Cytologia **44**: 457–466, 1979

Cytogenetic Studies in North American Minnows (Cyprinidae) VI. Karyotypes of thirteen species in the genus *Notropis*

J. R. Gold, C. W. Whitlock, W. J. Karel, and J. A. Barlow, Jr.

Genetics Section, Texas A&M University,
College Station, Texas 77843, U. S. A.

Received September 28, 1977

The cyprinid fishes (minnows) endemic to the North American continent comprise an ecologically diverse array of 35–40 genera and some 250 species. Most of these are currently placed in a single subfamily, the Leuciscinae, and are thought to have originated from a single or few ancestors that migrated to North America from Eurasia not prior to the Miocene (Miller 1959). Most leuciscine fossils in North America, however, date from the Pliocene and Pleistocene periods (Miller 1965, Kimmel 1975, Smith 1975), suggesting that many species are of recent origin. Gold *et al.* (1978) estimated the rate of speciation within living North American cyprinid genera to be approximately 0.7 new species per lineage (genus) per million years. This rate is considerably higher than speciation rates estimated for many lower vertebrate taxa (Bush *et al.* 1977).

In this study, we continue our search for genetic changes whose evolutionary rate corresponds with the relatively rapid rate of speciation exhibited by these fishes. Those genetic changes whose evolutionary rate correlates with rates of speciation and organismal evolution may lie at the basis of progressive evolution (Wilson *et al.* 1975, Wilson 1976). Herein, we describe karyotypes of thirteen species from the genus *Notropis,* the most speciose of all North American cyprinid genera containing well over 100 living representatives (Miller 1965). The lone *Notropis* fossil found in North America is tentatively dated to the Pliocene (Miller 1965), which suggests that most of the evolution in the genus has taken place during the last ten million years. Thus, if gross chromosomal rearrangement has contributed to speciation in *Notropis* (as suggested for other vertebrate groups —Wilson *et al.* 1975, Bush *et al.* 1977), we might expect to find extensive chromosomal rearrangement within the genus. Karyotypes of six species of *Notropis* have been reported previously (Denton and Howell 1969, Lieppman and Hubbs 1969, Campos and Hubbs 1973, Greenfield *et al.* 1973), and all six had essentially similar karyotypes of 50 (diploid) chromosomes.

Materials and methods

The thirteen *Notropis* species were collected by seining in Texas and Louisiana, and returned live to Texas A&M University. The species (collection sites) were

as follows: *N. lutrensis, N. oxyrhynchus, N. shumardi,* and *N. venustus* (Brazos R., Brazos Co., Tx.); *N. fumeus* and *N. umbratilis* (Sabine R., Jaspar Co., Tx.); *N. sabinae* and *N. texanus* (Neches R., Hardin Co., Tx.); *N. volucellus* (Brazos R., Bell Co., Tx.); *N. chrysocephalus, N. longirostris* and *N. roseipinnis* (Tangipahoa R., Tangipahoa Par., La.); and *N. signipinnis* (Pearl R., Washington Par., La.). All specimens were deposited in reference collections at the Department of Wildlife and Fisheries Science, Texas A & M University.

Details of the chromosome preparation technique may be found in Gold (1974). Chromosome number counts were made from negatives; those showing the best-spread metaphases were developed into positives and arranged into karyograms. Measurements of relative chromosome lengths were made using precision calipers, and each chromosome was scored by its centromeric index using the four long arm-short arm ratio (r) groupings of Levan *et al.* (1964): i. e., median,

Table 1. Chromosome number counts from 13 *Notropis* species

Taxon	Number of individuals	Number of cells counted	% Modal counts	Modal 2n number
N. chrysocephalus	4	74	61	50
N. fumeus	4	70	59	50
N. longirostris	2	40	60	50
N. lutrensis	5	102	63	50
N. oxyrhynchus	3	70	56	50
N. roseipinnis	3	61	71	50
N. sabinae	4	67	58	50
N. shumardi	3	59	68	50
N. signipinnis	3	76	62	50
N. texanus	6	89	68	50
N. umbratilis	4	78	56	50
N. venustus	5	98	63	50
N. volucellus	3	63	78	50

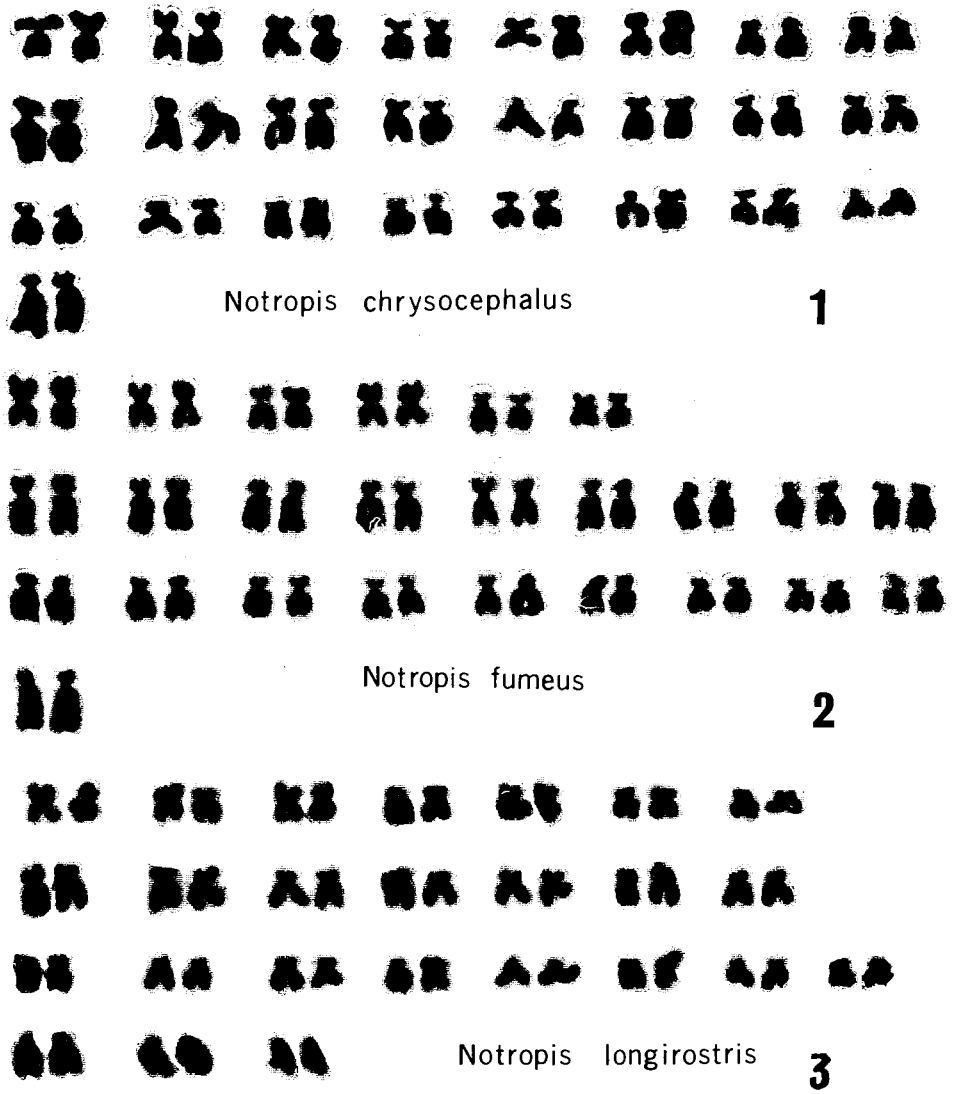
r=1.0–1.7; submedian, r=1.7–3.0; subterminal r=3.0–7.0; termimal, r=7.0–∞. Chromosome pairs were arranged into groups as described by Bickham (1975): group A chromosomes have centromeres median to submedian (*msm*); group B chromosomes have centromeres subterminal to terminal (*stt*). Fundamental chromosome arm numbers for each species were estimated by scoring group A (*msm*) chromosomes as bi-armed, and group B (*stt*) chromosomes as uni-armed.

Results

Chromosome count data are listed in Table 1. Usually, at least 10–15 cells were counted from each individual. Distinct modes of 2n=50 chromosomes (between 56–78% of counts) were found for each species. Chromosome numbers of *N. lutrensis, N. texanus,* and *N. venustus* were described previously (Lieppman and Hubbs 1969, Campos and Hubbs 1973), and were the same as found here.

Cells not yielding modal counts were invariably short by one or more chro-

mosomes, and were presumed to result from chromosome loss during preparation, overlap, or miscounting. Cells with more than 50 chromosomes were exceedingly infrequent (<1% of all counts), and likely stemmed from premature chromatid

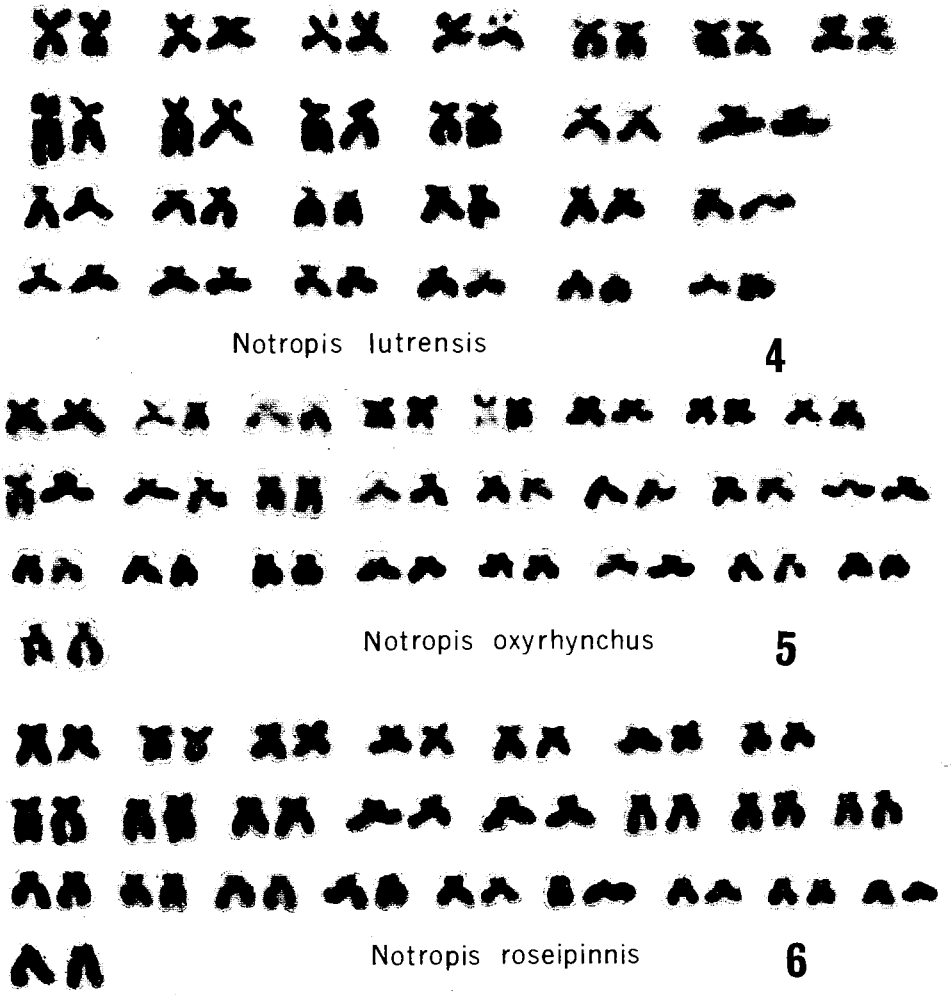


Figs. 1–3. 1, somatic metaphase chromosomes (from kidney) of *Notropis chrysocephalus* (2n=50). 2, somatic metaphase chromosomes (from kidney) of *Notropis fumeus* (2n=50). 3, somatic metaphase chromosomes (from kidney) of *Notropis longirostris* (2n=50).

separation or miscounting. No indication of chromosomal polymorphism was found. Except for *N. longirostris,* at least one male and female were examined from each species. Sex chromosomes were not identified. Modal karyograms for each species are shown in Figs. 1–13. A fortuitous but infrequent shading

effect of phase contrast microscopy accounts for the unusual photograph of the *N. umbratilis* karyotype (Fig. 11).

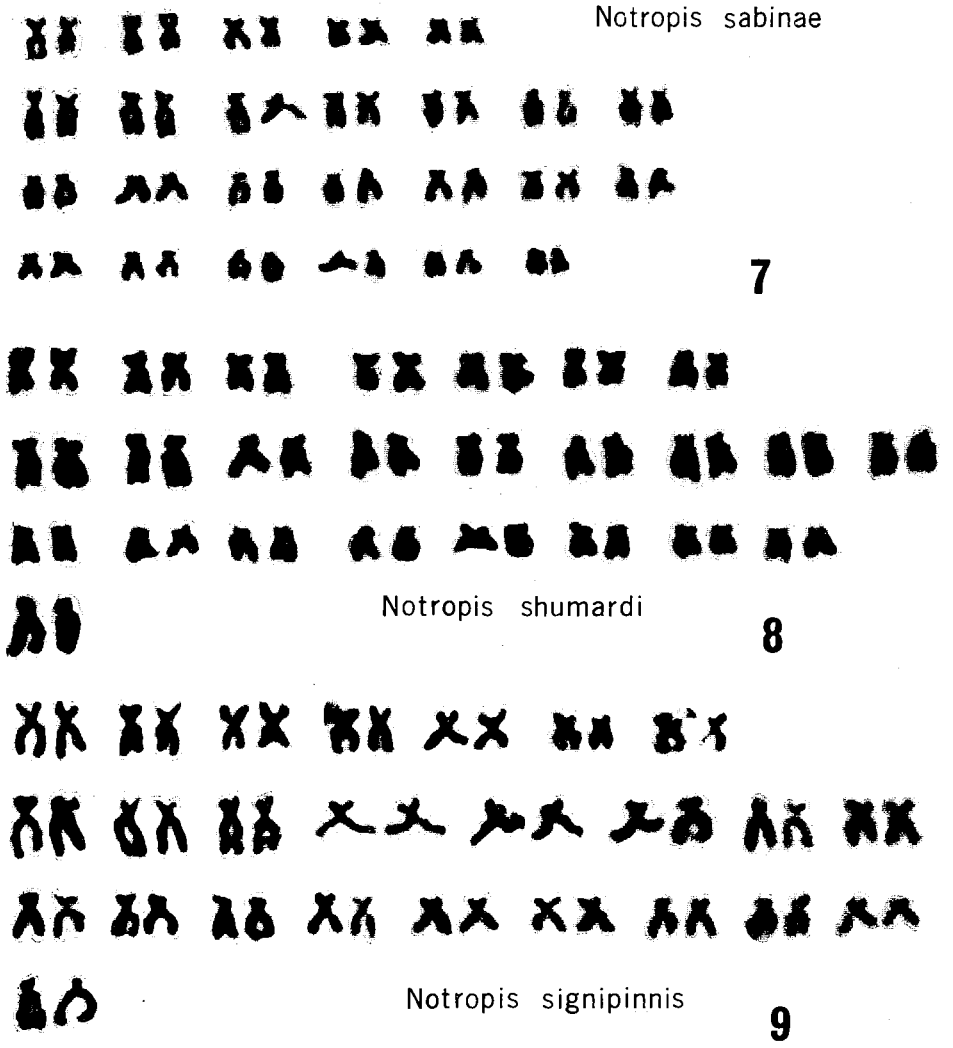
Karyotypes of all thirteen *Notropis* species were highly asymmetric, typical of most cyprinids (Avise and Gold 1977). Each contained a continuum of chromosomes graded in size, with measured centromere locations ranging from approxi-



Figs. 4–6. 4, somatic metaphase chromosomes (from kidney) of *Notropis lutrensis* (2n=50). 5, somatic metaphase chromosomes (from kidney) of *Notropis oxyrhynchus* (2n=50). 6, somatic metaphase chromosomes (from kidney) of *Notropis roseipinnis* (2n=50).

mately median to nearly terminal. Attempts to classify chromosomes by centromere location within size groupings were unsuccessful. Most species were scored as having between 10–18 chromosomes with median centromeres, and between 24–38 chromosomes with submedian centromeres. Between 2–8 chromosomes in each species were too close to the median-submedian border to classify unambiguously.

For that reason, chromosomes with median or submedian centromeres were summed into one category (group A=*msm*=bi-armed chromosomes), and chromosomes with subterminal or terminal centromeres were summed into a second (group B=*stt*=uni-armed chromosomes).



Figs. 7–9. 7, somatic metaphase chromosomes (from kidney) of *Notropis sabinae* (2n=50). 8, somatic metaphase chromosomes (from kidney) of *Notropis shumardi* (2n=50). 9, somatic metaphase chromosomes (from kidney) of *Notropis signipinnis* (2n=50).

Chromosome formulae and haploid arm number estimates for each species appear in Table 2, and show differences which reflect varying numbers of measured *stt* chromosomes: *N. longirostris* was scored as having three *stt* pairs (47 arms); *N. volucellus,* two *stt* pairs (48 arms); and *N. chrysocephalus, N. fumeus, N. oxyrhynchus, N. roseipinnis, N. shumardi, N. signipinnis, N. texanus,* and *N. venustus,* one *stt*

pair (49 arms). Three species (*N. lutrensis, N. sabinae,* and *N. umbratilis*) were scored as having all *msm* chromosomes (50 arms).

Arm number variation among these species, and among all North American cyprinids, should be interpreted with caution. With currently applied tech-

Figs. 10–12. 10, somatic metaphase chromosomes (from kidney) of *Notropis texanus* (2n=50). 11, somatic metaphase chromosomes (from kidney) of *Notropis umbratilis* (2n=50). 12, somatic metaphase chromosomes (from kidney) of *Notropis venustus* (2n=50).

niques of fish chromosome preparation (Gold 1979), it is not possible to ascertain unequivocally which chromosomes are homologous across species; nor is it possible to determine with certainty that chromosomes paired in karyograms are homologues. Further, in most cyprinids examined, a few chromosomes invariably fall close to the *msm–stt* border making classification to either group A or

group B at best partially subjective. Nonetheless, there is evidence (variation in estimated arm number and karyotype asymmetry) that rearrangements affecting centromere position, but not chromosome number, have occurred during the history of North American cyprinid evolution.

Table 2. Chromosome arm number estimates from modal karyotypes of 13 *Notropis* species

Taxon	Chromosome formula† (A: B)	Fundamental arm number (haploid)
N. chrysocephalus	24: 1	49
N. fumeus	24: 1	49
N. longirostris	22: 3	47
N. lutrensis	25: 0	50
N. oxyrhynchus	24: 1	49
N. roseipinnis	24: 1	49
N. sabinae	25: 0	50
N. shumardi	24: 1	49
N. signipinnis	24: 1	49
N. texanus	24: 1	49
N. umbratilis	25: 0	50
N. venustus	24: 1	49
N. volucellus	23: 2	48

† A=*msm* chromosomes; B=*stt* chromosomes.

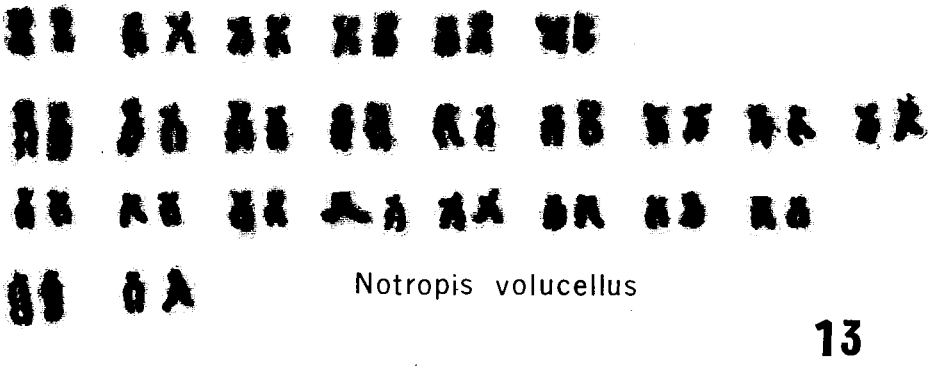


Fig. 13. Somatic metaphase chromosomes (from kidney) of *Notropis volucellus* (2n=50).

Gold and Avise (1977) noted that several North American cyprinids possessed an unusually long *stt* chromosome pair which might be useful in systematics. Of the thirteen *Notropis* species examined, all but four (*N. lutrensis, N. sabinae, N. umbratilis,* and *N. venustus*) had at least one long *stt* chromosome pair; *N. longirostris* was scored as having two long *stt* pairs. Whether or not this particular chromosome is homologous across species, and hence of use in cyprinid systematics, remains to be seen.

Discussion

Chromosomal evolution in the genus *Notropis* appears to have been no more extensive than among other North American cyprinids. All sixteen *Notropis* species karyotyped (including *N. callistius, N. cornutus,* and *N. stilbius*) have 2n=50 chromosomes, and estimated (haploid) chromosome arm numbers vary only slightly, from 46–50 (Denton and Howell 1969, Greenfield *et al.* 1973, this paper). Chromosome numbers are known for 34 other North American cyprinid species (21 genera), and all but five have 2n=50 chromosomes (references in Gold *et al.* 1978). The reported variation in (haploid) arm number of 23 species (19 genera) which have 2n=50 chromosomes ranges from 43–50, but only one species (*Richardsonius egregius*) falls outside the range 46–50 (Greenfield and and Greenfield 1972, Greenfield *et al.* 1973, Uyeno and Miller 1973, Howell and Villa 1976, Gold and Avise 1977, Gold *et al.* 1978).

The apparent conservatism of gross chromosomal evolution in *Notropis,* and in North American Cyprinidae, is of significance in view of recent studies which show that in several plant and animal taxa rates of gross karyotypic change are highly correlated with rates of speciation (Wilson 1976, Wilson *et al.* 1975, Levin and Wilson 1976, Bush *et al.* 1977). Briefly, rapidly evolved taxa appear to have undergone more extensive and rapid chromosomal rearrangement than slowly evolved taxa, and it has been postulated that "genetic revolutions" important to speciation may occur through gene rearrangement rather than through changes in structural genes. It is thought that chromosomal rearrangement could facilitate speciation either by functioning as a cytogenetic reproductive isolating mechanism, by altering patterns of gene expression (regulation), or by creating adaptive "supergenes" which cannot easily be broken up by recombination (White 1968, 1973, Grant 1973, Bush *et al.* 1977).

Cyprinids are highly speciose in North America (250 species in 35–40 genera), and judging from the fossil record, have evolved relatively recently. The oldest fossils date to the Miocene (Miller 1965), but most are found in Pliocene and Pleistocene deposits (Miller 1965, Kimmel 1975, Smith 1975). *Notropis* emphasizes the relatively rapid speciation in these fishes since the oldest (and only) *Notropis* fossil is tentatively dated to the Pliocene, and today there are well over 100 living species in the genus (Miller 1965).

The present data suggest that cyprinids have evolved rapidly in North America in the virtual absence of gross chromosomal rearrangement. Gold *et al.* (1978) estimated rates of speciation and chromosome number change within living North American cyprinid genera, and found that fewer chromosome number changes occur per speciation event than in many vertebrates. This is most clearly demonstrated by the evidence from *Notropis*. That so few gross changes in karyotype have occurred in a group where speciation has been so extensive is striking, and clearly indicates that gross chromosomal rearrangement has played only a very minor role in the evolution of these fishes. Howell and Villa (1976) reached a similar conclusion regarding two sympatric, but reproductively well-isolated species in the genus *Rhinichthys*.

The nature of the genetic changes which underlie speciation in North American Cyprinidae is unknown. Studies by Avise (1977) on allozyme differentiation among cyprinids suggest that structural gene evolution is independent of speciation. Changes in regulating genes may be important (Wilson 1976), but not to the extent that gross karyotypic evolution reflects regulatory evolution.

Summary

We have examined karyotypes from thirteen species of the North American cyprinid genus *Notropis: N. chrysocephalus, N. fumeus, N. longirostris, N. lutrensis, N. oxyrhynchus, N. roseipinnis, N. sabinae, N. shumardi, N. signipinnis, N. texanus, N. umbratilis, N. venustus,* and *N. volucellus.* All thirteen species have 2n=50 chromosomes. Estimated haploid arm numbers among the thirteen species ranged from 46–50; one-armed chromosomes (centromeres subterminal to terminal) comprised only a small portion of the karyotype of each species. Chromosomal evolution in *Notropis,* and in North American Cyprinidae, has been remarkably conservative. Almost all species karyotyped have 2n=50 chromosomes, and with one exception, cyprinid species with 50 chromosomes vary in estimated haploid chromosome arm number from only 46–50. In contrast, speciation in these fishes appears to have been rapid. Thus, the present data suggest that gross chromosomal rearrangement has played only a very minor role in the speciation and evolution of these fishes.

Acknowledgments

We thank F. H. Deal and W. D. Womac for their assistance in collecting specimens; and N. M. Kieffer and R. S. Halliwell for the use of their microscopes. Work was supported by funds administered through the Dean's Office of the College of Agriculture, and through the Texas Agricultural Experiment Station of Texas A&M University.

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