

Xiphinema bernardi n. sp. (Nematoda: Longidoridae) from the Great Smoky Mountain National Park

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Abstract: In October 1985 during a survey of fauna of the Great Smoky Mountains National Park, Ernest Bernard recovered a limited number of specimens of a non-described species of *Xiphinema* (Nematoda: Longidoridae) and sent them to the senior author. The species is distinct from other species by its large size and having Z-organs in the genital tract. During July 2006, Dr. Bernard's survey crew took samples in the area where the species was first found and was successful in finding it again. Without Dr. Bernard's efforts, this species could not have been described and thus the new species is named *X. bernardi* n. sp. in his honor. Several female and juvenile specimens of the new species were recovered in a sample from a mixed forest of maple, hemlock, and silverbell. It is distinct from all others in *Xiphinema* group 4 species (with Z-organs) by having a longer total stylet length, 259.8 to 284.2 μm vs < 253 μm for all other species in this group. *Xiphinema bernardi* n. sp. is distinctive because of its long body length (4.45 to 6.00 mm), tail shape, and c' ratio. Of the group 4 species, it most closely resembles *X. phoenicis*. Second, third and fourth stage juvenile descriptions and morphometrics are included. The polytomous key code for *X. bernardi* n. sp. is A4-B1-C6-D56-E56-F(4)5-G4-H2-I34-J5-K2-L1. Molecular approaches using the internal transcribed spacer 1 sequences of nuclear ribosomal DNA suggested that *X. bakeri* and *X. diversicaudatum* are the most closely related species from the species examined.

Key words: DNA sequencing, ITS, juveniles, molecular phylogeny, morphometrics, nematode, Smoky Mountains National Park, taxonomy, *Xiphinema bernardi* n. sp.

During an October 1985 survey of fauna of the Great Smoky Mountains National Park, Ernest Bernard recovered a limited number of specimens of a non-described species of *Xiphinema* (Nematoda: Longidoridae) and sent them to the senior author. This species is distinct from other *Xiphinema* species presented in the area by its very long body and by having z-differentiation in the genital tract. During July 2006, Dr. Bernard's survey crew took samples in the area where the species was originally found and was successful in recovering it. Several female and juvenile specimens were recovered from a mixed forest sample of maple (*Acer* sp.), hemlock (*Tsuga* sp.), and silverbell (*Halesia carolina* L.).

Xiphinema species with Z-differentiations (Z-organs, pseudo-Z-organs, uterine spines) have been rarely reported in the North American continent. The only *Xiphinema* species with Z-organs reported are *X. ebriense* Luc, 1958 from an unconfirmed Florida report and *X. tropicale* Zullini, 1973 from a tropical rainforest in Mexico (Norton et al., 1984). Both species are in *Xiphinema* group 4 in the polytomous key of Loof and Luc (1990). *Xiphinema* species with pseudo-Z-organs with or without uterine spines reported from North America are also limited (Robbins and Brown, 1991). Those reported species are *X. basiri* Siddiqi, 1959 from Mexico and Florida (Norton et al., 1984), *X. coxi coxi* Tarjan, 1964 from Florida (Cho and Robbins, 1990), *X. diversicaudatum* (Micoletzky, 1927) Thorne 1939 from various US locations (Norton et al., 1984, Robbins and Brown, 1991), *X. smoliki* Luc and Coomans, 1988 from

Colorado (the only record of the species), and *X. thorneanum* Luc, Loof and Coomans, 1986 from South Dakota specimens originally identified as *X. vuittenezi* Luc, Lima, Weischer and Flegg, 1964 by Thorne in 1974 (Robbins and Brown, 1991). All the above species are readily distinguished from *X. bernardi* n. sp. by their polytomous key codes (Loof and Luc, 1990; Loof and Luc, 1993; Loof et al, 1996) and more recent code updates from newly described species (Robbins, unpublished).

MATERIALS AND METHODS

During July 2006, Dr. Bernard had a survey crew taking samples in the area where the species was first found and succeeded in finding it again. Several female and juvenile specimens were recovered. Nematodes were extracted from the soil by a combination of sieving-decanting and sucrose centrifugal-flotation and either, killed and fixed, processed to glycerin, and permanently mounted on slides as described by Ye and Robbins (2003) or placed in 1 molar NaCl for molecular study. Permanently mounted specimens were examined using a Nikon Optiphot II compound microscope with Nomarski interference contrast. Measurements were made by using either a Nikon drawing tube or an ocular micrometer. All measurements are expressed in micrometers, except for length (mm) and ratios. Data is expressed as mean \pm standard deviation with minimum to maximum range in parenthesis. Drawings were made with the aid of CorelDRAW.

DNA extraction, PCR, cloning and sequencing were prepared as described by Ye et al. (2004). Cloned plasmids were sequenced in both directions using vector primers T7 and SP6 for sequencing. DNA sequencing was performed by dideoxynucleotide chain termination using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) in an Applied Biosystems 377 automated sequencer (Applied

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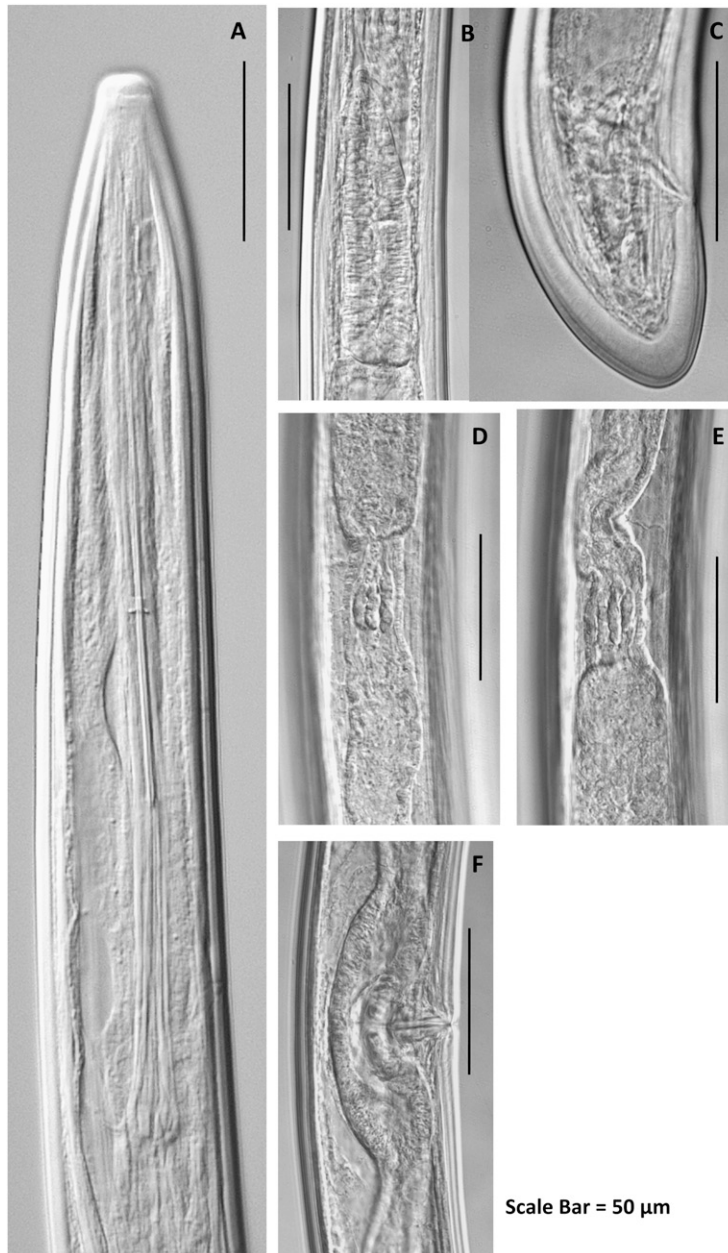


FIG. 1. Holotype female of *Xiphinema bernardi* n. sp. A) Head region with entire stylet. B) Posterior esophagus base. C) Tail. D) Anterior Z-organ. E) Posterior Z-organ. F) Vulval region. Scale bars in each picture.

Biosystems). The sequences were deposited into the GenBank database under accession numbers (EU375482-EU375484). DNA sequences were aligned by ClustalW (<http://workbench.sdsc.edu>, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). *Xiphinema bernardi* n. sp., *X. americanum* Cobb, 1913 and *X. chambersi* Thorne, 1939 were sequenced in this study. In order to investigate the phylogenetic relationships, the ITS1 DNA sequences of *Xiphinema bakeri* Xiph 47, *Xiphinema chambersi* Xiph 2, *Xiphinema americanum* Xiph 59, *Xiphinema americanum* Xiph 10, *Longidorus diadecturus* Long 146 were sequenced from previous study (Ye et al., 2004). The other ITS1 DNA sequences of *Xiphinema* species were

from genBank. *Longidorus diadecturus* Long 146 was selected as the outgroup as it is a species intermediate to *Xiphinema* and *Longidorus* (Ye et al., 2004). The model of base substitution was evaluated using MODELTEST (Posada and Crandall, 1998). The Akaike-supported model, the log likelihood (lnL), the Akaike information criterion (AIC), the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) running the chain for 1,000,000 generations and setting the “burnin” at 1,000. We used MCMC (Markov Chain Monte Carlo)



FIG. 2. Paratype females of *Xiphinema bernardi* n. sp. A-C) Head with entire stylet. D) Anterior genital region. E) Enlarged Z-organ region. F-H) Tail region different shapes.

methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using 50% majority-rule.

SYSTEMATICS

Xiphinema bernardi n. sp.
(Figs. 1-4)

Measurements: Listed in Tables 1 and 2.

Description

Female ($n = 25$ paratypes): Body an open spiral, almost J shaped when heat relaxed and killed, tapering at both

ends. Cuticle appears smooth when examined by light microscope. Head rounded. Amphid opening about 2/3 of head width, amphideal pouch normal stirrup shape. The neck tapers noticeably and increases from the anterior end to about mid-odontostyle. The odontostyle is long and slender, 3 μm wide at junction with the odontophore. Odontophore 4 μm wide at junction with odontostyle, flange 10 μm at widest point. Guide ring about 6 μm wide. Nerve ring around slender anterior esophagus about midway between the odontophore flanges and the expanded esophagus base. Esophagus dorylaimoid with a cylindrical base



FIG. 3. Paratype juveniles of *Xiphinema bernardi* n. sp. A) J2 head. B) J2 tail. C) J3 head. D) J3 tail. E) J4 head. F-G) J4 tails. Scale bars in each picture.

139 (89-156) μm in length, 32 (24-51) μm in width, with one dorsal gland nucleus 19% (14-22%) of the esophageal base length and two subventral nuclei (SV 1 = 50.3% (41.2-51.5%); SV 2 = 53.0% (41.2-53.0%)). The cardia is conoid (almost hemispherical) at the junction of the esophagus base and intestine, about half as wide as esophagus base, rounded posterior portion extending a similar distance into the intestine. The reproductive system is amphidelphic, didelphic, with reflexed ovaries, with no sperm observed in female genital tracts. Three or four sclerotized pieces (z-differentiation), spindle shaped, about 20 μm in length, 4 μm in width, located about 20 μm before the

uterus-oviduct junction, distance from vulva to uterus-oviduct junction 477 (250-440) μm for both anterior and posterior reproductive branches. Vulva a transverse slit in ventral view. Vagina perpendicular to the body axis and extends inward to about one third of the body width. The pre-rectum is generally distinct, 339 (199-408) μm in length. Tail conical-rounded and about as long as wide.

Juveniles: J1 not found. The J2, J3, and J4 measurements are in table 2. The J2 body is almost arcuate with a greater posterior curvature, with a long and conical tail. The J3 body is spiral in shape, similar to that of a female, the tail is conical with a definite ventral mucro

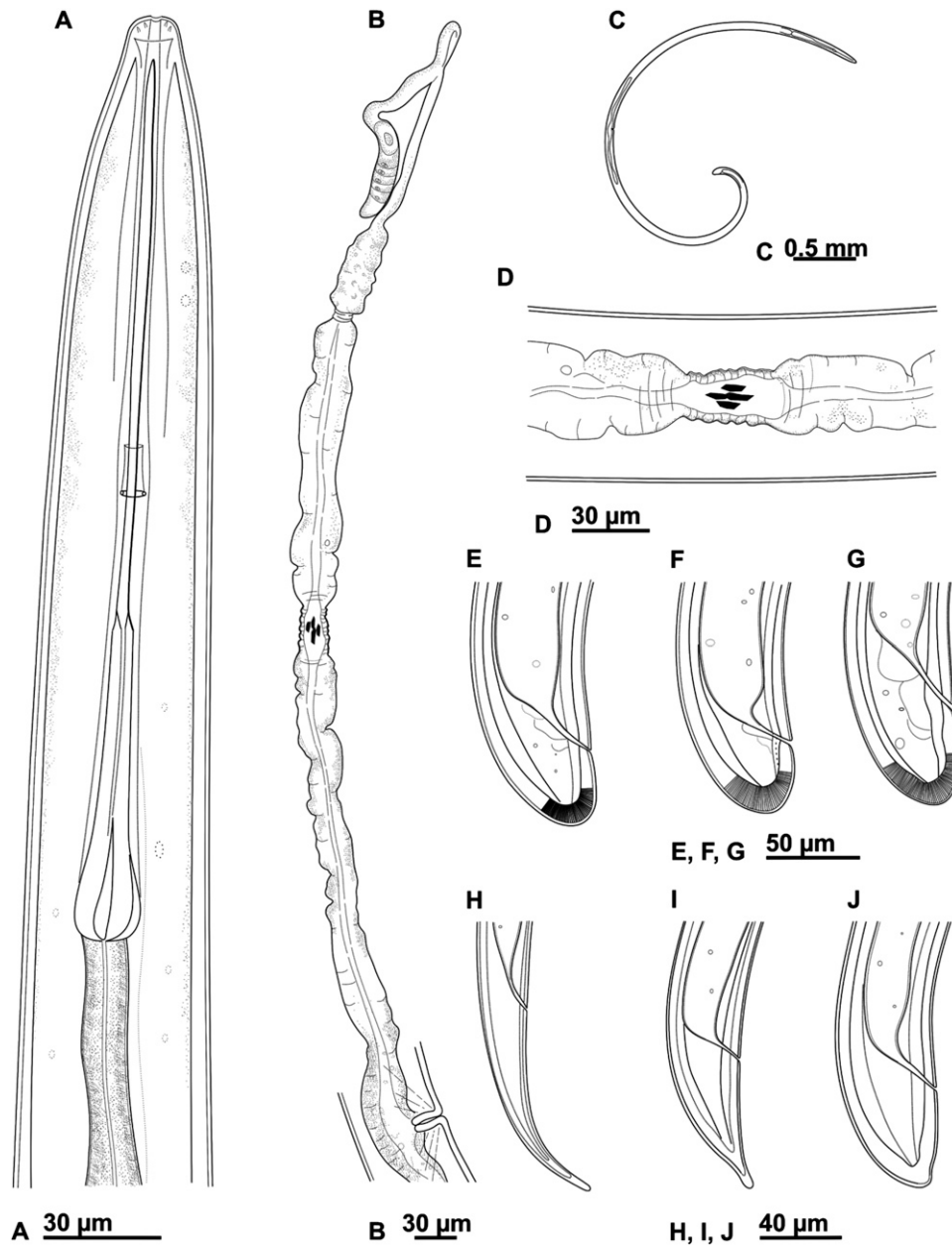


FIG. 4. Drawings of *Xiphinema bernardi* n. sp. paratype females A-G) and juveniles H-J). A) Female head region with entire stylet. B). Anterior genital tract. C). Entire female. D). Genital region showing Z-organ. E-G). Female tails. H) J2 tail. I). J3 tail. J). J4 tail.

or digit. The J4 body spiral is similar to that of a female, the tail is conical and slightly longer than wide. The odontostyle length of juveniles increases with progression of stages from J2 to J4, as does the replacement odontostyle length.

Males: Not found.

Type locality and habitat: Great Smoky Mountains National Park, Sevier County, TN, USA, Laurel Falls Trail, at an elevation of 3,307 ft, in a mixed maple, hemlock and silverbell forest. Specimens collected by Kelly Felderhoff, Monica MacCarroll, and Mark Moore on 6 July 2006. GPS coordinates N 35° 40.874, W83° 36.149.

Type specimens: Holotype female deposited in the Nematology Laboratory Collection, USDA, ARS, Beltsville,

Maryland. Two paratype females are deposited as follows: Department of Nematology, University of California, Riverside; Department of Nematology, University of California, Davis; CABI Bioscience, UK Centre, Surrey, UK; Department of Nematology, Agricultural University, Wageningen, the Netherlands; Institute of Parasitology Collection, Moscow, Russia; and the Canadian National Collection of Nematodes, Ottawa, Ontario, Canada. Remaining paratype specimens (female and all juveniles) are deposited in the Nematology Laboratory Collection, USDA, ARS, Beltsville, Maryland.

Etymology: Named for Dr. Ernest Bernard who first found this new species and made more specimens available.

TABLE 1. Morphometrics of *Xiphinema bernardi* n. sp. females from the Great Smoky Mountain National Park.

Character*	Paratypes ♀♀	1985 survey ♀♀ Laurel Falls	1985 survey ♀♀ Ramsey's Cascades	Holotype ♀
n	25	2	2	1
Body length (L) (mm)	5.19 ± 0.35 (4.45-6.00)	5.69, 5.67	5.41, 5.18	4.66
a	76.3 ± 4.5 (71-93)	76, 77	68, 62	71.7
b	8.6 ± 0.8 (6.4-10.2)	8.8, 8.7	9.1, 9.0	8.2
c	104.8 ± 11 (89-131)	133, 107	140, 122	92
c'	0.9 ± 0.1 (0.8-1.1)	0.8, 1.0	0.8, 0.8	1.0
V	50.1 ± 1.6 (47-55)	52.8, 52.2	50.5, 49.4	55.0
Distance from anterior end to guide ring	160 ± 8.2 (144-173)	151, 147	144, 138	155
Odontostyle length	172 ± 5.4 (164-185)	181, 169	183, 177	173
Odontophore length	98 ± 2.9 (91-104)	102, 101	102, 102	97
Total stylet length	270 ± 6.2 (260-284)	282, 269	284, 278	270
Lip width	16.8 ± 0.8 (16.2-18.3)	16.2, 17.3	17.3, 16.2	16.2
Body width	68 ± 3.9 (59-77)	75, 74	80, 83	65
Anal body width	53 ± 2.6 (49-59)	55, 55	51, 51	51
Tail length	50 ± 4.9 (41-63)	43, 53	39, 43	51
Distance from anterior end to vulva (mm)	2.60 ± 0.16 (2.30-3.02)	3.00, 2.96	2.74, 2.56	2.56
Hyaline tail length	11.4 ± 1.6 (9.1-16.2)	12.2, 14.2	14.2, 13.2	12.2
Anterior genital tract length	0.89 ± 0.02 (0.63-1.34)	0.88	0.96, 1.02	849
Posterior genital tract length	0.86 ± 0.02 (0.56-1.33)	0.86	0.97, 0.95	812
Distance from anterior end to esophagus-intestine junction	606 ± 63.5 (471-808)	643, 651	597, 577	568
J' (Hyaline length/hyaline width)	0.4 ± 0.04 (0.32-0.47)	0.35, 0.41	0.39, 0.38	0.40

*All measurements in μm unless noted otherwise.

Diagnosis: *Xiphinema bernardi* n. sp. is assumed to be a parthenogenetic species because of the absence of sperm in the female genital tract and no males. It is characterized by its body length (4.45-6.00) mm, long odontostyle (164-185) μm, the presence of Z-differentiation, and a short conically rounded tail (c' = 0.8-1.1). The females most closely resemble those of *X. phoenicis* Loof, 1982, a group 4 amphimictic species. The code for identifying the new species according to the polytomous key of Loof and Luc (1990) is: A4-B1-C6-D56-E56-F(4)5-G4-H2-I34-J5-K?-L1.

Relationships: *Xiphinema bernardi* n. sp. is most similar to all North American species reported with Z-

differentiation, groups 4 and 5 of the polytomous key of Loof and Luc (1990). *Xiphinema* species with Z-differentiations (Z-organs, pseudo-Z-organs, uterine spines) have been rarely reported in North America. *Xiphinema* species with Z-organs reported (group 4) are *X. ebriense* and *X. tropicale* by Norton et al. (1984). *Xiphinema* species with pseudo-Z-organs with or without uterine spines (group 5) reported are *X. basiri* (Norton et al., 1984), *X. coxi coxi* (Cho and Robbins, 1990), *X. diversicaudatum* Thorne 1939 (Norton et al., 1984, Robbins and Brown, 1991), *X. smoliki* and *X. thorneanum* from South Dakota. The closest species not found in North America are *X. phoenicis* Loof, 1983, and *X. rotundatum*

TABLE 2. Morphometrics of *Xiphinema bernardi* n. sp. juveniles from the Great Smoky Mountain National Park.

Character	J2	J3	J4
n	4	3	5
Body length (L) mm	1.72 ± 0.03 (1.71-1.77)	2.84 ± 0.028 (2.60-3.15)	3.67 ± 0.042 (3.36-4.37)
a	42.3 ± 2.0 (40-44)	60.1 ± 3.9 (56-63)	65.0 ± 3.3 (62-69)
b	5.0 ± 0.1 (4.9-5.0)	6.3 ± 0.4 (5.9-6.8)	6.6 ± 0.7 (5.8-7.4)
c	16.5 ± 1.1 (15-18)	43.8 ± 7.4 (36-50)	71.9 ± 4.2 (66-76)
c'	4.7 ± 0.3 (4.3-4.8)	1.7 ± 0.3 (1.5-2.0)	1.1 ± 0.1 (1.0-1.2)
Distance anterior end to guide ring	85.3 ± 9.5 (75-97)	92.7 ± 4.2 (89-97)	128.7 ± 12.0 (112-142)
Odontostyle length	97.4 ± 1.7 (95-100)	119.8 ± 7.3 (118-122)	144.9 ± 2.3 (142-148)
Odontophore length	63.9 ± 1.2 (63-65)	66.3 ± 2.3 (65-69)	83.6 ± 1.7 (81-85)
Total stylet length	161.4 ± 1.2 (160-162)	186.1 ± 1.2 (185-187)	228.6 ± 3.1 (225-234)
Replacement odontostyle length	121.8 ± 1.2 (120-124)	144.1 ± 7.3 (138-152)	167.7 ± 4.4 (162-175)
Lip width	10.7 ± 0.6 (10.2-11.2)	11.8 ± 0.6 (11.2-12.2)	14.0 ± 0.5 (13.2-14.2)
Body width	40.9 ± 1.7 (38-43)	47.4 ± 3.1 (45-51)	56.4 ± 5.8 (49-65)
Anal body width	22.6 ± 0.5 (22.3-23.3)	38.2 ± 3.8 (35.5-42.6)	47.5 ± 3.4 (45-53)
Tail length	105.1 ± 6.9 (95-112)	65.6 ± 6.5 (61-73)	51.2 ± 6.9 (45-63)
Hyaline tail length	28.7 ± 1.2 (28-30)	18.3 ± 3.5 (56-63)	11.0 ± 1.8 (8-12)
Distance from anterior end to esophagus-intestine junction	347.8 ± 6.8 (340-356)	452.6 ± 26.6 (422-472)	554.8 ± 60.8 (480-629)
J' (Hyaline length/hyaline width)	4.9 ± 0.7 (4.3-5.6)	1.3 ± 0.3 (1.0-1.5)	0.5 ± 0.1 (0.4-0.5)

nematodes is shown in Figures 3. The closest inferred relative of *X. bernardi* n. sp. in ITS1 analysis was *X. bakeri* with 99% support. The next closest relative is *X. diversicaudatum*. Although the 3 species comprised of a monophyletic clade with 100% support, they belong to 3 different groups defined by Loof and Luc (1993). *Xiphinema bernardi* n. sp. is in A4 group with the Z-organ, *X. bakeri* is in group A7 without Z-organ and *X. diversicaudatum* is in group A5 with a pseudo-Z-organ. The tree has resolved many highly supported monophyletic groups, including: 1). *X. bernardi* n. sp. and *X. bakeri*. 2). *X. bernardi* n. sp., *X. bakeri* and *X. diversicaudatum*. 3). *X. insigne* Loos, 1949, *X. elongatum* Schuurmann-Stekhoven and Teunissen, 1938 and *X. setariae* Luc, 1958. 4). *X. chambersi* Thorne, 1939, *X. insigne*, *X. elongatum* and *X. setariae*. 5). *X. chambersi*, *X. insigne*, *X. elongatum*, *X. setariae* and *X. italiae* Meyl, 1953. 6). *X. bernardi* n. sp., *X. bakeri*, *X. diversicaudatum*, *X. chambersi*, *X. insigne*, *X. elongatum*, *X. setariae* and *X. italiae*. 7). *X. hunaniense* Wang and Wu, 1992 and *X. index* Thorne and Allen, 1950. 8). *X. bernardi* n. sp., *X. bakeri*, *X. diversicaudatum*, *X. chambersi*, *X. insigne*, *X. elongatum*, *X. setariae*, *X. italiae*, *X. hunaniense* and *X. index*. 9). *X. americanum*, *X. thornei* Lamberti and Golden, 1986, *X. oxycaudatum* Lamberti and Bleve-Zacheo, 1979, *X. rivesi* Dalmasso, 1969, *X. diffusum* Lamberti and Bleve-Zacheo, 1979, *X. brevicollum* Lordello and Da Costa, 1961 and *X. incognitum* Lamberti and Bleve-Zacheo, 1979. 10). *X. brasiliense* Gonzaga and Lordello, 1951 and *X. krugi* Lordello, 1955. 9). *X. brasiliense*, *X. krugi* and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (Siddiqi and Lamberti, 1977). 11). *X. americanum*, *X. thornei*, *X. oxycaudatum*, *X. rivesi*, *X. diffusum*, *X. brevicollum*, *X. incognitum*, *X. brasiliense*, *X. krugi*, *X. brasiliense*, and *X. pachtaicum*. Thus, the molecular data provided additional information to the groupings and phylogeny mainly defined by morphology (Loof and Luc, 1990, Coomans et al., 2001).

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