. agrostis* probably has six in males or, according to Thorne (1961), four bordered by refractive lines, and *A. graminis* males probably have four, at least posteriorly. Price, Fisher and Kerr (1979) reported five or six incisures in *Anguina funesta* and Stynes and Bird (1981 *b*) stated that the number was variable. According to Norton (1965) *A. agropyronifloris* has " 8-10 minute incisures ". In the present study four incisures were observed in specimens of both sexes from *Dactylis* (populations 13 a, b), one male from *Puccinellia* (14) and one male from *Agrostis* from New Zealand. Four may be the basic number in the genus with occasional branching or additional lines. This is not at present a useful character for species identification.

Form of spicules and gubernaculum

Goodey (1927, 1930, 1932, 1933) described and illustrated the spicules of *A. tritici* males as not only larger and more heavily built than those of *A. graminis* and *A. agrostis*, but differing also in having the dorsal proximal edge of the capitulum bent or rolled towards the ventral side compared with no more than slight folding of this area in *A. agrostis* and a simple open-ended appearance in *A. graminis*. This difference was not confirmed in the present study. A tendency to ventral bending, rolling or hooking of the capitulum was observed in some specimens but not others of populations from *Agrostis* spp., *Puccinellia maritima* and *Holcus mollis* as well as *Triticum aestivum* (populations 1, 3, 6, 14, 15, 16) (Fig. 7). Generally two types of spicule could be distinguished though there is variation and intermediates occur : (1) the *graminis* type, typically as illustrated by Goodey (1927), simpler in shape with capitulum open-ended and merging with shaft to form a more or less oblong structure proximally, occurring in populations from *Festuca* (11) and *Dactylis* (13 a, b); (2) the *agrostis/tritici* type, occurring in the remaining populations, usually
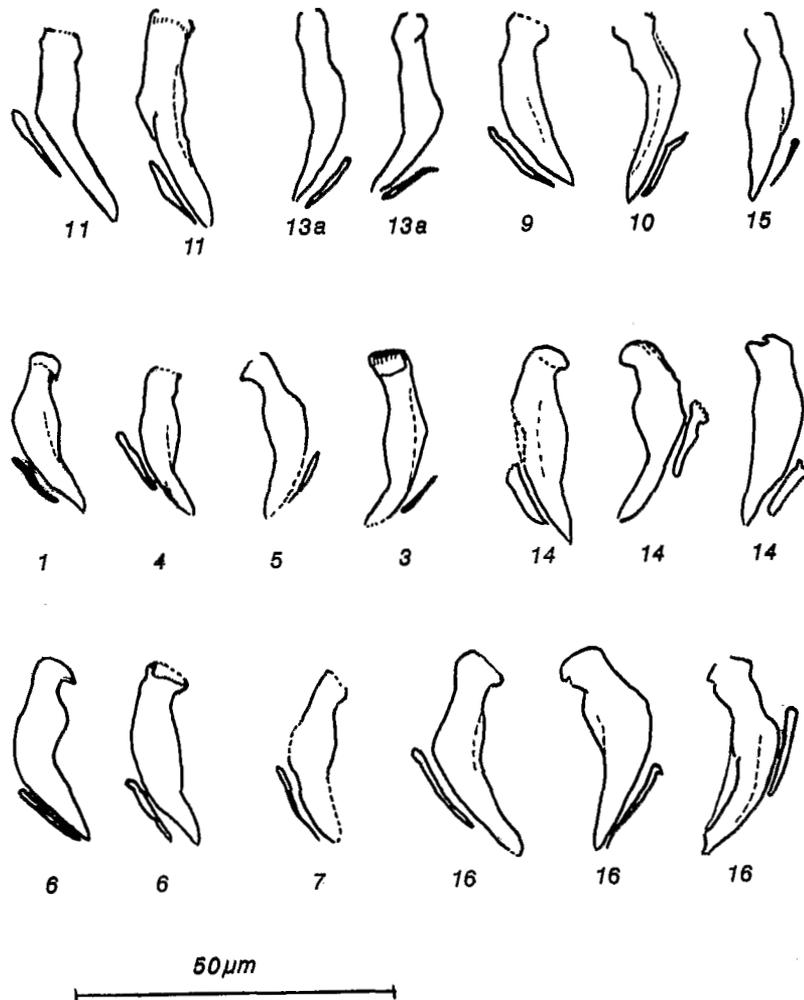
Fig. 7. Male spicule (one of a pair) and gubernaculum from *Anguina* populations. (Population numbers as in Table 1. Host genera : 1, 3, 4, 5, 6 : *Agrostis;* 7 : *Arctagrostis;* 9, 10 : *Lolium;* 11 : *Festuca;* 13 : *Dactylis;* 14 : *Puccinellia;* 15 : *Holcus;* 16 : *Triticum*).

with more clearly differentiated capitulum narrowing to short shaft, and dorsal edge often bent or rolled in ventral direction or capitulum as a whole appearing hooked ventrally as seen in profile.

The gubernaculum showed little variation. It is a curved trough-like plate; in lateral profile or optical section it appeared narrow and linear in most populations, often thickening dorsally; in the specimens from *Puccinellia* (population 14) it is thicker generally. Small processes, possibly for muscle attachment, are sometimes seen at the dorsal (proximal) end; in some of the *Puccinellia* specimens these were particularly marked giving the gubernaculum the appearance of a foot with 2-5 toes (Fig. 7). Spicules and gubernaculum are three-dimensional structures whose apparent shape in the optical microscope is affected by orientation. SEM

studies are needed to clarify their form and variability in the genus *Anguina.*

Presence of a knob of vestigial oviduct tissue on post-uterine sac

This female character (PUS-knob ±) was recorded and used in the statistical analyses; in the analysis of variance (see above) it showed significant differences among populations. This feature appeared to be lacking in some populations (6, 8, 15) but too few satisfactory specimens were available to draw conclusions as to its taxonomic value.

Tail-tip shape

The tail of both sexes of all *Anguina* species is tapered and pointed as seen under low magnification. However,

at high magnification (× *c.* 1 000) the posterior extremity usually appears at least minutely rounded, occasionally truncate or lobed. Three tail-shape groups could be distinguished even under lower magnification (× 40) (Figs 1-3) : obviously blunt, rounded or lobed (w = wide), more or less acute or very narrowly rounded (n = narrow), and attenuated (vn = very narrow). The last type occurred in three populations from Canadian arctic grasses and distinguished them from all others studied. The second type (n) was found in most populations from grasses of genus *Agrostis*. Population 3, from *A. stolonifera* was an exception in that most specimens were classed as having a bluntly rounded tail tip (w).

The more detailed form of the tail tip may sometimes be important. In the population from *Puccinellia* (14) the tail tip (classed as w) was usually irregular or lobed. The specimens from *Holcus mollis* (population 15) were classed as having a narrow-pointed tail tip (n), but unlike those from *Agrostis* spp. the extremity of the tail narrowed suddenly to a mucro-like projection which was usually offset ventrally. Specimens of an Australian *Anguina* population from *Holcus lanatus* (see below) consistently showed a precisely similar tail-tip form.

Cuticular annulation

The fine cuticular annulation of *Anguina* spp. is observable mainly in the tail region, sometimes also in the head region. Annules are of the order of 1 μm or less in width. They were recorded for usually few specimens of populations 1, 2, 3, 4, 9, 10, 11, 12, 14 and 16 (Table 1). There was no evidence of difference between populations other than 16 *(A. tritici)*. Numbers of annulations per 10 μm of body length in the tail region for *A. tritici* (population 16) were : males 10-12; females 7-11, and for the other populations : males 12-16, females 10-14.

Status of *Anguina* species

STUDIED SPECIES AND POPULATIONS

Anguina tritici *(Steinbuch, 1799) Chitwood, 1935, type species* : Steinbuch (1799) was the first to name this species and is the accepted authority for the specific name. However, the main purpose of his paper was to describe *Vibrio (= Anguina) agrostis;* he provided no illustration of *A. tritici* and gave only sufficient information to distinguish it from *A. agrostis* on the appearance of the worms and their effects on the host plant. For this purpose he quoted an earlier detailed description of *A. tritici* by the Abbé Roffredi (1775 *a;* possibly also 1775 *b* and 1776) which he regarded as authoritative. Since Steinbuch's name was based on Roffredi's description and material, the type locality for *A. tritici* is in Piedmont (then part of France) where Roffredi worked and several cantons of which he referred to as infested at the time (Roffredi, 1775 *a*, p. 4) and not Germany.

A. tritici is usually recognized by its situation — in characteristic ear-cockle galls on the type host (wheat) — and by the large size of the adults compared with related species. Several of the studied populations overlap with it in size (L measurement) and rather few other differences were found. Nevertheless it is accepted as a distinct species, with hosts confined to the genera *Triticum, Secale* and possibly *Hordeum* (see page 141). It differs from all other studied populations in the form of the procorpus and median bulb of the pharynx (page 136). The population from *Puccinellia* (14) is otherwise very similar; L measurements overlap and tail tip is similarly variable, often irregular or lobed (Figs 1-3). Two Canadian arctic populations, from *Arctagrostis* (cf. Mulvey, 1963) and *Dupontia* (populations 7 and 8), also overlap in L measurement but are distinguished by tail-tip shape. *A. tritici* differs from the remaining studied populations in L measurement, from *A. agrostis* also on tail-tip shape and heat-relaxed curvature of males, and from *A. graminis* from *Festuca* spp. and the form on *Dactylis* (populations 13 a, b) on spicule shape. According to Triantaphyllou and Hirschmann (1966), *A. tritici* and *A. agrostis* are cytologically distinct with haploid chromosome number 19 and 22 respectively.

Anguina agrostis *(Steinbuch, 1799) Filip'ev, 1936* : This species appears to be restricted to *Anguina* populations causing characteristic elongate galls and abnormally elongated floral structures in grasses of the genus *Agrostis* (Goodey, 1930), as suggested by Southey (1973). Populations 1, 2, 4 and 5 (see Table 1) are confirmed as *A. agrostis*. This species differs from the other studied populations by the males being curved with the ventral surface inwards when relaxed by heat, and by the tail tip in both sexes (Figs 1-3), which is conically tapered to an acute point (usually seen as minutely rounded only under high magnification, × *c.* 1 000). The best UK reference material is on slides 54/4/36-38 in the RES Collection (Table 1, population 1). It would be desirable to establish a neotype from the German Federal Republic as near as possible to Steinbuch's original type locality in the Forest of Erlangen, though reportedly much of the original area has been built on; attempts about 1975 to find *Anguina* material near there were unsuccessful (Weischer, pers. comm.).

Populations from Canadian arctic grasses : The specimens in the RES Collection of three populations from Canadian arctic grasses (Table 1, nos. 6, 7 and 8) were identified by T. Goodey as *A. agrostis* (as recorded by Mulvey, 1963). They appear close to that species, but the distinctive, very finely pointed and attenuated tail tips of most of them suggest that they may represent one or more undescribed species. However, their condition is too poor to confirm this. The direction of curvature of the males could not be definitively deter-

mined, though a few males of population 6 and the solitary male of population 8 showed signs of dorsal curvature (i.e. contrary to *A. agrostis*). Populations 7 and 8, from *Arctagrostis latifolia* and *Dupontia fisheri*, both from Northwest Territories, are large forms, comparable in size with *A. tritici*. Mulvey (1963) described a population from *Arctagrostis latifolia* from another locality in Northwest territories, which he identified as *A. agrostis,* but also noted that adults of both sexes were larger than in Goodey's (1930) description. Population 6 (from *Agrostis borealis* collected in Quebec) had L measurements comparable with *A. agrostis* but most males, at least, showed very narrow attenuated tail tips like the other two. Unless there is better material in Canadian nematode collections, fresh collections should be made from the localities concerned to confirm the identity of *Anguina* populations occurring on the native grasses.

Anguina graminis *(Hardy, 1850) Filip'ev, 1936 :* This species is usually recognised by its elongate purplish galls on the leaves of fine-leaved grasses of the genus *Festuca* (Goodey, 1927; Southey, 1974). The type host is *F. ovina* (Hardy, 1850 *a, b*) but, in Britain at least, it is probably more common on *F. rubra* and its variants; it occurs mostly in coastal turf. The type locality is on the north-east coast of Britain near the Scottish/English border (Southey, 1974) where neotype material should be sought. Currently the best reference specimens in UK are on slides 54/3/11-14 in the RES Collection (Table 1, population 11). *A. graminis* is distinguished from *A. tritici* by its smaller size, the form of the male spicules (Fig. 7), and usually smaller V value of females, and from *A. agrostis* by the form of the spicules, the curvature of males when heat-relaxed, and the shape of the tail tip (Figs 1-3).

Population from Dactylis glomerata : No consistent differences could be found between the specimens from this host (Table 1, populations 13 a and b) and those of *A. graminis* from *Festuca* spp. They are therefore regarded as conspecific. Unlike *A. graminis* from *Festuca* spp., the *Dactylis* population occurred naturally in flowers of its host but was shown experimentally to form galls and reproduce on young shoots of the plants before flowering (Southey, 1969). This raises the question whether records of *A. agrostis (sens. lat.)* in flowers of *Festuca* spp. (Southey, 1974; Jensen, 1961) might in fact refer to *A. graminis*. Specimens from *Festuca* flowers need to be examined, and experiments done, to establish whether different generations of the same population can infest leaves and flowers of *Festuca* spp. *Anguina* species on grass flowers, other than *A. agrostis* on *Agrostis* spp., do not always affect the external appearance of the inflorescence so infestation is easily overlooked. On *Festuca* species in the field, leaf galls are more likely to be noticed than flower galls. Galls from grass flowers are usually found first in seed samples and attention has been directed to them especially where

associated toxicity to livestock has occurred (Jensen, 1961; Price, Fisher & Kerr, 1979; Stynes & Bird, 1981 *b*).

Anguina funesta *Price, Fisher & Kerr, 1979 :* Stynes and Bird (1981 *b*) synonymised this species with *A. agrostis* after comparison of South and Western Australian populations from the type host *(Lolium rigidum)* with specimens identified as *A. agrostis* from England (= population 1 in present paper), New Zealand and USA. Fisher, McKay and Dubé (1984) subsequently argued for reinstatement of *A. funesta* on grounds of biological distinctness, accepting Southey's (1973) suggestion that the concept of *A. agrostis* be limited to populations reproducing on *Agrostis* spp. and pending clearer redefinition of *A. agrostis*. Few reliable measurements were possible from the few specimens of the two Australian *Lolium* populations in the RES collection (Table 1) which are in too poor condition to help settle this question. The tail tip of the males was classed as narrow-pointed (i.e. like *A. agrostis*) and that of the females was variable. Examination of the holotype and paratypes of *A. funesta* in the collection of the Waite Agricultural Research Institute, Adelaide (courtesy of Dr J. M. Fisher) has since confirmed that tail-tip shape varied; some specimens of both sexes had a bluntly rounded tip (i.e. like *A. graminis*). Holotype and paratypes showed clear dorsal curvature, as illustrated by Price, Fisher and Kerr (1979, Fig. 1), unlike *A. agrostis*. The male spicules, however, appear to be of *agrostis/ tritici* type (see page 136). Thus *A. funesta* shows similarities to *A. tritici, A. agrostis* and *A. graminis;* it differs from the first in body size and form of pharynx (but little else), from the second in heat-relaxed curvature of the males and in the more variable tail-tip shape (sometimes blunt-rounded as in *A. graminis*), and from the last possibly in shape of spicules and more variable tail tip. Fisher, McKay and Dubé (1984) drew attention to biological features in common between *A. funesta* and the Oregon (USA) population on *Festuca* spp., both associated with toxicity to livestock, and to the botanical affinity between the genera *Festuca* and *Lolium*, implying that the same nematode may be involved on both hosts. If the nematodes causing galls on *Festuca* flowers prove to be *A. graminis* (see page 139) then *A. funesta* may be synonymous with *A. graminis*. For the present it seems best to follow Fisher, Mc Kay and Dube (1984) in accepting *A. funesta* as a valid species.

Population from Puccinellia maritima : The 5 females and 12 males of this population in the RES Nematode Collection (Table 1, population 14) were identified by T. Goodey as *Anguina agrostis*. We believe it to be an undescribed species clearly different from *A. agrostis* but close to *A. tritici* and *A. graminis*. It appears to differ from *A. tritici* in form of the pharynx and smaller L measurement (though the ranges overlap), and from *A. graminis* in larger size, form of male spicules (Fig. 7) and

more frequently lobed or irregular tail tip (Fig. 1). The form of the gubernaculum may be unique to the species (see page 138). Examination of herbarium material shows that, like *A. agrostis*, it causes elongation of host floral-parts. Further study, preferably of fresh material, is needed to clarify the differences and permit detailed description.

Population from Holcus mollis : This is probably also an undescribed species close to *A. agrostis* but differing, for example, in the detailed shape of the tail tip (Fig. 2 and page 138) and the heat-relaxed shape of the males (page 136). The specimens used in the present study were not in good enough condition for detailed description but an English source of fresh material exists (Table 1) and an Australian population on *Holcus lanatus* is believed to be the same species (see pages 136 and 138).

UNEXAMINED SPECIES

The following comments are based on published information.

Anguina poophila Kir'yanova, 1952 was synonymised with *A. agrostis* by Brzeski (1981) after study of types and other material, and Fortuner and Maggenti (1987) agreed. However Kir'yanova (1952) illustrated a male with dorsal curvature (though it may not have been heat-relaxed) and referred to a difference in tail-tip shape from *A. agrostis*. These features may be important taxonomically and should be re-examined.

Anguina phalaridis (Steinbuch, 1799) Chizhov, 1980. Steinbuch (1799) described and illustrated *A. (= Vibrio) phalaridis* from galls in flowers of *Phalaris phleoides vivipara*, an early name for *Phleum phleoides*. He distinguished *A. (= Vibrio) phalaridis* from *A. (= Vibrio) agrostis* on the gross appearance and colour of the worms, different host plant, different shape of the gall, and different effects on host flowers. Goodey (1930) considered that Steinbuch's criteria were of little value and that *A. (= Vibrio) phalaridis* was probably synonymous with *A. agrostis*, though subject to examination of fresh material from galls on the type host. Goodey's suggested synonymization was taken as definite by later authors. Goodey (1930) also referred to Horn (1889) who described and illustrated a species of *Anguina* (then included in *Tylenchus*) from galls in flowers of *Phleum boehmeri (= P. phleoides)* near Waren, Germany (now DDR). Horn assumed that his nematode was identical with Steinbuch's *Vibrio phalaridis* but (incorrectly) renamed it *Tylenchus phlei* because of the change in name of host genus; he also mentioned the finding (by Prof. Münster, of Greifswald) of the same parasite on *Koeleria glauca*, but it remains unconfirmed that the nematodes were the same species. Horn's excellent drawings show typical coiled females (one with twice-flexed ovary), dorsally curved male, juvenile, and details

of anterior and posterior ends. The tail-tips of both sexes appear minutely rounded rather than sharp-pointed which, together with the male curvature, suggest that Horn's species was not *A. agrostis sens. strict.* Chizhov (1980) reinstated Steinbuch's name *(phalaridis)* for *Anguina* populations from *Phleum phleoides, Poa angustifolia* and *P. pratensis* from the Moscow region, which he distinguished from *A. agrostis* on *Agrostis tenuis (= A. capillaris)* and *A. stolonifera* on the basis of differences in size of all stages and shape of adults, and showed that the two groups did not cross-infect. However, Chizhov's action is questionable because (a) differences in size and shape of *Anguina* adults, especially females, are not necessarily specific but may be related to nutrition and degree of maturity and (b) material from the type locality was not available for comparison. To confirm the status of *A. phalaridis, Anguina* on *Phleum phleoides* should be collected as near as possible to the type locality (Erlangen). Alternatively Horn's (1889) locality (neighbourhood of Waren, DDR) might be chosen, especially as Horn's description is much better than Steinbuch's. However, if further studies confirm that Chizhov's Russian population on *Phleum phleoides* differs from *A. agrostis* on its type host, then there would in any case be as much justification for using the name *A. phalaridis* as *A. agrostis* for which equally no types exist and no material from the type locality is known. Meanwhile *Anguina phalaridis* is regarded as a *species inquirenda.*

Other species : The following are accepted on the basis of the published descriptions, but further study is desirable; information is often lacking on important characters, e.g. heat-relaxed posture of male and form of tail extremity under high magnification : *A. agropyri* Kir'yanova, 1955 (for comment on authority see Brzeski, 1981; redescribed by Kir'yanova and Ivanova, 1968); *A. agropyronifloris* Norton, 1965; *A. amsinckiae* (Steiner & Scott, 1935) Thorne, 1961 (description supplemented by Thorne, 1961); *A. australis* Steiner, 1940 (redescribed by van den Berg, 1986); *A. balsamophila* (Thorne, 1926) Filip'ev, 1936; *A. caricis* Solov'eva & Krall, 1982; *A. cecidoplastes* (Goodey, 1934) Flip'ev, 1936; *A. guizotiae* van den Berg, 1986; *A. microlaenae* (Fawcett, 1938) Steiner, 1940; *A. pacificae* Cid del Prado Vera & Maggenti, 1984 (the last apparently with fine attenuated tail-tip like the Canadian arctic populations). *A. pharangii* Chizhov, 1984 was transferred to *Subanguina* by Siddiqi (1986). *A. amsinckiae, A. balsamophila* and *A. guizotiae* are unusual in the genus in having dicotyledonous hosts; all other species are parasites of Gramineae.

Remarks

USE OF MEASUREMENTS

Body measurements and proportions have limited value in taxonomy and identification of *Anguina* species,

especially those of females whose dimensions, proportions and anatomy are much affected by state of development (Fisher, McKay & Dubé, 1984), nutrition-related factors (including probably host species and growing conditions) and method of killing and fixation (Stynes & Bird, 1981 *a*). As a result of sampling adults of *A. funesta* at weekly intervals during spring, Fisher, McKay and Dubé (1984) found that female length varied by a factor of 2 and female width by a factor of 3. After mating and egglaying, the adults soon die as associated plant parts ripen or senesce; too often, mounted specimens have been collected after onset of these processes. Males are more stable in size and proportions. There may be a case for selecting a male holotype, neotype, etc. when describing or redefining species of *Anguina* and related genera; certainly more attention should be paid to male characters. Of the more stable cuticular structures, spicules and gubernaculum show some interspecific differences but the stylet varies little (length about 10 μm) throughout the genus.

HOST SPECIFICITY - SPECIES OR HOST RACES?

Anguina populations show marked host specificity. Within the Gramineae, specificity is commonly at host-genus level though cross-infection between botanically related genera may occur. In the Pacific Northwest, USA, separate populations of *Anguina,* identified as *A. agrostis,* occur on *Agrostis* and *Festuca* species, sometimes in the same field, but will not cross-infect (Jensen, 1961). Courtney and Howell (1952) successfully transferred *A. agrostis* from *Agrostis tenuis* (= *A. capillaris*) (the type host) to four other species of *Agrostis* and back, but they failed to infect eight other grasses in genera *Phleum, Festuca, Poa, Holcus* and *Anthoxanthum.* In Czechoslovakia, Klumpar (1986) showed that *Anguina* populations (identified as *A. agrostis*) from *Trisetum flavescens* and *Phleum boehmeri* (= *phleoides* - see page 140) failed to cross-infect and also failed to transfer to grasses of genera *Agrostis, Lolium, Poa, Arrhenatherum* and *Dactylis.* In Iraq, Al-Talib *et al.* (1986) reported that a population from barley, identified as *A. tritici,* infected all tested barley *(Hordeum)* cultivars but failed to infect wheat *(Triticum)* cultivars.

Where such biologically separate populations are morphologically indistinguishable or differ only slightly, the question arises whether they are host races or sibling species. In practice the genus *Anguina* may be a complex of host races within a few closely related species, for example the *Festuca* and *Dactylis* populations studied here may be host races within *A. graminis* (page 139). Such questions can only be resolved by biological and genetic studies. Meanwhile it is best to identify, and where possible name as species, morphologically distinct forms which can be related to one or more host genera. With current knowledge of the taxonomy of this group, excessive lumping could create more problems for fu-

ture workers than naming species on rather few, constant differences.

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