A molecular perspective on the genera *Paragonimus* Braun, *Euparagonimus* Chen and *Pagumogonimus* Chen

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Abstract

The status of the genera *Euparagonimus* Chen, 1963 and *Pagumogonimus* Chen, 1963 relative to *Paragonimus* Braun, 1899 was investigated using DNA sequences from the mitochondrial cytochrome *c* oxidase subunit I (CO1) gene (partial) and the nuclear ribosomal DNA second internal transcribed spacer (ITS2). In the phylogenetic trees constructed, the genus *Pagumogonimus* is clearly not monophyletic and therefore not a natural taxon. Indeed, the type species of *Pagumogonimus*, *P. skrjabini* from China, is very closely related to *Paragonimus miyazakii* from Japan. The status of *Euparagonimus* is less obvious. *Euparagonimus cenocopiosus* lies distant from other lungflukes included in the analysis. It can be placed as sister to *Paragonimus* in some analyses and falls within the genus in others. A recently published morphological study placed *E. cenocopiosus* within the genus *Paragonimus* and probably this is where it should remain.

Introduction

Lung flukes (family Paragonimidae Dollfus, 1939) occur as adults in the lungs of mammals in Asia, Africa and the Americas (reviewed in Blair *et al*., 1999). Snail hosts occur in fresh and brackish waters. Mammals are usually infected by eating freshwater crustaceans containing metacercariae. Several species of lungflukes infect humans in various parts of the world. The taxonomy of the family presents some problems. At least fifty species have been named, about half of these from China alone. Several generic groupings have been proposed for the family.

The type genus, *Paragonimus* Braun, 1899, was erected to contain *P. westermani* (Kerbert, 1878), the human lung fluke from Asia. In the 1960s, Chen, working in China, proposed two additional genera. For *Paragonimus skrjabini* Chen, 1959 from Guangdong Province, he erected the genus *Pagumogonimus* Chen, 1963. Chen (1963, 1964a,b) distinguished *Pagumogonimus* from *Paragonimus* primarily by the fact that the metacercaria of the former possessed 72 flame cells (60 in *Paragonimus*). Later (Chen, 1977), he placed more emphasis on adult characters and in

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particular the elongate body shape and the location of the ventral sucker in the anterior one-third of the body. On the basis of these adult characters, he (1977) added to *Pagumogonimus* the species *P. yunnanensis*, *P. harinasutai*, *P. proliferus*, *P. bangkokensis*, *P. cheni* and *P. macrorchis*. Of these, at least two species, *P. macrorchis* and *P. cheni* have metacercariae with 60 flame cells. Chen was the editor of a monograph on paragonimids published in 1985. In the introductory section of this, he provided a key distinguishing between *Paragonimus* and *Pagumogonimus* solely on the basis of the adult characters mentioned above. The name *Pagumogonimus* continues to be used by Chinese workers but tends not to be used by taxonomists outside China (e.g. Miyazaki, 1974; Kurochkin, 1987) who place all species in the genus *Paragonimus*.

The genus *Euparagonimus* was erected by Chen (1963) for *E. cenocopiosus* Chen, 1962 from Guangdong Province. Metacercariae, juvenile and adult worms all possess an excretory bladder extending anteriorly only as far as the ventral sucker (Chen, 1962, 1965). This is a very distinctive feature. The excretory bladder in *Paragonimus* species extends to well in front of the ventral sucker. Apart from this, there are no major differences between *Euparagonimus* and *Paragonimus*. The genus was placed in a new subfamily, Euparagoniminae, by Chen in 1963. A second species, *E. hongzesiensis*, was described by Fu *et al*. (1990) from Jiangsu Province.

Most authors have retained *Euparagonimus* as a genus distinct from *Paragonimus*. There has been little critical discussion. Zhong *et al*. (1981) commented on some apparently unpublished work by Liu in saying 'Recently,Liu *et al*. (July 1980) have shown that the naming of *Euparagonimus cenocopiosus* Chen, 1962 as a new subfamily and new genus was erroneous. Liu *et al*. (July 1980) have found evidence to designate it *Paragonimus brevivesicarius*'. The specific name, *brevivesicarius*, clearly cannot be used even if the species is transferred to the genus *Paragonimus*: the name *cenocopiosus* has priority. Apart from this, the genus *Euparagonimus* has been retained as a distinct entity in the literature. Metacercariae resembling those of *Euparagonimus* have been found in the Americas and Africa (Miyazaki *et al*., 1980; Ollivier *et al*., 1995).

Chen (1963) proposed two new subgenera within *Paragonimus*. These he called *Rodentigonimus* and *Megagonimus*. Later he proposed (Chen, 1985) to abandon the use of these names. They will not be considered further here.

In this study we investigate the relationships of *Pagumogonimus* and *Euparagonimus* to *Paragonimus* using DNA sequence data. We chose partial mitochondrial cytochrome c oxidase subunit I (COI) and complete nuclear rDNA internal transcribed spacer 2 (ITS2) gene.

Materials and methods

Many sequences used in this study have been reported elsewhere (Blair *et al*., 1997, 1998) and are given in table 1. Representatives of the nominal genus *Pagumogonimus* were *P. skrjabini* (Chen, 1959) from China and *P. macrorchis* (Chen, 1962) from Thailand. Sequences of these species were reported in Blair *et al*. (1998). The specimens of *P. mexicanus* sequenced were raised to adulthood in experimental hosts using metacercariae collected from freshwater crabs (*Hypolobocera* sp.) from El Cañero River, La Concordia, Quinindé, Esmeraldas Province, Ecuador and prepared by J.C. Vieira. Metacercariae of *E. cenocopiosus* were collected from the heart of the freshwater crab *Sinopotamon fujianense* (identification by Fujian Institute of Parasitic Diseases) from small streams in Qujiang Administrative Village, Shuyang Township, Nanjing County in southern Fujian Province, China and from East Chenyang Stream, Chendong Natural Village, Chenyang Administrative Village, Langu Township, Wuyishan City in the north of the same Province. The two localities are about 350 km apart. Adult worms were raised in experimentally infected dogs and frozen at –70°C until required for PCR and sequencing. These adult worms were identified morphologically as *E. cenocopiosus* according to Chen & He (1965). Slide-mounted specimens

Table 1. Sources of material and published partial CO1 and ITS2 gene sequences for members of the nominal genera *Paragonimus* (*Pa.*), *Pagumogonimus* (*Pg.*) and *Euparagonimus*.

Species	Origin	ITS2	COI
Pa. westermani	Hyogo, Japan	U96907	U97205
	Leyte, Philippines	U96910	U97213
	Thailand	AF159604	U97212
	Malaysia	U96909	U97211
Pa. siamensis	Thailand	AF159605	AF159599
Pa. heterotremus	Thailand	AF159603	AF159597
Pa. harinasutai	Thailand	AF159609	AF159600
Pa. miyazakii	Miyazaki, Japan	U96912	U97215
Pa. ohirai	Tanegashima, Japan	U96911	U97214
Pa. mexicanus	Ecuador	AF159607	AF159596
Pa. kellicotti	USA	AF159606	–
Pg. skrjabini	Sichuan, China	U96913	U97216
Pg. macrorchis	Thailand	AF159608	AF159598
E. cenocopiosus	Nanjing, Fujian, China	AF159601	AF159595
E. cenocopiosus	Wuyishan, Fujian, China	AF159602	AF159594
Fasciola hepatica	USA	–	M93388

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Table 2. Pairwise differences among CO1 nucleotide sequences (ignoring an insertion three nucleotides long in *Fasciola hepatica*). Values above the diagonal are transitions/transversions. Those below are amino acid differences. Abbreviations of generic names as in table 1.

	Species	Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*Pa. westermani*	Japan	–	37/3	36/3	40/5	48/14	51/23	51/26	51/28	55/26	62/21	54/29	52/26	39/28	41/26	56/47
2	*Pa. westermani*	Malaysia	1	–	28/4	29/4	47/17	44/26	44/23	44/25	44/23	55/22	41/28	43/25	33/29	34/27	48/48
3	*Pa. westermani*	Philippines	1	1	–	23/2	38/17	37/24	37/25	40/27	38/25	36/22	37/28	40/27	26/27	26/25	36/48
4	*Pa. westermani*	Thailand	1	1	0	–	40/17	42/24	44/23	45/27	46/25	43/22	44/30	46/27	36/27	37/25	49/48
5	*Pa. siamensis*	Thailand	3	2	3	3	–	38/27	33/32	33/32	34/30	34/25	32/31	30/32	32/30	34/28	32/47
6	*Pa. heterotremus*	Thailand	2	3	3	3	5	–	22/25	33/13	35/23	29/14	34/14	30/15	33/23	33/21	33/46
7	*Pa. harinasutai*	Thailand	2	3	3	3	5	1	–	34/18	39/10	32/23	29/23	31/28	33/24	35/22	31/51
8	*Pa. miyazakii*	Japan	2	3	3	3	5	1	0	–	38/14	38/15	30/5	33/14	36/26	37/24	30/51
9	*Pa. ohirai*	Japan	1	2	2	2	4	2	1	1	–	35/21	30/17	30/24	38/22	38/20	33/53
10	*Pa. mexicanus*	Ecuador	2	3	3	3	5	0	1	1	2	–	34/18	31/19	37/21	37/21	27/46
11	*Pg. skrjabini*	China	3	2	3	3	4	2	1	1	2	2	–	23/17	36/27	36/25	25/50
12	*Pg. macrorchis*	Thailand	2	3	3	3	5	1	0	0	1	1	1	–	31/28	33/26	31/47
13	*E. cenocopiosus*	Wuyishan,China	3	4	4	4	6	1	2	2	3	1	3	2	–	2/2	39/45
14	*E. cenocopiosus*	Nanjing, China	3	4	4	4	6	1	2	2	3	1	3	2	0	–	40/43
15	*F. hepatica*	USA	21	21	21	21	20	19	21	20	21	19	20	20	20	20	–

of *E. cenocopiosus* are held at the Institute for Parasitic Diseases, Shanghai.

Total genomic DNA was prepared from frozen adult worms using standard extraction techniques. A single adult worm was used in each case. The two gene regions were amplified by the polymerase chain reaction (PCR) from 20~50 ng of worm DNA. For the CO1 region, the primers used were JB3 and JB4.5 as described by Bowles *et al*. (1993). For the ITS2, primers used were 3S (Bowles *et al*., 1995) and A28 (Blair *et al*., 1997). All sequence data from adult worms were determined directly from the PCR products. Cycle sequencing reactions were run on a Licor automated sequencer twice in both directions to confirm the sequence. In the case of *P. mexicanus*, sequencing reaction products were separated on an ABI 373 automated sequencer. PCR primers were used as sequencing primers (after fluorescent-labelling if appropriate).

Sequence alignments were performed manually using the sequence editor ESEE3 (Cabot & Beckenbach, 1989). Codon usage (CO1 gene) was derived from Garey & Wolstenholme (1989), except that the codon ATA was translated to isoleucine rather than methionine (Bowles *et al*., 1992) and AAA was translated to asparagine rather than lysine (Ohama *et al*., 1990). Phylogenetic trees were constructed in PAUP 3.1 using heuristic searches. Bootstrapping was done (1000 resamplings). For the CO1 data set, a sequence from *Fasciola hepatica* was used as an outgroup. For the ITS2 data set, no suitable outgroup was available. The trees inferred from ITS2 data were therefore rooted at the midpoint. Numbers of transitions, transversions and amino acid differences in pairwise comparisons among CO1 sequences and numbers of nucleotide differences in pairwise comparison among ITS2 sequences were calculated in MEGA (Kumar *et al*., 1993). Insertions and deletions were omitted from calculations.

Results and discussion

The CO1 alignment was 393 bases long including an insertion of one codon (3nt) in the outgroup, *F. hepatica*, relative to all the *Paragonimus* species/strains. Table 2 gives additional information about the CO1 sequences. The single most-parsimonious tree constructed using all sites in the alignment had a length of 431, a consistency index of 0.543 (0.494 when uninformative characters were omitted). The same tree topology was found by bootstrapping, and the bootstrap values are shown on the tree (fig. 1, right hand side). The third codon position is expected to be the most variable in a protein coding gene. Removal of all third codon positions from the analysis might increase the consistency index. When this was done, 30 trees of length 58 and consistency index of 0.69 were found. The strict consensus tree collapsed all branches indicated by an asterisk in fig. 1. This indicates that much of the phylogenetic information is contained in sites at third codon positions. PAUP found 3 equally most parsimonious trees (length 27 and consistency index 0.963) using inferred amino acid sequences. The strict consensus tree derived from these collapsed many internal branches among the lungflukes and therefore provided little information about relationships among

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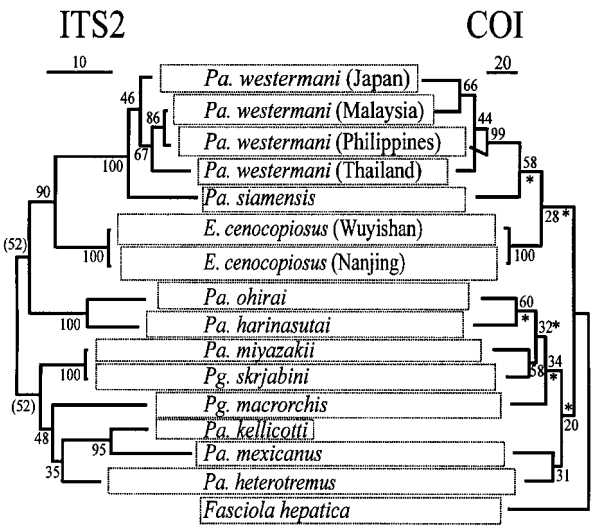

Fig. 1. Tree depicting the phylogenetic position of *Euparagonimus cenocopiosus* and *Pagumogonimus* (abbreviated to *Pg*.) species among *Paragonimus* (abbreviated to *Pa*.) using ITS2 (left side of figure) and CO1 sequences (right side of figure). Note that no CO1 sequence was available for *P. kellicotti*. Analyses were done using heuristic searches in PAUP 3.1. In each case, the tree found after bootstrap resampling (1000 replicates) was the same as the single most-parsimonious tree found using a heuristic search. Bootstrap values are shown on the trees. Branch lengths are those assigned by PAUP. Asterisks indicate branches that collapse in the strict consensus tree resulting after a search with third codon positions omitted.

them. Sequences of *E. cenocopiosus* appeared on the trees nested among sequences from *Paragonimus* species (fig. 1) or could not be resolved from these (trees constructed from nucleotide sequences omitting the third codon position or from amino acid sequences). *Paragonimus miyazakii* and *P. skrjabini* formed a well supported clade in trees constructed from nucleotide sequences (all codon positions (fig. 1), and when third codon positions were omitted).

Table 2 shows transition/transversion ratios in pairwise comparisons for the CO1 sequences. These suggest that transitions are approaching saturation, even among the lungflukes. Weighting transversions more heavily than transitions (various different weighting schemes, results not shown) yielded trees very similar to those constructed from first and second codon positions, or from amino acid sequences.

The ITS2 gene alignment is 364 bases in length ignoring a few short insertions/deletions. All the insertions and deletions were excluded from any calculation (or treated as 'missing data' in parsimony analyses). Table 3 provides other statistics concerning the alignment. The single most-parsimonious tree (fig. 1, left hand side), which was midpoint rooted because of the lack of an appropriate outgroup, had a length of 139 and consistency index of 0.784 (0.674 when uninformative characters excluded). The same tree was found as the consensus tree after 1000 rounds of bootstrapping. In this tree, *Euparagonimus cenocopiosus* was placed among *Paragonimus* species. It is possible to arrange the ITS2 tree to present *E. cenocopiosus* as if it were an outgroup. However, pairwise distances among the taxa (table 3) are such as to indicate that this would not be appropriate.

The two species assigned by Chen and others to the genus *Pagumogonimus* (*P. skrjabini* and *P. macrorchis*) are well separated on the trees. In each case, their closest relatives are undisputed members of the genus *Paragonimus*. This is particularly striking for *P. skrjabini*, which is very closely related to *P. miyazakii* from Japan (and see Blair *et al*., 1997). The former species has a metacercaria with 72 flame cells – the original diagnostic feature of *Pagumogonimus*. The latter has 60 flame cells in the metacercaria (Maejima *et al*., 1971), a feature in common with many other species of *Paragonimus*. A morphometric analysis of adult specimens of paragonimids reported by Zhan *et al*. (1997) also included *P. skrjabini* and *P. macrorchis*. In his tree, these species were well separated and the closest sister species of each was a member of *Paragonimus* or of *Euparagonimus*. The non-monophyly of representatives of *Pagumogonimus* on our trees, and the close relationship between *P. skrjabini* and *P. miyazakii* indicate that *Pagumogonimus* is not a natural taxon.

Table 3. Pairwise differences among ITS2 nucleotide sequences (ignoring the few short deletions/insertions). Abbreviations of generic names as in table 1.

	Species	Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Pa. westermani*	Japan	–	6	7	10	16	27	34	25	35	31	27	25	34	23
2	*Pa. westermani*	Malaysia		–	1	8	16	31	36	28	37	32	30	28	38	23
3	*Pa. westermani*	Philippines			–	9	17	32	37	29	38	32	31	29	37	24
4	*Pa. westermani*	Thailand				–	20	34	39	32	40	33	33	32	42	26
5	*Pa. siamensis*	Thailand					–	37	43	33	41	42	36	33	44	30
6	*Pa. heterotremus*	Thailand						–	28	16	30	26	20	16	23	22
7	*Pa. harinasutai*	Thailand							–	25	17	34	28	25	34	29
8	*Pa. miyazakii*	Japan								–	27	30	23	0	22	21
9	*Pa. ohirai*	Japan									–	35	28	27	36	28
10	*Pa. mexicanus*	Ecuador										–	18	30	33	29
11	*Pa. kellicotti*	USA											–	23	28	27
12	*Pg. skrjabini*	China												–	22	21
13	*Pg. macrorchis*	Thailand													–	29
14	*E. cenocopiosus*	China														–

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Sequences from the sole representative of *Euparagonimus*, *E. cenocopiosus*, were rather distant from all other lung flukes in the trees in fig. 1. However, in all trees, the *Euparagonimus* sequences are nested among the lungflukes in a way that would render *Paragonimus* paraphyletic if *Euparagonimus* were to be recognized as a distinct taxon. Although the metacercariae and adults of *Euparagonimus* differ from *Paragonimus* species in the length of the excretory bladder, their anatomy does not otherwise set them apart from *Paragonimus*. Indeed, in a recent morphometric analysis of lungflukes (Zhan *et al*., 1997), *E. cenocopiosus* was nested among species assigned to the genera *Paragonimus* and *Pagumogonimus*. It seems that this species should not be placed in a distinct genus.

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References

Blair, D., Agatsuma, T., Watanobe, T., Okamoto, M. & Ito, A. (1997) Geographical genetic structure within the human lung fluke, *Paragonimus westermani*, detected from DNA sequences. *Parasitology* **115,** 411–417.

Blair, D., Waikagul, J., Honzako, Y. & Agatsuma, T. (1998) Phylogenetic relationships among the Thai species of *Paragonimus* inferred from DNA sequences. *Proceedings of the 9th International Congress of Parasitology, Chiba, Japan, 24–28 August 1998*. Monduzzi International, Bologna.

Blair, D., Xu, Z. B. & Agatsuma, T. (1999) Paragonimiasis and the genus *Paragonimus*. *Advances in Parasitology* **42,** 113–222.

Bowles, J., Blair, D. & McManus, D.P. (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54,** 165–174.

Bowles, J., Hope, M., Tiu, W.U., Liu, X. & McManus, D.P. (1993) Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica* **55,** 217–229.

Bowles, J., Blair, D. & McManus, D.P. (1995) A molecular phylogeny of the human schistosomes. *Molecular Phylogenetics and Evolution* **4,** 103–109.

Cabot, E.L. & Beckenbach, A.T. (1989) Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Computer Applications in the Biosciences* **5,** 233–234.

Chen, H.T. (1962) Some new Chinese paragonimid trematodes: with notes on certain technical problems in the study of the group. *Acta Scientia Naturae Univsitas Sunyatseni* (3), 58–64 (in Chinese).

Chen, H.T. (1963) Taxonomic system of Family Paragonimidae Dollfus, 1939. Abstracts of Papers for the 1963 Science Conference of Chung Shan Medical College, p. 45, Canton, Nov. 1963 (in Chinese).

Chen, H.T. (1964a) The Chinese species of paragonimid trematodes, their phylogenetic relationship and faunal distribution. *Acta Parasitologica Sinica* **1,** 53–68 (in Chinese, English abstract).

Chen, H.T. (1964b) Morphological studies on the adult *Paragonimus* (*M.*) *macrorchis* Chen, 1962, with a consideration on the taxonomy of Paragonimidae. *Acta Zoologica Sinica* **16,** 381–392.

Chen, H.T. (1965) *Paragonimus*, *Pagumogonimus* and a *Paragonimus*-like trematode in man. *Chinese Medical Journal* **84,** 781–791.

Chen, H.T. (1977) *Abstracts of Academic Conferences in Guangzhou, Trematode Section.* pp. 109–122, Guangzhou, Zhong Shan Medical College.

Chen, H.T. (Ed.) (1985) Paragonimidae Dollfus, 1939. pp. 19–107 in *Fauna Sinica: Platyhelminthes, Trematoda, Digenea*. Beijing, Science Press (in Chinese).

Chen, H.T. & He, G.T. (1965) Observations on adult and metacercariae of *Euparagonimus cenocopiosus* Chen 1962. *Acta Parasitologica Sinica* **2,** 257–264 (in Chinese).

Fu, A.Q., Lu, X.S., Tao, J.P., Du, Q.L. & Zhang, G.X. (1990) [Note on a new trematode of the genus *Euparagonimus* in Hongze, China]. *Journal of Jiangsu Agricultural College* **11,** 39–40 (in Chinese).

Garey, J.R. & Wolstenholme, D.R. (1989) Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a tRNA ser AGN that contains a dihydrouridine-arm replacement loop, and of serine-specifying AGA and AGG codons. *Journal of Molecular Evolution* **28,** 374–387.

Kumar, S., Tamura, K. & Nei, M. (1993) *MEGA: Molecular Evolutionary Genetics Analysis v 1.0*. Pennsylvania State University, University Park, Pa 16802, USA.

Kurochkin, Y.V. (1987) [*Trematodes of the fauna of the SSSR, Paragonimidae.*] Moscow, Nauka (in Russian).

Maejima, J., Kamo, H. & Hatsushika, R. (1971) Studies on *Paragonimus miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961. 2. Second intermediate host and intracrab stage. *Yonago Acta Medica* **15,** 35–44.

Miyazaki, I. (1974) Lung flukes in the world – morphology and life history. pp. 101–135 *in* Sara, M. *et al*. (*Eds*) *A symposium on epidemiology of parasitic diseases*. Tokyo, International Medical Foundation of Japan.

Miyazaki, I., Kifune, T. & Lamothe-Argumedo, R. (1980) Taxonomical and biological studies on the lung flukes of Central America. *Occasional Publications of the Department of Parasitology, School of Medicine, Fukuoka University* **2,** 1–28.

Ohama, T., Osawa, S., Watanabe, K. & Jukes, T.H. (1990) Evolution of the mitochondrial genetic code IV. AAA as an asparagine codon in some animal mitochondria. *Journal of Molecular Evolution* **30,** 329–332.

Ollivier, G., Boussinesq, M., Albaret, J.L., Cumberlidge, N., Farhati, K., Chippaux, J.P. & Bayssade-Dufour, C. (1995) Étude épidémiologique d'une distomatose à *Paragonimus* sp. au sud-Cameroun. *Bulletin de la Société de Pathologie Exotique* **88,** 164–169 (in French, English abstract).

Zhan, X.M., Li, Z.Y., Xu, X.C. & Li, Q.Y. (1997) Studies on the numerical taxonomy of lung flukes and their application. *Supplement to the Journal of Sun Yatsen University*, no. 1, 6–10.

Zhong, H.L., He, L.Y., Xu, Z.B. & Cao, W.J. (1981) Recent progress in studies of *Paragonimus* and paragonimiasis control in China. *Chinese Medical Journal* **94,** 483–494.

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